BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	R 2022-018
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	
(35 ILL. ADM. CODE 620)	)	

NOTICE OF FILING

PLEASE TAKE NOTICE that I have today filed with the Office of the Clerk of the Illinois Pollution Control Board, the **ILLINOIS ENVIRONMENTAL PROTECTION AGENCY'S POST HEARING COMMENTS**, a copy of which is served upon you.

Respectfully submitted,

Dated: March 3, 2023

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,

Sara Terranova
Division of Legal Counsel
Illinois Environmental Protection Agency
1021 North Grand Avenue East
P.O. Box 19276
Springfield, IL 62794-9276
(217) 782-5544

BY: /s/ Sara Terranova

THIS FILING IS SUBMITTED ELECTRONICALLY

SERVICE LIST

ILLINOIS POLLUTION CONTROL BOARD Don Brown, Clerk Don.Brown@illinois.gov Vanessa Horton, Hearing Officer Vanessa.Horton@illinois.gov James R. Thompson Center 100 W. Randolph, Suite 11-500 Chicago, IL 60601	ILLINOIS ENVIRONMENTAL PROTECTION AGENCY Sara Terranova, Assistant Counsel Sara.Terranova@illinois.gov 1021 North Grand Avenue East Springfield, IL 62794-9276
ILLINOIS ATTORNEY GENERAL Ellen F. O'Laughlin, Asst Attorney General Ellen.Olaughlin@ilga.gov Jason James, Asst. Attorney General Jason.james@ilag.gov 69 W. Washington, Suite 1800 Chicago, IL 60602	ILLINOIS DEPARTMENT OF NATURAL RESOURCES Renee Snow, General Counsel Renee.Snow@illinois.gov One Natural Resources Way Springfield IL 62702-1271
Metropolitan Water Reclamation District Jorge Mihalopoulos, Head Asst. Attorney Jorge.mihalopoulos@mwrd.org Susan Morkalis morkaliss@mwrd.org, General Counsel J. Mark Powell, Senior Attorney PowellJ@mwrd.org 100 E. Erie Street Chicago, IL 60611	Melissa Brown, Melissa.Brown@heplerbloom.com Illinois Environmental Regulatory Group 4340 Acer Grove Drive Springfield, IL 62711
Joshua R. More Josh.More@afslaw.com Bina Joshi Bina.Joshi@afslaw.com Sarah Lode Sara.lode@afslaw.com Arent,Fox Schiff LLP 233 South Wacker Drive, Suite 7100 Chicago, IL 60606	Fredric Andes fandes@btlaw.com Barnes & Thornburg LLP 1 North Wacker Drive Suite 4400 Chicago, IL 60606
Nessa Coppinger ncoppinger@bdlaw.com Daniel Schulson Dschulson@bdlaw.com Matthew Schneider mschneider@bdlaw.com Beveridge & Diamond 1900 N. St. NW Washington D.C., 20036	Stephen Risotto-Senior Director srisotto@americanchemistry.com Aleacia Chinkhota Aleacia_chinkhota@americanchemistry.com American Chemistry Council 700 2nd Street, NE Washington, DC 20002

Brown, Hay & Stephens LLP Claire Manning cmanning@bhslaw.com aschuering@bhslaw.com 205 S. Fifth Street, Suite 1000 P.O. Box 2459 Springfield, IL 62705	Sorling Northrup James Morphew Jmmorphew@sorlinglaw.com 1 North Old Capitol Plaza Suite 200 P.O. Box 5131 Springfield, IL 62705

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ILLINOIS ENVIRONMENTAL PROTECTION AGENCY'S RESPONSE, POST HEARING COMMENTS, AND ERRATA SHEET

NOW COMES the Illinois Environmental Protection Agency ("Illinois EPA or Agency"), by and through one if its attorneys, and submits the following comments:

Post Hearing Comments

Comment 1: Class I: Potable Resource Groundwater IS Drinking Water.

Though groundwater may be used for drinking, groundwater is not the same as drinking water. Drinking water is water that has been treated and is being served to customers of a Public Water Supply. Groundwater quality standards are designed for the protection of human health during the consumption of groundwater under circumstances when no treatment is provided, such as in private wells, but also for the protection of other groundwater uses, such as irrigation or livestock use, if that use has a more sensitive end point than human consumption. More importantly, groundwater standards are designed to protect the groundwater resource for future use.

In its Final Order, the Board discusses the bases and reasoning behind the structure of Part 620. *In the Matter of Groundwater Quality Standards (35 Ill. Adm. Code 620)* R89-14(B) (Nov. 7, 1991). Just because groundwater quality standards have never previously been proposed for PFAS chemicals, does not change the fact that the same regulatory precepts apply to any constituent being evaluated for a groundwater quality standard. The Board highlights that Part 620

originates from Section 8 of Illinois Ground Water Protection Act ("IGPA"). R89-14(B) at 3. In addition, before quoting the policy statement of the IGPA, the Board states "[T]he IGPA is a multi—faceted policy and program statement designed to provide that protection and to assure the continued viability of the State's groundwater resources." *Id*.

The Board made it clear when discussing Class I Groundwater, that it should be maintained in a potable state. *Id.* at 10-12. Further, the Board discusses that non-degradation provisions are limited to the high-quality Class I and Class III resource groundwater. *Id.* 15-18. Therefore, from the initial adoption of Part 620, the goal of groundwater standards has been to protect the resource from contaminants at concentrations that allow its use without treatment whenever possible.

Comment 2: Illinois EPA's Use of U.S. EPA's Toxicity Hierarchy for the Selection of Toxicity Values

Pages 6 - 9 of Ms. Hawbaker's written testimony (p. 45 - 48 of the December 7, 2021, initial filing) discusses the basis for Illinois EPA's selection of toxicity values for calculating health-based concentrations pursuant to Part 620, Subpart F and Appendix A. Illinois EPA's use of U.S. EPA's toxicity hierarchy to select toxicity values for Part 620 is first discussed in PCB R08-18. As stated in Ms. Hawbaker's testimony, the Board's R08-18 final opinion and order, dated October 4, 2012, affirmed that the Agency appropriately relied on U.S. EPA's toxicity hierarchy in proposing updates to the Part 620 groundwater quality standards. Since 2012, U.S. EPA has issued updates to its toxicity hierarchy on two occasions: May 16, 2013, and May 26, 2021.

U.S. EPA's May 16, 2013, Tier 3 Toxicity Value White Paper (paper), included as Attachment 1C 2 (p. 518), of the December 7, 2021, initial filing, provided a ranking of Tier 3 toxicity sources preferred by U.S. EPA. The Tier 3 toxicity sources listed in the paper are ranked as follows:

1) United States Health and Human Services Agency for Toxic Substances and Disease Registry ("ATSDR") Dose Minimal Risk Levels ("dose MRLs").
2) California EPA, Office of Environmental Health Hazard Assessment ("OEHHA").
3) Provisional Peer-Reviewed Toxicity Values ("PPRTV") "Appendix".
4) Health Effects Assessment Summary Table ("HEAST").

The paper also states, "These sources are credible (rely on best available science, have undergone a high degree of scrutiny and peer review, are often considered by other Agencies)." (p. 14 of the paper).

On May 26, 2021, U.S. EPA's Office of Land and Emergency Management ("OLEM") issued a Memorandum providing recommendations on the use of chronic versus subchronic noncancer values for Superfund human health assessments, included as Attachment 1C 3 (p. 603) of the December 7, 2021, Initial Filing. The purpose of the memo was to recommend the use of subchronic toxicity values in lieu of chronic values for five inhalation and 14 oral toxicity values of the 32 toxicity values selected for review. OLEM selected the subchronic toxicity values for update based on newer chemical studies and because these subchronic toxicity values are more stringent than older chronic toxicity values derived from a toxicity source listed higher on the Tier.

Most recently, in its May 2022 update, U.S. EPA updated the Regional Screening Level ("RSL") User's Guide to allow the use of U.S. EPA Office of Water PFAS toxicity values when calculating RSLs.

At the December 7, 2022, IPCB hearing, three witnesses provided testimony regarding Illinois EPA's use of U.S. EPA's toxicity hierarchy when selecting toxicity values for the calculation of health-based Class I potable resource groundwater standards: Ms. Carey, Dr. Prueitt, and Mr. Risotto.

Testifying on behalf of the International Molybdenum Association, Ms. Carey's testimony focuses on Illinois EPA's selection of U.S. EPA's Integrated Risk Information System ("IRIS") chronic molybdenum oral reference dose, published in November of 1992, listing a critical effect of increased uric acid levels, instead of ATSDR's intermediate dose MRL, published in May of 2020, listing a critical effect of renal proximal tubule hyperplasia, when calculating health-based standards. Although her testimony stated the IRIS value is outdated, during questions about U.S. EPA's use of the IRIS value for calculating health-based standards, Ms. Carey acknowledged that U.S. EPA did utilize the IRIS value over the ATSDR value for the calculation of chronic health-based screening levels.

Illinois EPA selected U.S. EPA's IRIS toxicity value for calculating a health-based concentration for multiple reasons:

- IRIS is the Tier 1 toxicity source listed in U.S. EPA's hierarchy; whereas the toxicity value recommended by Ms. Carey is from a Tier 3 ranked toxicity source (ATSDR).
- The IRIS toxicity value is based on chronic exposure, which is the exposure type used in calculating health-based standards for noncancer health effects for residential populations. The ATSDR toxicity value is based on intermediate (subchronic exposure). In 2021, U.S. EPA updated its hierarchy to select subchronic values for certain chemicals when more recent data is available from Tier 3 sources. Molybdenum's subchronic toxicity value was not reviewed to replace the present IRIS chronic toxicity value.
- U.S. EPA uses the IRIS toxicity value for developing chronic health-based screening levels for residential populations. U.S. EPA uses the ATSDR toxicity value for

developing subchronic health-based screening levels for construction worker populations.

- ATSDR's subchronic toxicity value is not derived from benchmark dose (BMD) or pharmacokinetic (PK) models using time-weighted averages. ATSDR calculated its intermediate dose MRL by dividing the selected study's NOAEL (17 mg/kg-day) by uncertainty/modifying factors equaling 300. As a result, it is not appropriate to use the subchronic value for evaluating chronic exposure without applying an additional uncertainty factor of 10 for subchronic to chronic extrapolation.
- IRIS molybdenum toxicity value has a critical effect of increased uric acid. Increased molybdenum ingestion results in decreased copper absorption. As a result, more copper is excreted from the body as higher amounts of molybdenum are ingested. Copper assists in the excretion of uric acid. When low dietary copper levels are present, uric acid builds up. The Koval'skiy, et al., study selected by IRIS is a human health study conducted in a region selected specifically for its high molybdenum content in plants and its low copper content due to this inverse relationship. For ATSDR's toxicity value, an assumption was made that the average copper intake of the U.S. population exceeds dietary requirements. Therefore, animal studies involving inadequate levels of copper were not considered relevant in the derivation of its toxicity value. Although ATSDR included a modifying factor of 3 to address a concern that reproductive/developmental effects may occur in populations with marginal copper intakes, the use of the IRIS toxicity value is specifically protective for those with marginal copper intakes for increased uric acid levels. ATSDR's molybdenum toxicity profile is included as Attachment 1.

Testifying on behalf of 3M Corporation, Dr. Prueitt's testimony characterizes Illinois EPA's use of U.S. EPA's toxicity hierarchy as, "an inappropriate and unsound methodology to develop proposed groundwater standards for six different per- and polyfluoroalkyl substances or PFAS," (p. 47:l. 21-24 of transcript of December 7, 2022, hearing). Dr. Prueitt further testifies, "State and federal agencies should follow established human health risk assessment practice in developing toxicity values for use in the derivation of regulatory standards such as groundwater standards." (p. 48:l. 2-7 of transcript of December 7, 2022, hearing).

Dr. Prueitt refers to Slide 3 in Exhibit No. 27 for practices for the development of toxicity values for use in deriving health-based standards. Illinois EPA notes the section of the slide, titled, "Use of Existing Toxicity Values," contains two items:

- Evaluate scientific rigor and appropriateness of available toxicity values
- Choose a value that is scientifically supported.

Per Dr. Prueitt's testimony, "To the extent that IEPA wishes to rely on toxicity values derived by other agencies, IEPA should first conduct an independent evaluation of the scientific rigor and appropriateness of the available toxicity values to ensure that the most scientifically supported toxicity values are chosen as the basis for the proposed groundwater standards. IEPA has not done that. Their failure to engage in such an evaluation resulted in proposed PFAS standards that are technically infeasible. They're overly conservative, unreliable, and inappropriate for groundwater standards." (p. 49:l. 2-14 of transcript of December 7, 2022, hearing).

Dr. Prueitt further states, "IEPA stated it chose the ATSDR minimal risk level or MRL for PFOS, P-F-O-S, because ATSDR relies on more recent toxicity studies that the USEPA's Office of Water's PFOS toxicity value derived in 2016. Just because a study is published more recently, however, does not necessarily mean it is more scientifically sound or a better choice for an

endpoint on which to derive a toxicity value." (p. 51:l. 22-24, p. 52:l. 1-6 of transcript of December 7, 2022, hearing) The Illinois EPA agrees that more recent studies are not necessarily better studies; however, in the case of Illinois EPA's selection of ATSDR's PFOS dose MRL over U.S. EPA's Office of Water toxicity value used for the development of its 2016 health advisory level ("HAL"), Illinois EPA notes that in June 2022, the Office of Water replaced its 2016 HAL with a more stringent interim HAL of 0.02 ng/L, stating, "The new published peer-reviewed data and draft EPA analyses (U.S. EPA, 2021a, b) indicate that the levels at which negative health outcomes could occur are much lower than previously understood when the Agency issued its 2016 HAs for PFOA and PFOS (70 parts per trillion or ppt). EPA's 2021 draft non-cancer reference doses (RfDs) based on human epidemiology studies for various effects (e.g., developmental/growth, cardiovascular health outcomes, immune health) range from ~10-7 to 10-9 mg/kg/day. These draft RfDs are two to four orders of magnitude lower than EPA's 2016 RfDs of 2 x 10-5 mg/kg/day (U.S. EPA, 2021a, b)." (U.S. EPA Technical Fact Sheet: Drinking Water Health Advisories for Four PFAS (PFOA, PFOS, GenX chemicals, and PFBS, June 2022, included as Attachment 2) U.S. EPA recognizes that the Office of Water's 2016 HAL toxicity value is no longer technically sound for calculating a PFOS health-based standard.

Dr. Prueitt states in her testimony that ATSDR only considered studies with animal strains that had pharmacokinetic model parameters available for predicting serum concentrations of PFAS in the animals from the administered dose, stating it was "scientifically inappropriate". (p. 53: l. 1-7 of transcript of December 7, 2022, hearing). However, when evaluating animal toxicity to convert to a human equivalency dose using a pharmacokinetic model, animal strains with established pharmacokinetic model parameters are used. Without pharmacokinetic model

parameters from an animal strain, a pharmacokinetic model cannot be used to predict serum concentrations to calculate human-equivalency doses.

Illinois EPA notes that Dr. Prueitt does not further discuss why the toxicity values selected by Illinois EPA are not scientifically supported toxicity values, nor does she recommend alternative toxicity values available that have undergone peer-review and public comment. PPRTV, ATSDR, California EPA, and U.S. EPA Office of Water are all accepted sources by U.S. EPA. U.S. EPA considers these sources as credible, as relying on best available science, and as having undergone a high degree of scrutiny and peer review. Therefore, the toxicity values selected by Illinois EPA are scientifically supported toxicity values.

Since Illinois EPA's Part 620 amendments initial filing, U.S. EPA has added the six proposed PFAS to its RSL tables. With the exception of the PFOA cancer toxicity value (oral slope factor or "CSF"), Illinois EPA selected the same PFAS toxicity values included in the RSL tables. Page 11 of Ms. Hawbaker's written testimony (p. 50 of the December 7, 2021, initial filing) discusses the Agency's basis for selecting the CSF derived by California EPA over the 2016 U.S. EPA Health Advisory CSF selected by RSL. Since U.S. EPA's publication of its 2016 Lifetime Health Advisory, additional human and animal studies reported more susceptibility to cancer than previously thought. First, regarding pancreatic tumors from the National Toxicology Program ("NTP") and second, more recent studies providing evidence that liver tumors may be formed via multiple modes of action, in addition to the PPARα response in rats, as noted in evaluations conducted by International Agency for Research on Cancer ("IARC"). U.S. EPA's June 2022, PFOA interim health advisory document discusses its derivation of multiple draft candidate CSFs indicating that PFOA is a more potent carcinogen than described in the 2016 health advisory document. The interim health advisory states U.S. EPA did not select one overall draft CSF to

determine a cancer risk candidate drinking water value; however, an initial evaluation of the multiple candidate CSFs indicates the 1.0E-06 cancer risk drinking water concentrations are either "comparable to or greater than the lifetime iHA value for PFOA" of 0.004 ppt.

Dr. Prueitt also states, "This hierarchy is not intended for choosing a toxicity value as the basis for an enforceable groundwater standard and it is not appropriate to use it for this purpose." (p. 49:l. 19-23 of transcript of December 7, 2022, hearing). Dr. Prueitt testifies U.S. EPA's hierarchy is intended for use in selecting toxicity values for the derivation of RSLs, further stating RSLs are not intended to be legally enforceable standards, but instead are guidance values used for screening purposes to determine if further investigation is warranted.

Illinois EPA wishes to clarify that RSLs are health-based levels calculated for the protection of human health. If the health-based screening levels are not met, additional action is warranted because the concentrations found may not be protective of human health. RSLs are the equivalent of Illinois EPA's Part 742 Tier 1 health-based remediation objectives, Tier 1 remediation objectives are enforceable standards in Illinois. Illinois EPA first proposed the use of U.S. EPA's toxicity hierarchy in 2008 (*see* PCB. R08-18) for selecting toxicity values for the development of Part 620 Class I potable resource groundwater quality standards. The hierarchy is also used for calculating Part 742 remediation objectives. Pages 8-12 of Ms. Hawbaker's written testimony (p. 48-51 of December 7, 2021, initial filing), discussed the history of Illinois EPA's use of the toxicity hierarchy and the Board's support of its use in calculating health-based potable resource groundwater standards.

Class I potable resource groundwater standards calculated using the methods at Appendix A are based on the protection of human health when ingesting groundwater. Whether a health-based objective is called a screening level, remediation objective, or a standard, the premise is the

same: it is a value intended for the protection of human health. As noted in Ms. Hawbaker's written testimony, submitted in the December 7, 2021, initial filing, the Board's R89-14(B) final opinion and order dated November 7, 1991, stated:

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

As Class I potable resource groundwater quality standards are intended to preserve drinkability of the State's groundwater, health-based standards are the appropriate standards.

Testifying on behalf of American Chemistry Council, Mr. Risotto also discusses U.S. EPA toxicity hierarchy, but his testimony fails to mention the 2013 and 2021 updates to the hierarchy, specifically the 2013 toxicity hierarchy white paper ranking Tier 3 toxicity sources. Mr. Risotto's testimony regarding the shelf life of toxicity values, does not discuss that RSL updates toxicity values within its tables every six months (May and November). Therefore, when newer data is more appropriate to use, RSL updates its toxicity values to remain protective of human health.

Comment 3: The Use of Background Concentrations for Setting Groundwater Quality Standards.

The Agency has addressed background concentrations relative to groundwater quality standards previously. The Agency addressed background concentrations of PFAS and other chemicals in its pre-filed answer to the PFAS Regulatory Coalition Question 17 and further addressed how background concentration of PFAS and other chemicals are dealt with in testimony. Trans. June 21, 2022, Pgs. 19-31. The IGPA states a preference for numerical standards rather than narrative standards. 415 ILCS 55/8(b)(3). Further, the Board supports groundwater quality

standards that maintain Class I groundwater in a drinkable state without treatment. R89-14(b) at 10-12. Finally, the Board makes the following statement in relevant part regarding Part 620 "…the instant regulations do not create or require any new corrective action program; all such programs are part of other regulations…" (emphasis original). *Id*. at 25. This statement by the Board is consistent with testimony provided by the Agency, cited above and has not changed from R89-14(B) to R22-18.

Comment 4: Illinois EPA's Proposed Update of Exposure Factors to Account for Child Exposure

Illinois EPA evaluated different receptor populations when determining the appropriate exposure factors, calculating drinking water ingestion rates for five populations:

- The average adult water ingestion rate currently in Part 620, Appendix A.
- The average adult water ingestion rate based on adult exposure factors used by U.S. EPA RSL. U.S. EPA updated the adult exposure factors in 2014. The update is included as Attachment 1B 1 (p. 505 of the December 7, 2021, initial filing).
- An average pregnant woman water ingestion rate, based on U.S. EPA's Exposure Factors Handbook.
- An average lactating woman water ingestion rate, based on U.S. EPA's Exposure Factors Handbook.
- An average child (0 – 6 years of age) water ingestion rate. This is the exposure population selected by RSL to account for childhood exposure to chemicals for both its cancer and noncancer calculations and used by Illinois EPA Part 742 (TACO) for developing noncancer remediation objectives in other media.

Below is a table depicting the daily water ingestion rates for the populations noted above.

Exposure Population	Daily Water Ingestion Rate (L/kg bw-day)
Average Adult (Current Part 620)	0.0286
Average Adult (RSL)	0.0313
Pregnant Women[1]	0.0333
Lactating Women[1]	0.0469
Child (0 – 6 years of age)	0.0520

[1] The pregnant women and lactating women water ingestion rates are protective only of the pregnant or lactating women. The water ingestion rates do not consider the protection of a fetus or a breastfeeding infant.

Illinois EPA selected the child water ingestion rate of 0.052 L/kg bw-day because it accounts for the highest water ingestion rate of the populations evaluated and is protective for all populations noted above.

Further, the use of a child exposure population (0.78 L/day for 15 kg body weight, equaling 0.052 L/kg bw-day) is consistent with RSL and with Illinois EPA Part 742 TACO noncancer remediation objectives for other media. Mr. Risotto's testimony discusses the application of different exposure factors depending on particular endpoints. However, the consistent application of exposure factors protective of all populations noted above is the most reasonable when dealing with a large number of chemicals such as Part 620, Part 742, and U.S. EPA's RSLs.

Comment 5: Relative Source Contribution Value Selection

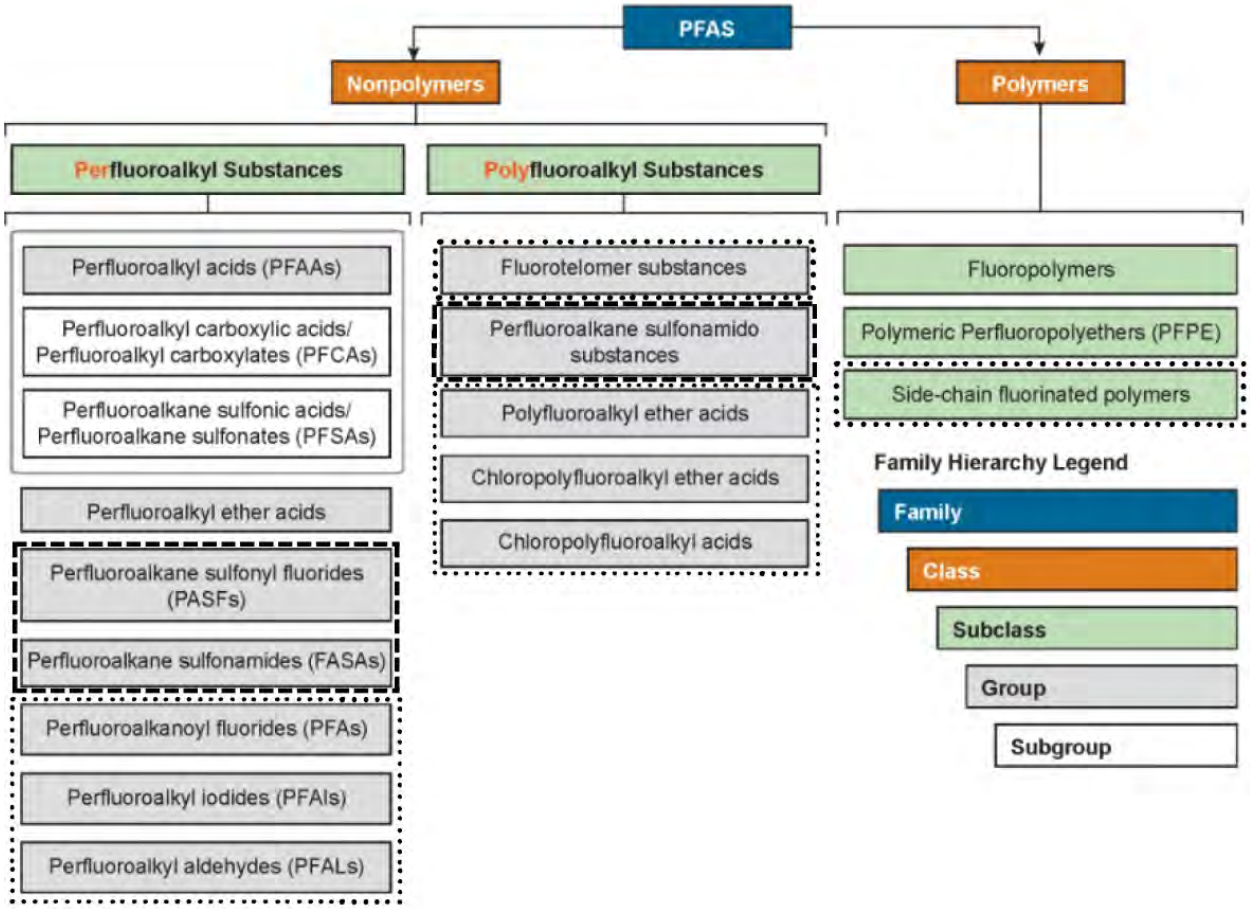
The relative source contribution ("RSC") is the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion when calculating a health-based noncancer drinking water level. RSCs do not apply when calculating health-based cancer drinking water levels. Therefore, Illinois EPA's proposed PFOA groundwater quality standard does not apply for an RSC for its calculation to determine a health-based cancer level.

Illinois EPA, U.S. EPA, and other states use U.S. EPA's October 2000, Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. *See* Attachment 3. This document discusses and provides a figure of the exposure decision tree discussed in both Dr. Prueitt's and Mr. Risotto's prefiled testimonies and responses to questions, and in Dr. Prueitt's testimony before the Board. This Decision Tree (figure 4-1) is located on page 4-8 U.S. EPA's October 2000 document and is included as a separate attachment. *See* Attachment 4.

Illinois EPA's process through the exposure decision tree is as follows:

1. Identify a population(s) of concern. Illinois EPA selected a child 0 – 6 years of age as the population of concern.
2. Identify relevant exposure sources/pathways. The general population is exposed to PFAS through food and water ingestion, particulate dust ingestion, indoor and outdoor inhalation exposure, dermal exposure, and hand-to-mouth transfers of materials and soil containing PFAS. Several types of products contain PFAS, such as home products/building materials, cleaning products, food packaging, cookware, personal care products, and water or stain resistant clothing and furniture. PFAS is also used in a multitude of industrial processes, releasing PFAS emissions into the air. PFAS is bioaccumulative and can transfer to food sources such as livestock or wildlife used for human consumption, dairy milk, and produce. Food packaging containing PFAS can transfer to the food product. PFAS is prevalent in air as dust particles and vapors, with higher levels of PFAS in air found inside buildings than outdoors. Infants and children have increased hand-to-mouth transfers of dust particles from household items treated with PFAS (carpets, furniture, textiles).

Five of the six PFAS proposed for addition to Part 620 are classified as perfluoroalkyl acids ("PFAAs"). PFAAs consist of perfluoroalkane sulfonic acids or PFSAs (PFBS, PFHxS, and PFOS), and perfluoroalkyl carboxylic acids or PFCAs (PFOA and PFNA). PFAAs are terminal degradation products of other PFAS, meaning other groups of PFAS can break down and transform into PFAAs. Below is a figure of the PFAS Family from Interstate Technology Regulatory Council ("ITRC") PFAS Fact Sheets at: https://pfas-1.itrcweb.org/2-2-chemistry-terminology-and-acronyms/.



According to the above-referenced ITRC fact sheet, the subclass and groups of PFAS outlined with dotted lines are potential PFCA precursors. The groups of PFAS outlined with hashmark lines are potential PFSA precursors. Approximately 4,700 PFAS have

CAS Registry Numbers. Therefore, many PFAS may become exposure sources for the proposed PFAS groundwater quality standards due to breakdown and transformation processes.

3. Are adequate data available to describe central tendencies and high-ends for relevant exposure sources/pathways?

   Illinois EPA selected "No" for this box. There is insufficient data to quantify exposures from various sources for the population selected. A "No" decision moves the user to Box 4.

4. Are there sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources?

   Illinois EPA selected "No" for this Box. There is insufficient information available to characterize the likelihood of exposure to relevant sources. Studies have shown exposure through sources other than drinking water is occurring via inhalation, ingestion, and dermal exposures; however, the likelihood of exposure for each source cannot be quantified. Further, due to the large number of PFAA precursors within the PFAS family, characterization of exposure to relevant sources becomes more complicated. A "No" decision moves the user to Box 5.

5. Box 5 concludes the tree with two boxes:

   Box 5A. Use 20% of the RfD or POD/UF.

   Box 5B. Gather more information and re-review.

Illinois selected the recommended RSC of 20% of RfD. The selection of the 20% RSC is consistent with U.S. EPA's RSC determination in its 2016 PFOA and PFOS health advisories, and

in its 2022 PFOA and PFOS interim health advisories and PFBS and HFPO-DA (GenX) final lifetime health advisories that data available to quantify exposures from other relevant sources is lacking.

Michigan and Minnesota, two states specifically mentioned in Dr. Prueitt's December 7, 2022, testimony, developed RSCs of 50% based on measured PFAS blood serum level data from the National Health and Nutrition Examination Survey ("NHANES") for children 3 – 11 and for participants 12 years old or older. Predicted serum concentrations were calculated based on the states' selected reference doses (RfDs). The NHANES 95th percentile value, used as a background value, was subtracted from each PFAS predicted RfD serum concentration to determine a serum level that could be apportioned due to water ingestion. The apportioned serum level is then divided by the RfD serum concentration to determine a percentage of serum levels attributed to water ingestion (Box 13 of the decision tree). Illinois EPA does not agree this method is sufficient to quantify exposure from other sources.

Studies have shown PFAS exists in the bloodstream for virtually all populations of the world. However, not every person with measurable PFAS blood serum levels are drinking water with PFAS detections present. In Illinois, the 2021 statewide PFAS sampling of community water supplies showed 84% of supplies use water, either directly or from water purchases, that had no detections of the PFAS analyzed using a minimum reporting level of 2 ng/L for each of the 18 analytes evaluated. The statewide sampling included surface water sources and groundwater sources. Therefore, populations are being exposed from sources other than drinking water. U.S. EPA and many states have selected to use the default RSC of 20% due to limitations in the quantification of relevant sources other than water ingestion. U.S. EPA discusses its use of the exposure decision tree to determine there is insufficient data to quantitatively derive an RSC in its

2022 interim PFOA and PFOS and final PFBS and HFPO-DA health advisories. The 2022 U.S. EPA health advisories are included as Attachments 5, 6, 7, and 8.

Comment 6: The Use of Irrigation and Livestock Values as Groundwater Quality Standards

In selecting the proposed groundwater quality standard for selenium, Illinois EPA chose a value representing the beneficial use of groundwater as a resource for irrigation of crops and produce from the National Academy of Sciences "Water Quality Criteria", 1972, because the irrigation value of 0.02 mg/L is more stringent than the drinking water MCL of 0.05 mg/L. The irrigation table is included as Attachment 1I 19 (p. 4,832) of the December 7, 2021, Initial Filing. The proposed value is taken from the same source for irrigation standards that has been relied upon since the Board's first promulgation of the 35 Ill. Adm. Code Part 620 groundwater quality regulations in 1991. The source identifies a protective irrigation value for selenium based on use of irrigation water for up to 20 years on fine textured soils with a pH between 6.0 and 8.5.

Irrigation has long been practiced in Illinois. Attachment 5 of Illinois EPA's May 6, 2022, pre-filed responses reports that farmers have relied on supplemental well water irrigation has been practiced in certain areas of Illinois since at least 1926. The practice of crop irrigation has significantly increased in Illinois since 2012, as shown in Attachment 6 of Illinois EPA's May 6, 2022, pre-filed responses. According to the Illinois State Water Survey ("ISWS") in Attachment 7 of the Illinois EPA's May 6, 2022, pre-filed responses, irrigation in Illinois will continue to increase due to concerns of drought and changes in farming practices where seed contracts require assured crop yields.

The ISWS map, included as Attachment 8 of Illinois EPA's May 6, 2022, pre-filed responses, shows that the majority of soils in the state have pH levels between 6.0 and 7.5. Illinois

groundwater quality standards are intended to be protective for groundwater throughout the entire state, rather than only being protective in certain areas with certain soil conditions.

When testifying before the Illinois Pollution Control Board on December 7, 2022, Ms. Yost stated at p.37:l.8-13 that the basis for the 1972 National Academy of Sciences "Water Quality Criteria" beneficial use value for selenium of 0.02 mg/L has two bases. "The first for continuous irrigation which Illinois EPA and I agree don't occur in Illinois. The second is for the use on fine-grained or alkaline soils. And while there are certain fine-grained soils, the soil in Illinois is predominantly acidic or neutral."

This statement by Ms. Yost in incorrect; the actual document, filed as Attachment 1I 19 (p. 4,832) of the December 7, 2021, Initial Filing, states that the selenium value of 0.02 mg/L is recommended "for use up to 20 years on fine textured soils of pH 6.0 to 8.5." According to ISWS in Attachment 8 of Illinois EPA's May 6, 2022, pre-filed responses, average soil pH values in Illinois vary from mildly alkaline (7.0-7.5) primarily in central west and northwest regions of the State to strongly acidic (5.2-5.5) in extreme southern Illinois. Contrary to Ms. Yost's statement, ISWS does not conclude that soil in Illinois in predominantly acidic. Further, Ms. Yost states starting at p.37:l.23 of the December 7, 2022, testimony that the value selected for selenium was based on geographical areas with range crops that grow in arid climates. The narrative in the 1972 "Water Quality Criteria" document (Attachment 1I 20 (p. 4,834) of the December 7, 2021, Initial Filing) states the 0.02 mg/L recommendation is for use on forage crops, not range crops, and explicitly states, "Selenium is toxic at low concentrations in nutrient solutions, and only small amounts added to soils increase selenium content of forages to a level toxic to livestock." Certain chemicals can adversely affect irrigated crops or livestock forage at levels lower than those causing adverse effects on human health from drinking water use. As discussed in Illinois EPA's May 6,

2022, pre-filed responses, Illinois agriculture supports crop yield for forage crops, such as corn, which are known to be grown in fine textured soils in Illinois. Class I potable resource groundwater is used for beneficial use in Illinois for both irrigation and drinking water and should be protected for both applications. At p.38:l.8-10 of the December 7, 2022, testimony, Ms. Yost states she found references indicating the need for selenium supplementation of food for animals, however, she provided no such references for review. Illinois EPA has appropriately proposed a Class I standard of 0.02 mg/L for selenium to protect groundwater throughout the state for the beneficial use of irrigation.

Further, the use of an irrigation value as a Class I standard is not unusual. The boron Class I: Potable Resource Groundwater Quality Standard is based on the beneficial use of groundwater for the irrigation of plants. Boron's Class I standard of 2 mg/L is based on irrigation for use up to twenty years on fine-textured soils of pH 6.0 – 8.5 from the same source as the proposed selenium standard. Although a toxicity value became available in 2004 to calculate a boron health-based standard, Illinois EPA did not propose to update the boron Class I standard in its Part 620 amendments proposal filed February 19, 2008 (PCB No. R08-18). The reason Illinois EPA did not update the value is because the calculated health-based concentration of 5.6 mg/L is less stringent than the irrigation value of 2 mg/L, and therefore, not protective of the resource for beneficial use. The calculated health-based concentration with the proposed child exposure factors is 3.1 mg/L, also less stringent than the irrigation value. An update to the boron Class I standard is not proposed, as a different use (irrigation) requires a more stringent standard, as affirmed by the Board below.

When discussing Class II: General Resource Groundwater Quality Standards, the Board stated the following in its final opinion in R89-14(B):

> "Section 620.420 establishes standards for Class II: General Resource Groundwater. Because groundwaters are placed in Class II because they are quality-limited, quantity-limited, or both . . ., *it is necessary that the standards that apply to these waters reflect this range of possible attributes*. Among the factors considered in determining the Class II numbers are the capabilities of treatment technologies to bring Class II waters to qualities suitable for potable use . . . . Thus, many Class II standards are based on MCLs as modified to reflect treatment capabilities. *For some parameters[,] the Class II standards are based on support of a use other than potability (e.g., livestock watering, irrigation, industrial use) where the different use requires a more stringent standard* . . . . (emphasis by Agency)."

This statement is applicable for Class I groundwater as well. In addition to its use as a drinking water resource, Class I groundwater is used for irrigation and livestock watering. In some cases, these uses require a more stringent standard. Boron and selenium for the beneficial use of irrigation and fluoride for the beneficial use of livestock watering are such cases. The proposed values are protective for both human health and beneficial uses. The Board's November 7, 1991, R89-14(B) final order is included in the Agency's December 7, 2021, Initial Filing as Attachment 1A 3, beginning on page 424, of the filing.

Comment 7: Other States Actions for PFAS

According to the Interstate Technology and Regulation Council's ("ITRC") PFAS Fact Sheets' water tables, 30 states established 52 actions regarding PFAS in groundwater and/or drinking water as of January 2023, as seen in the table below. While Illinois numerical standards are low, they are not the most stringent to date. Eleven promulgated rules include concentrations lower than Illinois EPA's proposed six PFAS standards. For example, in 2021, Michigan promulgated Maximum Contaminant Levels ("MCLs"), for PFNA (6 ng/L), PFBS (420 ng/L) and PFHxS (51 ng/L), all lower than those proposed in Illinois (12 ng/L, 1,200 ng/L, and 77 ng/L respectively). Washington's 2022 State Action Levels ("SALs) are also lower than Illinois for the

same three PFAS standards referenced above. Both examples are applicable to both groundwater and drinking water.

For states taking multiple actions, allowable concentrations tend to decrease with updated standards/guidance values with few exceptions. For example, Rhode Island's 2017 Groundwater Quality Standard, listed as both a groundwater and drinking water rule, is 70 ng/L for the individual and sum concentration of PFOA and PFOS; in 2022, the state promulgated MCLs at 20 ng/L for the individual or sum concentration of PFOA, PFOS, PFNA, PFHxS, PFHpA, and PFDA. In 2018, Minnesota promulgated chronic Health Risk Limits ("HRLs") for PFOA (35 ng/L), PFOS (300 ng/L), PFBA (7,000 ng/L), and PFBS (7,000 ng/L). In 2021, Minnesota added chronic Health-Based Values ("HBVs") for PFHxS (47 ng/L) and PFHxA (200 ng/L). In 2022, Minnesota issued updated chronic HBVs that decreased the health-based concentrations of PFOS (15 ng/L) and PFBS (100 ng/L). Minnesota's HBVs are not promulgated, but Minnesota Department of Health uses the same methodology for setting promulgated HRLs and anticipates the HBVs will become HRLs.

Twenty-five of the state actions are based on a sum of selected PFAS, which accounts for the overall trend of seemingly higher concentrations than Illinois EPA's proposed individual standards. Connecticut established a groundwater protection criterion in 2018 for the combined sum of PFOA, PFOS, PFNA, PFHxS, and PFHpA (70 ng/L), four of the six PFAS proposed by Illinois, plus PFHpA. The sum of Illinois EPA's proposed standards for the same four PFAS is 98.7 ng/L. Maine's Interim Drinking Water Standard includes a sum of PFOA, PFOS, PFNA, and PFHxS at 70 ng/L and an individual standard for PFBS at 400 ng/L. Both the sum of Illinois EPA's proposed standards for the four PFAS previously discussed (98.7 ng/L) and its individual proposed standard for PFBS (1,200 ng/L) are less stringent than Maine's.

Another factor is States use of the 2016 U.S. EPA Office of Water's Lifetime Health Advisory for PFOA and PFOS of 70 ng/L for individual or sum concentrations. Fifteen of the standards/guidance values are based on the 2016 health advisory level. In June 2022, U.S. EPA Office of Water issued updated interim health advisories of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS, replacing the 2016 health advisories. All but two of the 15 state standards using the 2016 health advisory were last updated prior to 2022.

The table below shows U.S. EPA's and individual State's actions taken to develop and promulgate PFAS standards. The information in the table comes from water tables presented by ITRC on its PFAS Fact Sheets located at: https://pfas-1.itrcweb.org/fact-sheets/ . ITRC updated its water tables in January 2023 and the table below includes the January 2023 updates.

U.S. EPA/State	**Year**	**Type**	**Promulgated Rule**	**Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)**
U.S. EPA	2009	DW	N (PHA)	PFOA: 400 PFOS: 400
	2016	DW	N (LHA)	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70
	2019	GW	N (IR)	PFOA: 40 PFOS: 40
	2022	DW/GW	N (RSL)	PFOA: 60 PFOS: 40 PFOS-K: 40 PFNA: 59 PFBS: 6,000 PFHxS: 390 HFPO-DA: 60
	2022	DW	N (IHA)	PFOA: 0.004 PFOS: 0.02
	2022	DW	N (LHA)	PFBS: 2,000 HFPO-DA: 10
Alaska	2016	GW	Y	PFOA: 400 PFOS: 400
	2018	DW/GW	N	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70

U.S. EPA/State	Year	Type	Promulgated Rule	Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)
California	2021	DW	Y	PFOA: 10 PFOS: 40 PFBS: 5,000
Colorado	2018	GW	Y	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70
	2020	GW/SW (HH DW)	Y	PFOA: 70 PFOS: 70 PFNA: 70 PFBS: 400,000 PFHxS: 700 PFOSA: 70 8:2 FTS: 70 NEtFOSAA: 70 NMeFOSAA: 70 Combined PFOA/ PFOS/ 8:2 FTS/ NEtFOSAA/ NMEFOSAA/ PFOSA/ PFNA: 70
Connecticut	2018	DW/GW	N	Combined PFOA/ PFOS/ PFNA/ PFHxS/ PFHpA: 70
	2020	GW	N	PFOA: 16 PFOS: 10 PFNA: 12 PFHxS: 49
Delaware	2016	GW	N	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70
Florida	2019	GW	N	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70

U.S. EPA/State	Year	Type	Promulgated Rule	Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)
Hawaii	2021	GW	N	PFOA: 40 PFOS: 40 PFNA: 4.4 PFBA: 7,600 PFBS: 600 PFHxS: 19 PFHxA: 4,000 PFPeA: 800 PFHpA: 40 PFHpS: 20 PFOSA: 24 PFDA: 4 PFDS: 20 PFUnDA: 10 PFDoDA: 13 PFTrDA: 13 PFTeDA: 130 HFPO-DA: 160
Indiana	2019	Protected GW	Y	PFBS: 400,000
Iowa	2016	Protected GW	Y	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70
	2016	Non-protected GW	Y	PFOS: 1,000
Maine	2021	GW	N	PFBS: 400 Combined PFOA/PFOS/ PFNA/ PFHpA/ PFHxS: 70
Maine cont.	2021	DW	N	PFOA: 20 PFOS: 20 PFNA: 20 PFHxS: 20 PFHpA: 20 PFDA: 20 Combined PFOA/ PFOS/ PFNA/ PFHxS/ PFHpA/ PFDA: 20
Maryland	2021	GW	N	PFHxS: 140
Massachusetts	2019	GW - 1	Y	PFOA: 20 PFOS: 20 PFNA: 20

U.S. EPA/State	Year	Type	Promulgated Rule	Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)
				PFHxS: 20 PFHpA: 20 PFDA: 20 Combined PFOA/ PFOS/ PFNA/ PFHxS/ PFHpA/ PFDA: 20
	2020	DW	Y	PFOA: 20 PFOS: 20 PFNA: 20 PFHxS: 20 PFHpA: 20 PFDA: 20 Combined PFOA/ PFOS/ PFNA/ PFHxS/ PFHpA/ PFDA: 20
Michigan	2019	DW	N	PFOA: 9 PFOS: 8 PFNA: 9 PFBS: 1,000 PFHS: 84
	2021	GW/GW	Y	PFOA: 8 PFOS: 16 PFNA: 6 PFBS: 420 PFHxS: 51 PFHxA: 400,000 HFPO-DA: 370
Minnesota	2018	DW/GW	Y	PFOA: 35 PFOS: 300 PFBA: 7,000 PFBS: 7,000
	2021	GW	N	PFHxA: 200
	2022	DW/GW	N	PFOS: 15 PFBS: 100 PFHxS: 47
	2023	DW	N	PFOA: 35 PFOS: 15 PFBA: 7,000 PFBS: 100 PFHxS: 47 PFHxA: 200
Nevada	2015	DW	N	PFOA: 667 PFOS: 667 PFBS: 667,000

U.S. EPA/State	Year	Type	Promulgated Rule	Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)
New Hampshire	2019	GW	Y	PFOA: 12 PFOS: 15 PFNA: 11 PFHxS: 18
	2020	DW	Y	PFOA: 12 PFOS: 15 PFNA: 11 PFHxS: 18
New Jersey	2020	DW	Y	PFOA: 14 PFOS: 13 PFNA: 13
	2022	GW	Y	PFOA: 14 PFOS: 13 PFNA: 13 CIPFPECA:2
New Mexico	2019	DW	N	PFOA: 70 PFOS: 70 PFHxS: 70 Combined PFOA/ PFOS/ PFHxS: 70
New York	2020	DW	Y	PFOA: 10 PFOS: 10
North Carolina	2006	GW	Y	PFOA: 2,000
	2017	DW	N	HFPO-DA: 140
Ohio	2022	DW	N	PFOA: 70 PFOS: 70 PFNA: 21 PFBS: 2,100 PFHxS: 140 HFPO-DA: 2 Combined PFOA/ PFOS: 70
Oregon	2022	DW	N	PFOA: 30 PFOS: 30 PFNA: 30 PFHxS: 30 Combined PFOA/ PFOS/ PFNA/ PFHxS: 30
Pennsylvania	2021	GW	N	PFOA: 70 PFOS: 70 PFBS: 10 Combined PFOA/ PFOS: 70
	2023	DW	Y	PFOA: 14 PFOS: 18

U.S. EPA/State	Year	Type	Promulgated Rule	Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)
Rhode Island	2017	DW/GW	Y	PFOA: 70 PFOS: 70 Combined PFOA/ PFOS: 70
Rhode Island	2022	DW	Y	PFOA: 20 PFOS: 20 PFNA: 20 PFHxS: 20 PFHpA: 20 PFDA: 20 Combined PFOA/ PFOS/PFNA/ PFHxS/ PFHpA/ PFDA: 20
Texas	2021	GW	Y	PFOA: 290 PFOS: 560 PFNA: 71,000 PFBS: 34,000 PFHxS: 93 PFHxA: 93 PFPeA: 93 PFHpA: 560 PFOSA: 290 PFDA: 370 Combined PFDS/ PFUnDA/ PFDoDA/ PFTrDA/ PFTeDA: 290
Vermont	2018, 2019, 2020	GW	Y	PFOA: 20 PFOS: 20 PFNA: 20 PFHxS: 20 PFHpA: 20 Combined PFOA/ PFOS/ PFNA/ PFHxS/ PFHpA: 20
Washington	2022	GW	Y	PFOA: 10 PFOS: 15 PFNA: 9 PFBS: 345 PFHxS: 65 HFPO-DA: 24
	2022	DW	Y	PFOA: 10 PFOS: 15 PFNA: 9 PFBS: 345 PFHxS: 65
Wisconsin	2022	DW	N	PFOA: 70

U.S. EPA/State	**Year**	**Type**	**Promulgated Rule**	**Acceptable PFAS Levels in Groundwater/Drinking Water (ng/L or ppt)**
				PFOS: 70 Combined PFOA/ PFOS: 70

COMMENT 8: PFAS Sampling Methods

PFAS sampling methods have been widely discussed throughout the rulemaking. Illinois EPA proposed to update its definitions to replace the practical quantitation limit ("PQL"), an outdated term no longer used by SW-846, with the terms Lower Limit of Quantitation ("LLOQ") and Lowest Concentration Minimum Reporting Level ("LCMRL"). These terms represent the lowest levels at which a chemical concentration can be quantified through analysis using a specific method. The table below represents the following methods available for PFAS analyses:

Analytical Method	Date	No. of Analytes	Matrices
U.S. EPA 533	November 2019	25	finished drinking water, source drinking water
U.S. EPA 537.1 v. 2	March 2020	18	finished drinking water, source drinking water
ASTM D7979-20	September 2020	21	non-potable groundwater, surface water, wastewater, sludge
U.S. EPA SW-846 8327	July 2021	24	non-potable groundwater, surface water, wastewater
U.S. EPA 1633 (Draft 3)	December 2022	40	non-potable groundwater, surface water, wastewater, landfill leachate, soil, sediment, sludge, fish tissue

Part 620.110 defines "potable" as "generally fit for human consumption in accordance with accepted water supply principles and practices." Pursuant to Part 620.210, Illinois EPA classifies Class I groundwater in the state as potable resource groundwater; to be preserved for use as a drinking water resource. Several witnesses have made statements implying that groundwater is not potable water, and either is not or should not be recognized as such. However, since the

Illinois General Assembly's adoption of the Groundwater Protection Act in 1985, Illinois has sought to prevent the degradation of groundwater as a resource for legitimate purposes (i.e., as a drinking water resource), and Part 620: Groundwater Quality, is regulated under the assumption of groundwater's use as a drinking water resource. Groundwater does not have to be presently in use as a drinking water supply; it is to be protected as a resource for future drinking water supplies.

Two methods are available for analysis of potable water: Method 537.1 and Method 533. Both methods include finished and raw (source) water as acceptable matrices. For its statewide PFAS sampling initiative, Illinois EPA selected Method 537.1 for drinking water analyses of community water supplies ("CWS"), with an MRL of 2 ng/L for all PFAS analytes proposed in the Part 620 amendments. In some cases, particularly smaller CWS[1], Illinois EPA collected samples directly from the source wells. Following Illinois EPA's PFAS sampling, the Agency requested that the CWS with confirmed detections of PFAS sample their finished water and raw (source) water quarterly using Method 537.1. This sampling includes both surface water intakes and groundwater wells. Results provided to Illinois EPA and available on Illinois EPA's Drinking Water Watch dashboard demonstrate an MRL of 2 ng/L can be met for all proposed PFAS.

The table below depicts the LCMRLs/LLOQs for Methods 533, 537.1, D7979-20, 8327, and Draft 3 Method 1633 in comparison to Illinois EPA's proposed PFAS Class I potable resource groundwater quality standards:

[1] Illinois EPA collected water samples from the source wells, as no treatment is applied prior to distribution, from the following CWS: Westwind Estates, Chain-O-Lakes MHP, Sylvan Lake 1st Subdivision, Barberry Acres MHP, Bradley Heights Subdivision, Family Manufactured Home Community, LLC, Oak Lawn MHP, Watch E Kee Manufactured Home Community, Pauls MHP, Youngs Hillcrest MHP, Iroquois Mobile Estates, Inc., Shangri-La MHP, Vietzen MHP, and Diamond Lake MHP.

PFAS	Proposed Class I Standard ng/L	Method 533 LCMRL ng/L	Method 537.1 LCMRL ng/L	Method D7979-20 LCMRL ng/L	Method 8327 LLOQ ng/L	Draft 3 Method 1633 LLOQ ng/L
PFBS	1,200	3.5	2	10	10	2
PFHxS	77	3.7	2	10	10	2
PFNA	12	4.8	2	10	10	2
PFOA	2	3.4	2	10	10	2
PFOS	7.7	4.4	2	10	10	2
HFPO-DA	12	3.7	2	---	10	5

Illinois EPA notes U.S. EPA's Draft 3 Method 1633 achieves minimum levels of quantitation generated from a multi-laboratory validated study conducted for wastewater; however, the Draft 3 Method 1633 Notice states, "Wastewater is generally a more difficult matrix to analyze than groundwater or surface water, and the wastewater data often drives the statistical determinations of the upper and lower limits of QC criteria. Preliminary review of the surface water and groundwater data indicates this may be the case for Method 1633 as well". The method states the wastewater levels are acceptable for aqueous matrices. These are the levels listed in the table for Draft 3 Method 1633. U.S. EPA recommends Draft 3 Method 1633 be used when analyzing for its applicable matrices. U.S. EPA anticipates a Draft 4 Method 1633 will be released in early 2023 that will incorporate final QC acceptance criteria for all aqueous matrices (surface, groundwater, and wastewater), and a final version to be released in 2023 with final QC acceptance criteria for all eight matrices derived from the multi-lab validation study. Illinois EPA's proposed Class I potable resource groundwater quality standards for PFBS, PFHxS, PFNA, and HFPO-DA (GenX) are greater than the LCMRLs/LLOQs for all analytical methods, potable and non-potable. Illinois EPA's Class I standard for PFOS is greater than the LCMRL/LLOQs for Methods 533, 537.1, and Draft 3 Method 1633, although Draft 3 Method 1633 is not applicable for analysis of potable resource groundwater. Illinois EPA's proposed

Class I standard for PFOA is set at an LCMRL of 2 ng/L in accordance with Part 620.605(b)(1), requiring that numerical standards be set to the LCMRLs/LLOQs if calculated health-based drinking water levels are less than the applicable LCMRLs/LLOQs. Illinois EPA selected the 2 ng/L standard because laboratories contacted by Illinois EPA could consistently meet the 2 ng/L MRL using Method 537.1 for all Method 537.1 PFAS analytes. PFOA's calculated health-based drinking water level equating to a one in one million (1.0E-06) target cancer risk is 0.54 ng/L, using the updated methods proposed in this amendment. The proposed PFOA standard of 2 ng/L equates to a target cancer risk of 3.7 in one million (3.7E-06).

Illinois EPA is attaching the following sample method documents for reference:

- Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry. U.S. EPA Office of Water. November 2019. (*See* Attachment 9).
- Method 537.1, Version 2.0: Determination of Selected Per- and Polyfluoroalkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. EPA Office of Research and Development. March 2020. (*See* Attachment 10).
- Method D7979: Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid

Chromatography Tandem Mass Spectrometry (LC/MS/MS). American Society for Testing and Materials ("ASTM"). August 2020. (*See* Attachment 11).

- Method 8327: Per- and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. EPA SW-846. July 2021. (*See* Attachment 12).
- Method 3512: Solvent Dilution of Non-Potable Waters. U.S. EPA SW-846. July 2021. (*See* Attachment 13).
- Additional Performance Data Associated with Multi-Laboratory Validation of SW-846 Methods 3512 and 8327. U.S. EPA SW-846. July 2021. (*See* Attachment 14).
- Draft 3 Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. U.S. EPA Office of Water. December 2022. (*See* Attachment 15).

COMMENT 9: Proposed Class I Cobalt and Vanadium MRLs are Less Than Proposed Standards

Dr. Hahn's pre-filed and hearing testimony discusses Illinois EPA's proposed updates to Class I potable resource groundwater quality standards for cobalt and vanadium. Dr. Hahn states in her hearing testimony that the proposed cobalt and vanadium health-based Class I groundwater standards are "perhaps below levels that laboratories can practically quantify" (p. 19:l. 5-7, of December 7, 2022, Hearing Transcript). This statement reiterates statements Dr. Hahn made in pre-filed testimony and responses.

Cobalt

Cobalt's current Class I groundwater standard of 1 mg/L, first promulgated in 1991, is based on beneficial use of Class I groundwater for watering livestock. In 2008, U.S. EPA issued a Provisional Peer-Reviewed Toxicity Value ("PPRTV") chronic oral reference dose ("RfD") of

0.0003 mg/kg-day. The proposed updated Class I standard of 0.0012 mg/L represents cobalt's noncancer health-based level. U.S. EPA's Third Unregulated Contaminant Monitoring Rule ("UCMR"), published May of 2012, listed a Minimum Reporting Level ("MRL") of 0.001 mg/L for cobalt using U.S. EPA Method 200.8, which is slightly less than Illinois EPA's proposed Class I potable resource groundwater standard of 0.0012 mg/L. Therefore, pursuant to Part 620.605(b), the health-based standard applies.

Vanadium

Vanadium's current Class I potable resource groundwater quality standard of 0.049 mg/L, first promulgated in 2012, is a health-based standard based on an RfD of 0.007 mg/kg-day from U.S. EPA's Health Effects Assessment Summary Table ("HEAST"). The HEAST vanadium toxicity value has since been retired and is no longer available. In 2009, U.S. EPA issued a vanadium chronic RfD PPRTV of 0.00007 mg/kg-day, a decrease of two orders of magnitude from the HEAST value. Using the PPRTV RfD and proposed noncancer child water ingestion rates, the calculated health-based standard is 0.00027 mg/L. U.S. EPA's Third UCMR, discussed above in cobalt, listed a MRL of 0.0002 mg/L for vanadium using U.S. EPA Method 200.8, which is less than Illinois EPA's proposed Class I potable resource groundwater standard of 0.00027 mg/L. Therefore, pursuant to Part 620.605(b), the health-based standard applies.

If a particular laboratory is unable to meet the EPA Method 200.8 MRLs listed in the Third UCMR, then another laboratory capable of meeting the MRLs should be selected for analyzing groundwater samples.

In addition, testimony and exhibits discussing dissolved or filtered metals sampling or analyses are not applicable when discussing health-based groundwater standards. Health-based

groundwater standards for metals are based on total, or unfiltered sampling; therefore, no insight can be gained with a discussion of dissolved or filtered samples.

COMMENT 10: Development of a GQS before an MCL

The IGPA recognizes that federal drinking water levels (MCLs) should be considered in the adoption of groundwater quality standards. This is no surprise because MCLs are health based and the use of groundwater for drinking is a recognized use in Illinois. Many of the Class I groundwater quality standards are based on MCLs. However, the development of groundwater quality standards for constituents with no MCL is a well-established practice in Part 620. For example: Combined, there are 20 inorganic and radionuclide constituents that have MCLs. There are 34 inorganic and radionuclide Class I groundwater standards. There are 52 organic constituents with MCLs while there are 85 organic constituents with Class I groundwater quality standards. In both instances, approximately 40% of Class I standards do not have a corresponding MCL. Therefore, adoption of groundwater quality standards without the existence of an MCL is hardly a rare or unique circumstance. Indeed, the bulk of 620, Subpart F is devoted to that very activity.

COMMENT 11: Economic Impact

Pursuant to 35 Ill. Adm. Code 102.202(b), the Agency provided a statement regarding technical feasibility and economic reasonableness. *See* Agency's Statement of Reasons at 22, R2022-018 (Dec. 7, 2021). Therefore, the Agency set forth the Board's prior evaluations and determinations and compared those evaluations and determinations with the current proposal. *Id*. at 22-26. Consistent with the Board's numerous prior evaluations and determinations, the Agency concluded and maintains the proposed groundwater quality standard amendments are technically feasible and economically reasonable. The Agency reiterates this point with the following:

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

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

As the Board stated in R08-18, there is no information suggesting that the proposed amendments would impose an economic or technical burden significantly different from that resulting from prior Part 620 rulemakings. *See* R08-18 at 27. Therefore, as previously determined by the Board, the groundwater quality standards proposed today are technically feasible and economically reasonable and will not adversely impact on the people of the State of Illinois. *Id*. at 25-26.

See Agency's Statement of Reasons at 22-26 , R2022-018 (Dec. 7, 2021).

WHEREFORE, the Illinois EPA asks the Board to accept these Post Hearing Comments.

Respectfully submitted,

Dated: March 3, 2023

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,

BY: /s/ Sara Terranova

Sara Terranova
Division of Legal Counsel
Illinois Environmental Protection Agency
1021 North Grand Avenue East
P.O. Box 19276
Springfield, IL 62794-9276
(217) 782-5544

CERTIFICATE OF SERVICE

I, the undersigned, on affirmation state the following:

That I have served the attached **NOTICE OF FILING** and **ILLINOIS ENVIRONMENTAL PROTECTION AGENCY'S POST HEARING COMMENTS** by e-mail upon the attached service list.

That my e-mail address is: Sara.Terranova@illinois.gov.

That the e-mail transmission took place before 4:30 p.m. on the date of March 3, 2023.

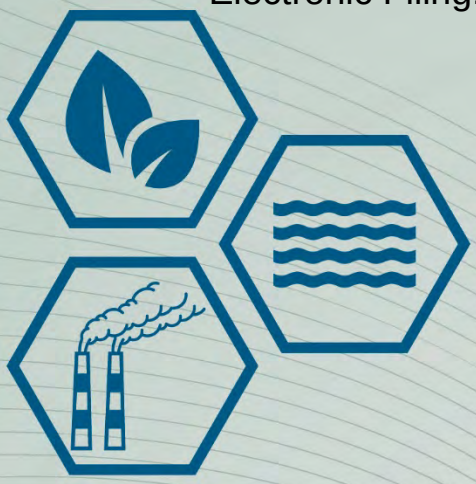
/s/ Sara Terranova

March 3, 2023

List of Attachments

Attachment 1	Final Brief ATSDR Molybdenum Toxicological Profile
Attachment 2	Final Brief EPA PFAS 4 Technical Fact Sheet
Attachment 3	Final Brief EPA Exposure Decision Tree
Attachment 4	Final Brief Fig. 4-1 Exposure Decision Tree
Attachment 5	Final Brief 2022-06-15 PFOA EPA Interim HA
Attachment 6	Final Brief 2022-06-15 PFOS EPA Interim HA
Attachment 7	Final Brief 2022-06-15 PFBS EPA Final HA
Attachment 8	Final Brief 2022-06-15 HFPO-DA EPA Final HA
Attachment 9	Method 533 FINAL
Attachment 10	Method 537.1 v 2 FINAL
Attachment 11	ASTM D7979-20
Attachment 12	Method 8327 Analysis
Attachment 13	Method 3512 Final extraction
Attachment 14	Method 3512 Final extraction
Attachment 15	Draft 3 Method 1633
Attachment 16	Appendix E Health Effects Table

Attachment 1



Toxicological Profile for Molybdenum

May 2020

ATSDR
U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Patrick N. Breysse, Ph.D., CIH
Director, National Center for Environmental Health and
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
May 2020	Final toxicological profile released
April 2017	Draft for public comment toxicological profile released

CONTRIBUTORS & REVIEWERS

CHEMICAL MANAGER TEAM

G. Daniel Todd, Ph.D. (Lead)
Sam Keith, M.S., C.H.P.
Obaid Faroon, D.V.M., Ph.D.
Melanie Buser, M.P.H.

Lisa Ingerman, Ph.D., D.A.B.T.
Mario Citra, Ph.D.
Gary L. Diamond, Ph.D.
Courtney Hard, B.A.
Julie M. Klotzbach, Ph.D.
Amy Nguyen, Ph.D.

ATSDR, Division of Toxicology and Human Health Sciences, Atlanta, GA

SRC, Inc., North Syracuse, NY

REVIEWERS

Interagency Minimal Risk Level Workgroup:

Includes ATSDR; National Center for Environmental Health (NCEH); National Institute for Occupational Health and Safety (NIOSH); U.S. Environmental Protection Agency (EPA); National Toxicology Program (NTP).

Additional reviews for science and/or policy:

ATSDR, Division of Community Health Investigations; ATSDR, Office of Science; NCEH, Division of Laboratory Science; NCEH, Division of Environmental Health Science and Practice; EPA, Office of Water.

PEER REVIEWERS

1. David C. Dorman, DVM, Ph.D., DABT, DABVT, Professor, Toxicology, North Carolina State University, Raleigh, North Carolina
2. Jonathan H Freedman, Ph.D., Professor, Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky
3. Michael Aschner, Ph.D., Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York
4. John Meeker, Sc.D., C.I.H., Professor, Environmental Health Sciences, Associate Dean for Research, School of Public Health, University of Michigan, Ann Arbor, Michigan
5. Alexander V. Lyubimov, M.D., Ph.D., D.A.B.T., Toxicology Research Laboratory, Chicago, Illinois
6. Dagobert Heijerick, ARCHE consulting, Gent, Belgium

These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Molybdenum (Mo) is a naturally occurring trace element that can be found extensively in nature. Molybdenum is a metal that exists as a dark-gray or black powder with a metallic luster or as a silvery-white mass (NLM 2020a). It does not occur naturally in the pure metallic form, but principally as oxide or sulfide compounds (Barceloux 1999; EPA 1979). Therefore, almost all exposure is to a molybdenum compound rather than the actual metal. Important naturally occurring molybdenum compounds are the minerals molybdenite, powellite, wulfenite, ferrimolybdite, and ilsemannite. In this toxicological profile, "molybdenum" is used to refer to the element (molybdenum metal) and generically for substances or compounds containing molybdenum. The most common forms used in commerce and found in the environment are molybdenum trioxide and molybdate salts (sodium molybdate or ammonium molybdate). Industrial applications of molybdenum nanoparticles have also been identified; however, molybdenum nanoparticle exposure is not discussed in this toxicological profile because their physical-chemical properties differ from that of larger molybdenum particles and the toxicological and toxicokinetic properties of nanoparticles can vastly differ from those of larger particles.

Biologically, molybdenum plays an important role as a micronutrient in plants and animals, including humans. It is used widely in industry for metallurgical applications; some of these applications include high temperature furnaces, as a support wire for tungsten filaments in incandescent light bulbs, and as a component of steel used in solar panels and wind turbines (EPA 1979; Stiefel 2011).

Molybdenum is more abundant in areas of natural mineral deposits and can be found in all environmental media. Higher concentrations in air, water, and soil can be found near industrial operations due to contamination. Molybdenum concentrations in ambient air have been reported to range from below detection limits to 0.03 mg/m^3 (EPA 1979). Concentrations of molybdenum in ambient air of urban areas, 0.01–0.03 $\mu g/m^3$, are higher than those found in rural areas, 0.001–0.0032 $\mu g/m^3$. It has been reported that concentrations of molybdenum in surface waters are generally <1.0 µg/L (USGS 2006) and drinking water (USGS 2011) and groundwaters contain about 1.0 µg/L (USGS 2011). Near mining activities, surface water molybdenum concentrations can be orders of magnitude higher (Frasacoli and Hudson-Edwards 2018). Concentrations as high as 1,400 µg/L have been detected in drinking waters in areas impacted by mining and milling operations (USGS 2011), far exceeding the U.S. Environmental Protection Agency (EPA) health-based screening level of 40 µg/L (EPA 2018a). Globally, most soils

contain molybdenum at concentrations between 0.6 and 3.5 ppm, although total concentrations in soils can vary widely depending on geological composition or industrial contamination. The average concentration of soils is generally 1–2 ppm. In the United States, it has been reported that the median concentration of molybdenum in soils is 1.2–1.3 ppm, with a range of 0.1–40 ppm (EPA 1979).

The exposure to molybdenum to the general population is almost entirely through food. Foods derived from above-ground plants, such as legumes, leafy vegetables, and cauliflower, generally have a relatively higher concentration of molybdenum in comparison to food from tubers or animals. Beans, cereal grains, leafy vegetables, legumes, liver, and milk are reported as the richest sources of molybdenum in the average diet (Barceloux 1999). Drinking water coming from sources close to areas with high molybdenum contamination from industrial effluents may contain a higher concentration of molybdenum.

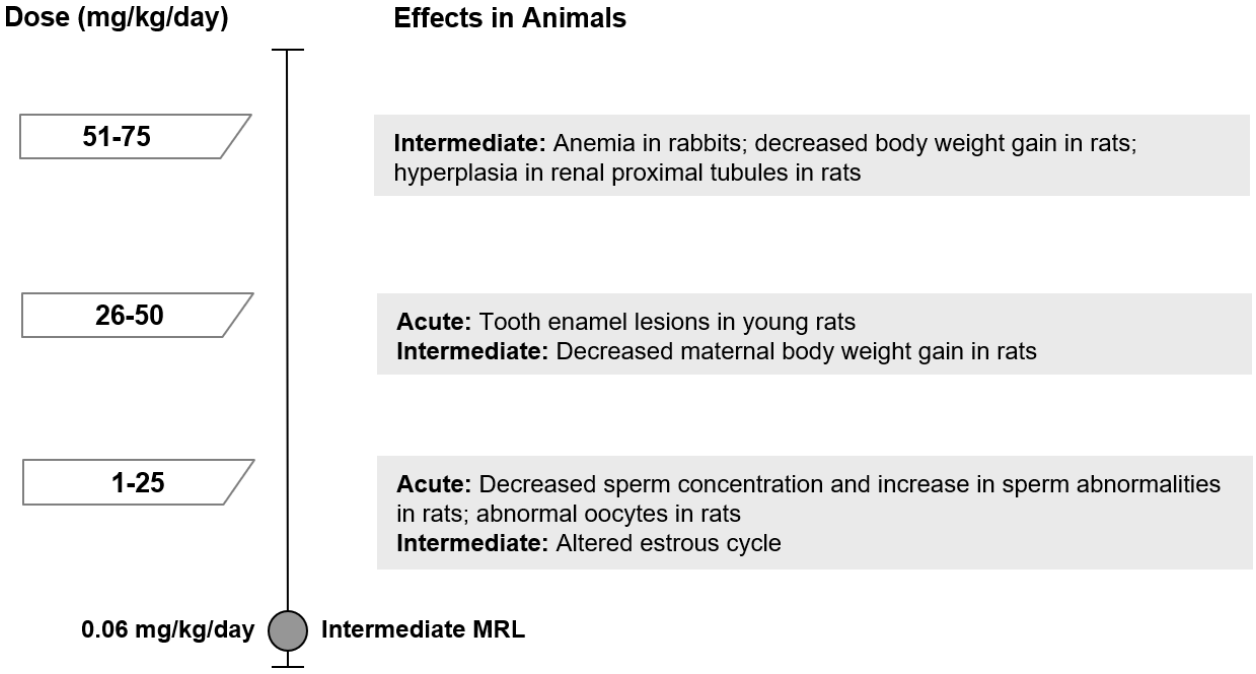
1.2 SUMMARY OF HEALTH EFFECTS

Molybdenum is an essential nutrient; the nutritional requirement for adults is 45 µg/day (0.64 µg/kg/day) (NAS 2001). Exposure to excess levels has been associated with adverse health outcomes. The most sensitive effects appear to be respiratory effects following inhalation exposure to molybdenum trioxide, and decreases in body weight, kidney damage, decreases in sperm count, and anemia following oral exposure (see Figure 1-1). A systematic review of the available human and laboratory animal health effects database resulted in the following hazard identification conclusions:

- Respiratory effects are a presumed health effect for humans for molybdenum oxides.
- Renal effects are a presumed health effect for humans.
- The data were inadequate to conclude whether hepatic, uric acid level, reproductive, or developmental effects will occur in humans.

Respiratory Effects. Decreases in lung function, dyspnea, and cough were reported in a study of workers exposed to fine or ultrafine molybdenum trioxide dust (Ott et al. 2004). Another study of workers at a molybdenite roasting facility exposed to molybdenum trioxide and other oxides did not have alterations in lung function (Walravens et al. 1979). In studies of rats and mice exposed to molybdenum trioxide for 2 years, hyaline degeneration of the nasal epithelium, squamous metaplasia of the epiglottis, and chronic inflammation (rats only) were observed (NTP 1997). However, no effects were observed following a 13-week exposure to similar concentrations (NTP 1997).

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to Molybdenum



Hepatic Effects. Liver effects, which consisted of decreases in glycogen content, increases in aminotransferase activities, and increases in lipid content, have been observed at higher doses (≥300 mg/kg/day) that are often associated with body weight losses (Rana and Chauhan 2000; Rana and Kumar 1980b, 1980c; Rana et al. 1980, 1985). No hepatic effects have been observed at lower (≤60 mg/kg/day) doses (Bersenyi et al. 2008; Murray et al. 2014a).

Renal Effects. Several studies have reported renal effects in rats exposed to ≥60 mg/kg/day (Bompart et al. 1990; Murray et al. 2014a; Rana and Kumar 1980c, 1983; Rana et al. 1980). The effects included hyperplasia of the renal proximal tubules, degeneration, increases in total lipid levels in the kidney, and diuresis and creatinuria.

Reproductive Effects. Cross-sectional epidemiological studies have reported significant associations between blood molybdenum levels and sperm concentration and morphology (Meeker et al. 2008) or testosterone levels (Lewis and Meeker 2015; Meeker et al. 2010). No significant alterations in sperm parameters or estrous cycling were observed in a 90-day rat study (Murray et al. 2014a) or in a 2-generation reproductive toxicity study (Murray et al. 2019). Studies providing limited information on molybdenum doses and/or the copper content of the diet have reported reproductive effects. Decreases in sperm motility and concentration and increases in sperm morphological changes have been observed in

rats exposed to approximately 25 mg molybdenum/kg/day as sodium molybdate (Pandey and Singh 2002; Zhai et al. 2013). Degeneration of the seminiferous tubules was also observed at similar molybdenum doses (Jeter and Davis 1954). Effects have also been observed in the female reproductive system (oocyte morphological alterations, abnormal rate of ovulation, and irregularities in the estrous cycle) at ≥1.5 mg molybdenum/kg/day in rats (Fungwe et al. 1990; Jeter and Davis 1954; Zhang et al. 2013).

Developmental Effects. Mixed results have been observed in animal developmental toxicity studies. Decreases in the number of live fetuses and fetal growth were observed in rats administered 14 mg molybdenum/kg as sodium molybdate (Pandey and Singh 2002). Interpretation of the results of this study is limited by the lack of information on the copper content of the diet and the lack of developmental effects reported in two high-quality studies in which rats were exposed to doses as high as 40 mg molybdenum/kg/day as sodium molybdate (Murray et al. 2014b, 2019).

Uric Acid Levels. A study of workers at a molybdenite roasting facility exposed to molybdenum trioxide and other oxides reported an increase in serum uric acid levels (Walravens et al. 1979). An increased occurrence of gout-like symptoms and increased blood uric acid levels were also observed in residents living in an area of high molybdenum levels in the soil (Koval'skiy et al. 1961); no alterations in urinary uric acid levels were found in a 10-day experimental study in men (Deosthale and Gopalan 1974).

Cancer Effects. No increases in the risk of lung cancer were reported in workers who self-reported exposure to molybdenum (Droste et al. 1999). An increase in alveolar/bronchiolar adenomas or carcinomas was observed in mice exposed to molybdenum trioxide for 2 years (NTP 1997); in rats chronically exposed to airborne molybdenum trioxide, the incidence of alveolar/bronchiolar adenoma/ carcinoma was within the range of historical controls (NTP 1997). The potential carcinogenicity of molybdenum in humans has not been evaluated by the Department of Health and Human Services or the EPA. The International Agency for Research on Cancer (IARC 2018) categorized molybdenum trioxide as possibly carcinogenic to humans (Group 2B).

1.3 MINIMAL RISK LEVELS (MRLs)

As summarized in Table 1-1, an inhalation MRL has been derived for chronic-duration exposure to molybdenum trioxide and an oral MRL has been derived for intermediate-duration exposure to molybdenum. As presented in Figure 1-2, available data have identified the kidney as a sensitive target of

molybdenum toxicity following oral exposure. The available data were not considered adequate for derivation of acute- or intermediate-duration inhalation MRLs or acute- or chronic-duration oral MRLs.

Table 1-1. Minimal Risk Levels (MRLs) for Molybdenum[a]

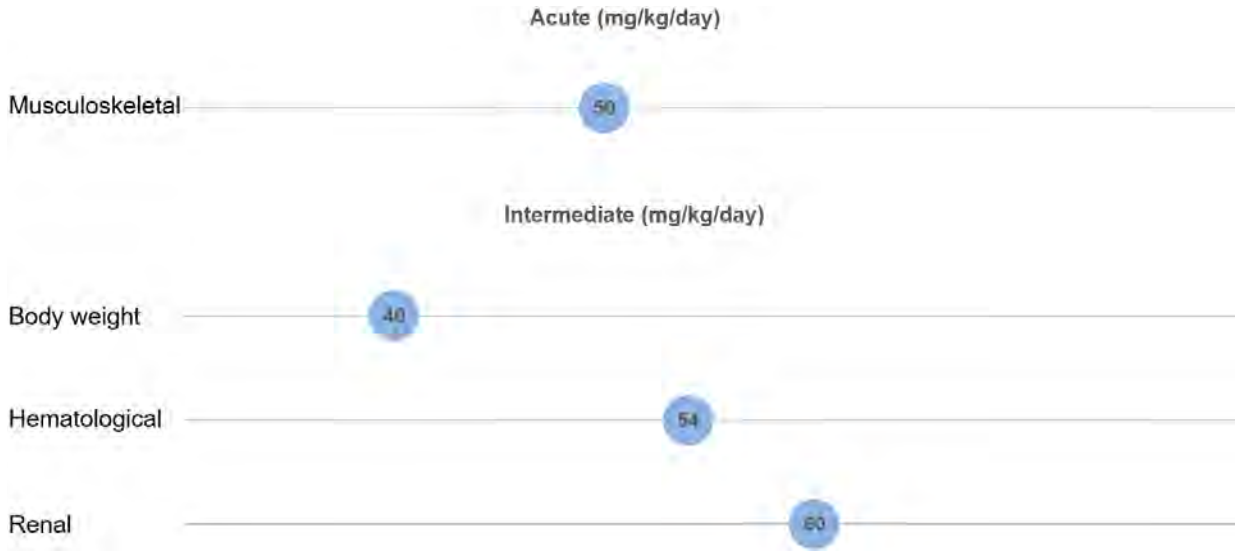
Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure (mg molybdenum/m^3)					
Acute	Insufficient data for derivation of an MRL				
Intermediate	Insufficient data for derivation of an MRL				
Chronic (molybdenum trioxide)	0.002	Squamous metaplasia of the epiglottis in female rats	0.071 ($BMCL_{HEC}$)	UF: 30	NTP 1997
Oral exposure (mg/kg/day)					
Acute	Insufficient data for derivation of an MRL				
Intermediate	0.06	Renal proximal tubule hyperplasia	17 (NOAEL)	UF: 100 MF: 3	Murray et al. 2014a
Chronic	Insufficient data for derivation of an MRL				

[a]See Appendix A for additional information.

BMCL = benchmark concentration lower confidence limit; HEC = human equivalent concentration; MF = modifying factor; NOAEL = no-observed-adverse-effect level; UF = uncertainty factor

Figure 1-2. Summary of Sensitive Targets of Molybdenum – Oral

The kidney is the most sensitive target of molybdenum oral exposure.
Numbers in circles are the lowest LOAELs for all health effects in animals.
No reliable dose response data were available for humans.



CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of molybdenum. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to molybdenum, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to molybdenum was also conducted; the results of this review are presented in Appendix C.

Human and animal inhalation studies are presented in Table 2-1 and Figure 2-2; animal oral studies are presented in Table 2-2 and Figure 2-3; and animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be

classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of molybdenum are indicated in Table 2-1 and Figure 2-2.

A User's Guide has been provided at the end of this profile (see Appendix D]). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

Molybdenum, as a component of pterin-based cofactor, is an essential element. Historically, three molybdenum cofactor-containing enzymes have been identified: sulfite oxidase, xanthine oxidase, and aldehyde oxidase (NAS 2001; Sardesai 1993). These enzymes are involved in the degradation of sulfur-containing amino acids and sulfatides, purine degradation pathway catalyzing the oxidation of hypoxanthine to xanthine and of xanthine to uric acid, and oxidation of aromatic and nonaromatic heterocycles and aldehydes to carboxylic acids (Wahl et al. 2010). Within the last 10 years, a fourth enzyme, mitochondrial amidoxime reducing component (mARC), has been identified in mammals (Wahl et al. 2010). Clear signs of molybdenum deficiency have not been found in healthy humans (NAS 2001). However, a deficiency in molybdenum cofactor has been observed in individuals with a severe metabolic defect. The lack of molybdenum cofactor and subsequent deficiencies in molybdoenzymes is manifested in central nervous system effects (Bayram et al. 2013). The effects that typically occur shortly after birth include intractable seizures and feeding difficulties; the patients develop severe psychomotor retardation due to progressive cerebral atrophy and ventricular dilatation (Bayram et al. 2013). The nutritional requirements for molybdenum are based on maintaining molybdenum balance; the Institute of Medicine has established the following age-specific Recommended Dietary Allowances (RDAs) (NAS 2001):

- 17 µg/day for 1–3 year olds
- 22 µg/day for 4–8 year olds
- 34 µg/day for 9–13 year olds
- 43 µg/day for 14–18 year olds

- 45 µg/day (0.64 µg/kg/day) for adults
- 50 µg/day in pregnant and lactating women

As illustrated in Figure 2-1, a number of human and laboratory animal studies have evaluated the toxicity of molybdenum following inhalation, oral, or dermal exposure; this toxicological profile on molybdenum does not include discussion of the health effects of molybdenum nanoparticles, which could have different toxicological and toxicokinetic properties than larger molybdenum particles. Of the 92 identified toxicity publications, 84% evaluated health outcomes in laboratory animals; most (74%) were conducted by the oral route of exposure. Inhalation studies primarily focused on the respiratory tract, although intermediate- and chronic-duration studies examined a wide range of endpoints in rats and mice exposed to molybdenum trioxide. Although a large number of laboratory oral exposure studies have been identified, most had a limited scope (examined one or two potential targets). However, a small number of studies evaluated a wide range of endpoints. The most studied endpoints following oral exposure were potential hematological, musculoskeletal, and reproductive outcomes. No human dermal exposure studies were identified; the animal studies primarily focused on dermal and immunological endpoints.

A number of factors can influence the toxicity of molybdenum including the animal species; previous dietary history; relative amounts of dietary molybdenum, copper, and sulfur; and the form of molybdenum. The oral toxicity of molybdenum has been well-established in ruminants, particularly cows and sheep. The toxicity is likely due to an interaction between molybdate and sulfide in the rumen, resulting in the formation of thiomolybdates (Gould and Kendall 2011). In the absence of adequate copper in the rumen, the thiomolybdate is absorbed through the rumen or small intestine and can bind to copper-containing compounds such as ceruloplasmin and cytochrome oxidase, resulting in symptoms resembling copper deficiency (a condition often referred to as molybdenosis). The observed effects can include decreases in weight gain, alterations in hair/wool texture and pigmentation, delayed puberty, and reduced conception rates. Molybdenum also interacts with copper in monogastric animals; however, the mode of interaction differs between the species. The available data suggest that the findings in ruminants do not appear to be relevant to humans or monogastric animals (NAS 2001). Thus, ruminant data will not be further discussed in the toxicological profile.

Studies in rats provide evidence that copper status, particularly the copper content of the diet, can influence the toxicokinetics and toxicity of molybdenum; see Section 3.4 for a more detailed discussion of the interaction between molybdenum and copper. Administration of 150 or 500 mg/kg molybdenum in

the diet for up to 6 weeks to rats fed a copper-deficient or copper-adequate diet resulted in profound differences in the distribution of copper and molybdenum in the plasma, liver, and kidneys (Nederbragt 1980, 1982). For example, at a molybdenum dietary concentration of 150 mg/kg, molybdenum levels in the liver and kidneys were 3.5 and 9 times higher than pre-exposure levels, respectively, in the copper-adequate rats as compared to 6 and 4 times higher, respectively, in the copper-deficient rats. Additionally, the relative increases in copper levels in the liver and kidneys associated with molybdenum exposure were greater in the rats fed the copper-deficient diet, as compared to those fed the copper-adequate diet. Exposure to elevated levels of dietary molybdenum in animals maintained on basal diets with inadequate copper levels resulted in marked toxicity (for example, Brinkman and Miller 1961; Johnson et al. 1969; Sasmal et al. 1968). Similar effects were not observed when animals were fed similar molybdenum levels and maintained on a copper-adequate diet (for example, Mills et al. 1958; Murray et al. 2014a; Peredo et al. 2013). In the United States, the average copper intake is 1.0–1.6 mg/day and the copper RDA is 0.9 mg/day (NAS 2001). Thus, studies in which laboratory animals were fed a copper-deficient diet may not be relevant to evaluating the risk of molybdenum toxicity to the general population with adequate copper intake. Studies in which the laboratory animals were fed a basal diet with inadequate copper levels are clearly identified in the text, are discussed separately from studies in which there were adequate dietary copper levels, and are not included in the LSE table or figure. The current recommended dietary copper concentrations of 5, 6, and 3 ppm have been established for rats, mice, and rabbits, respectively (NAS 1977, 1995); for rats and mice, a copper dietary level of 8 ppm has been established to support gestation and lactation (NAS 1995).

Ammonium tetrathiomolybdate is an experimental chelating agent used to decrease excess copper levels in individuals with Wilson's disease, a genetic disease that limits copper excretion resulting in an accumulation of toxic levels of copper in the liver, brain, and eyes. Administration of tetrathiomolybdate compounds, as compared to other molybdate compounds, results in more dramatic shifts in copper levels in rats fed copper-adequate diets (Mills et al. 1981a), and the toxicity may differ from other molybdenum compounds. Significant increases in serum and kidney copper levels, decreases in liver copper levels, and increases in serum, liver, and kidney molybdenum levels were found in rats exposed to ammonium tetrathiomolybdate as compared to rats receiving the same molybdenum dose as sodium molybdate (Mills et al. 1981a); these results suggest that the tetrathiomolybdate impaired utilization of dietary copper, utilization of stored copper, or both. A study in rats demonstrated that administration of supplemental copper could reverse the adverse effects observed following administration via gavage of 12 mg molybdenum/kg/day as ammonium tetrathiomolybdate (Lyubimov et al. 2004). This study suggests that ammonium tetrathiomolybdate may interfere with copper homeostasis. No studies evaluating whether

copper supplementation would reverse the toxicity of other molybdenum compounds were identified. Because tetrathiomolybdate compounds may not be representative of other molybdenum compounds, studies involving exposure to tetrathiomolybdate compounds are not included in the LSE table and figure, but are discussed in the text.

The human and animal studies suggest several sensitive targets of molybdenum toxicity:

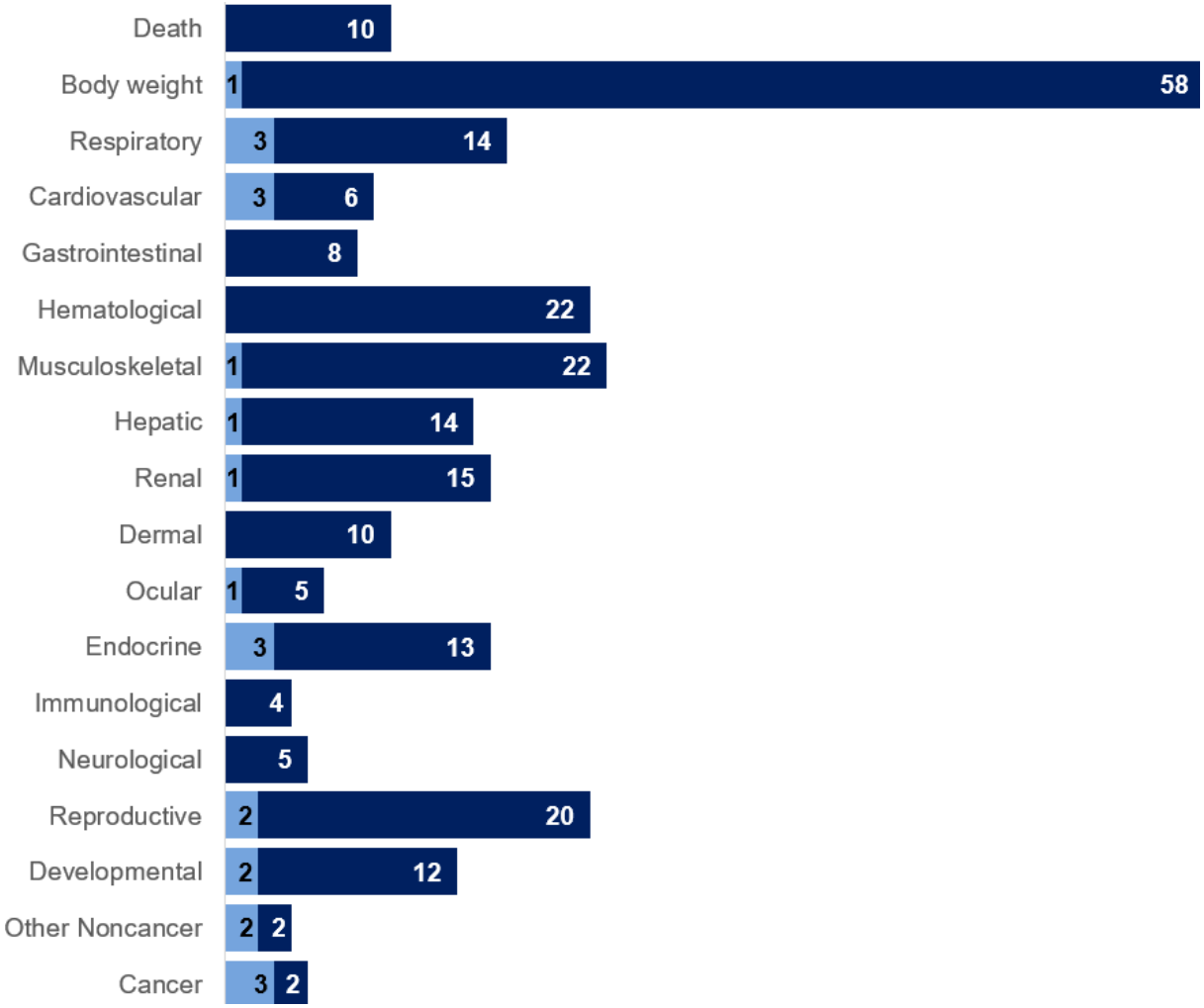
- **Respiratory Endpoints:** Respiratory effects are a presumed health effect for humans based on inadequate evidence in molybdenum oxide workers and a high level of evidence in rats and mice chronically exposed to airborne molybdenum trioxide.

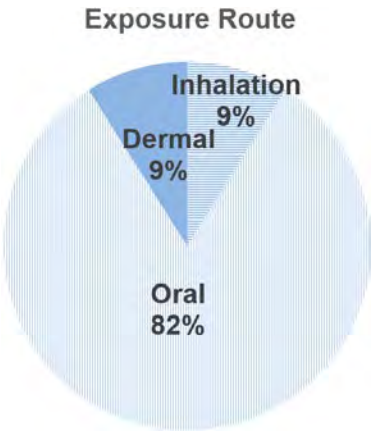
- **Renal Endpoints:** Renal effects are a presumed health effect for humans based on no data in humans and a high level of evidence in laboratory animals. The observed effects include histological alterations in the kidneys and alterations in renal function.

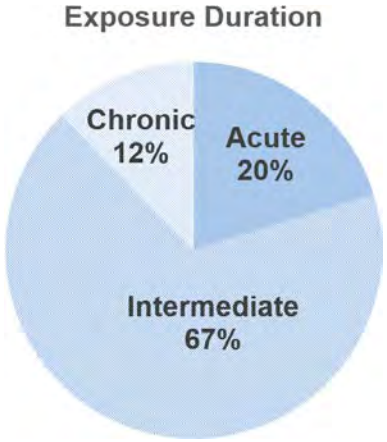
- **Other Endpoints:** Although there is some evidence that molybdenum exposure may result in hepatic, reproductive, or developmental effects, the data are not considered adequate to classify whether molybdenum is a hepatic or developmental hazard to humans.
  - **Hepatic Effects:** There is inadequate evidence of increased risk of liver disease in humans. There is high evidence that inhalation or oral exposure to molybdenum compounds will result in histological alterations in rats, mice, or rabbits. There is moderate evidence in rats that exposure may result in alterations in serum clinical chemistry parameters and/or lipid levels in laboratory animals.
  - **Reproductive Effects:** There is low evidence of male reproductive effects in cross-sectional studies that do not establish causality. Two high-quality animal studies have not found evidence of reproductive effects in rats. Several lower-quality studies have reported male and female reproductive effects; other studies have not reported any reproductive alterations.
  - **Developmental Effects:** There is low evidence of developmental effects in epidemiological studies that do not establish causality. There are mixed results in laboratory animal studies, with most studies not finding evidence of developmental toxicity.

Figure 2-1. Overview of the Number of Studies Examining Molybdenum Health Effects

Most studies examined the potential body weight, hematological, musculoskeletal, and reproductive effects of molybdenum
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 91 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to Molybdenum – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg Mo/m^3)	Less serious LOAEL (mg Mo/m^3)	Serious LOAEL (mg Mo/m^3)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 5 M, 5 F	4 hours	0, 1,200	CS, BW, FI, WI, OW, HP	Bd wt		1,200		Weight loss or no body weight gain during first 2–3 post-exposure days; thereafter, weight gain was similar to controls
					Resp	1,200			
Ammonium dimolybdate Jackson et al. 1991a									
2	Rat (Sprague-Dawley) 5 M, 5 F	4 hours	0, 3,890	CS, BW, FI, WI, OW, HP	Bd wt		3,890		Weight loss during first 2–3 post-exposure days; thereafter, weight gain was similar to controls
					Resp	3,890			
Molybdenum trioxide Jackson et al. 1991b									
3	Rat (Sprague-Dawley) 5 M, 5 F	4 hours	0, 899	CS, BW, FI, WI, OW, HP	Bd wt		899		Weight loss during first 2–3 post-exposure days; thereafter, weight gain was similar to controls
					Resp	899			
Sodium molybdate Jackson et al. 1991c									
4	Rat (Sprague-Dawley) 5 M, 5 F	4 hours	0, 2,613	CS, BW, FI, WI, OW, HP	Bd wt		2,613		14% decrease in body weight gain on post-exposure day 3
					Resp	2,613			
Molybdenum trioxide Jackson et al. 1991d									
5	Rat (CD) 3 M, 3 F	4 hours	3,360	CS, GN, HP	Resp	3,360			
Molybdenum trioxide Leuschner 2010									

Table 2-1. Levels of Significant Exposure to Molybdenum – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg Mo/m^3)	Less serious LOAEL (mg Mo/m^3)	Serious LOAEL (mg Mo/m^3)	Effects
6	Rat (Fischer-344) 5 M, 5 F	6 hours/day 5 days/week 14 days	0, 2, 6.7, 20, 67, 200	CS, BW, HP	Bd wt		67	200	Decreased body weight gain in males at 67 mg/m^3 (10%) and females exposed to 200 mg/m^3 (13%); weight loss in males at 200 mg/m^3 (terminal weight 5% less than initial weight)
					Resp	200			
Molybdenum trioxide NTP 1997									
7	Mouse (B6C3F1) 5 M, 5 F	6 hours/day 5 days/week 14 days	0, 2, 6.7, 20, 67, 200	CS, BW, HP	Bd wt			200	Body weight loss in males and decrease in body weight gain in females
					Resp	200			
Molybdenum trioxide NTP 1997									
INTERMEDIATE EXPOSURE									
8	Rat (Fischer-344) 10 M, 10 F	6.5 hours/day 5 days/week 13 weeks	0, 0.67, 2, 6.7, 20, 67	CS, BW, OW, HP, RX	Bd wt	67			
					Resp	67			
					Cardio	67			
					Gastro	67			
					Hemato	67			
					Musc/skel	67			
					Hepatic	67			
					Renal	67			
					Endocr	67			
					Repro	67 M			
Molybdenum trioxide NTP 1997									
9	Mouse (B6C3F1) 10 M, 10 F	6.5 hours/day 5 days/week 13 weeks	0, 0.67, 2, 6.7, 20, 67	CS, BW, OW, HP, RX	Bd wt	67			
					Resp	67			
					Cardio	67			
					Gastro	67			

Table 2-1. Levels of Significant Exposure to Molybdenum – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg Mo/m^3)	Less serious LOAEL (mg Mo/m^3)	Serious LOAEL (mg Mo/m^3)	Effects
					Hemato	67			
					Musc/skel	67			
					Hepatic	67			
					Renal	67			
					Endocr	67			
					Repro	67 M			
Molybdenum trioxide NTP 1997									
CHRONIC EXPOSURE									
10	Human 25 M	Occupational	0, 9.47	BI, OF	Resp	9.47			
					Other noncancer		9.47		Increased serum uric acid levels
Molybdate Walravens et al. 1979									
11	Rat (Fischer-344) 50 M, 50 F	6 hours/day 5 days/week 105 weeks	0, 6.7, 20, 67	CS, BW, HP	Bd wt	67			
					Resp		6.7[b]		Hyaline degeneration of nasal respiratory and olfactory epithelium (females only), squamous metaplasia of the epiglottis, and chronic lung inflammation (only significant at 20 and 67 mg/m^3 concentrations); $BMCL_{HEC}$ of 0.071 mg/m^3
					Cardio	67			
					Gastro	67			
					Musc/skel	67			
					Hepatic	67			
					Renal	67			
					Endocr	67			
Molybdenum trioxide NTP 1997									

Table 2-1. Levels of Significant Exposure to Molybdenum – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg Mo/m^3)	Less serious LOAEL (mg Mo/m^3)	Serious LOAEL (mg Mo/m^3)	Effects
12	Mouse (B6C3F1) 50 M, 50 F	6 hours/day 5 days/week 105 weeks	0, 6.7, 20, 67	CS, BW, HP	Bd wt	67			
					Resp		6.7		Squamous metaplasia of the epiglottis, histiocytic cellular infiltration in the lungs, and alveolar epithelial metaplasia were observed at ≥6.7 mg/m^3; nasal suppurative inflammation in males at 20 or 67 mg/m^3 and hyaline degeneration of nasal respiratory and olfactory epithelium (females only) at 67 mg/m^3
					Cardio	67			
					Gastro	67			
					Musc/skel	67			
					Hepatic	67			
					Renal	67			
					Endocr	67			
					Cancer			6.7	Alveolar/bronchiolar carcinoma in males at ≥6.7 mg/m^3 and increased incidence of alveolar/bronchiolar adenoma in females at ≥20 mg/m^3; an increase in alveolar/ bronchiolar adenoma or carcinoma in male mice exposed to 6.7 or 20 mg/m^3

Molybdenum trioxide
NTP 1997

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive a chronic-duration oral MRL for molybdenum trioxide of 0.002 mg molybdenum/m^3 based on a $BMCL_{10}$ human equivalent concentration (HEC) of 0.071 mg molybdenum/m^3 and an uncertainty factor of 30.

Bd wt or BW = body weight; BI = biochemical changes; BMCL = 95% lower confidence limit on the benchmark concentration; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GN = gross necropsy; Hemato = hematological; HP = histopathology; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = minimal risk level; Musc/skel = muscular skeletal; NOAEL = no-observed-adverse-effect level; OF = organ function; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive effects; WI = water intake

Figure 2-2. Levels of Significant Exposure to Molybdenum – Inhalation
Acute (≤14 days)

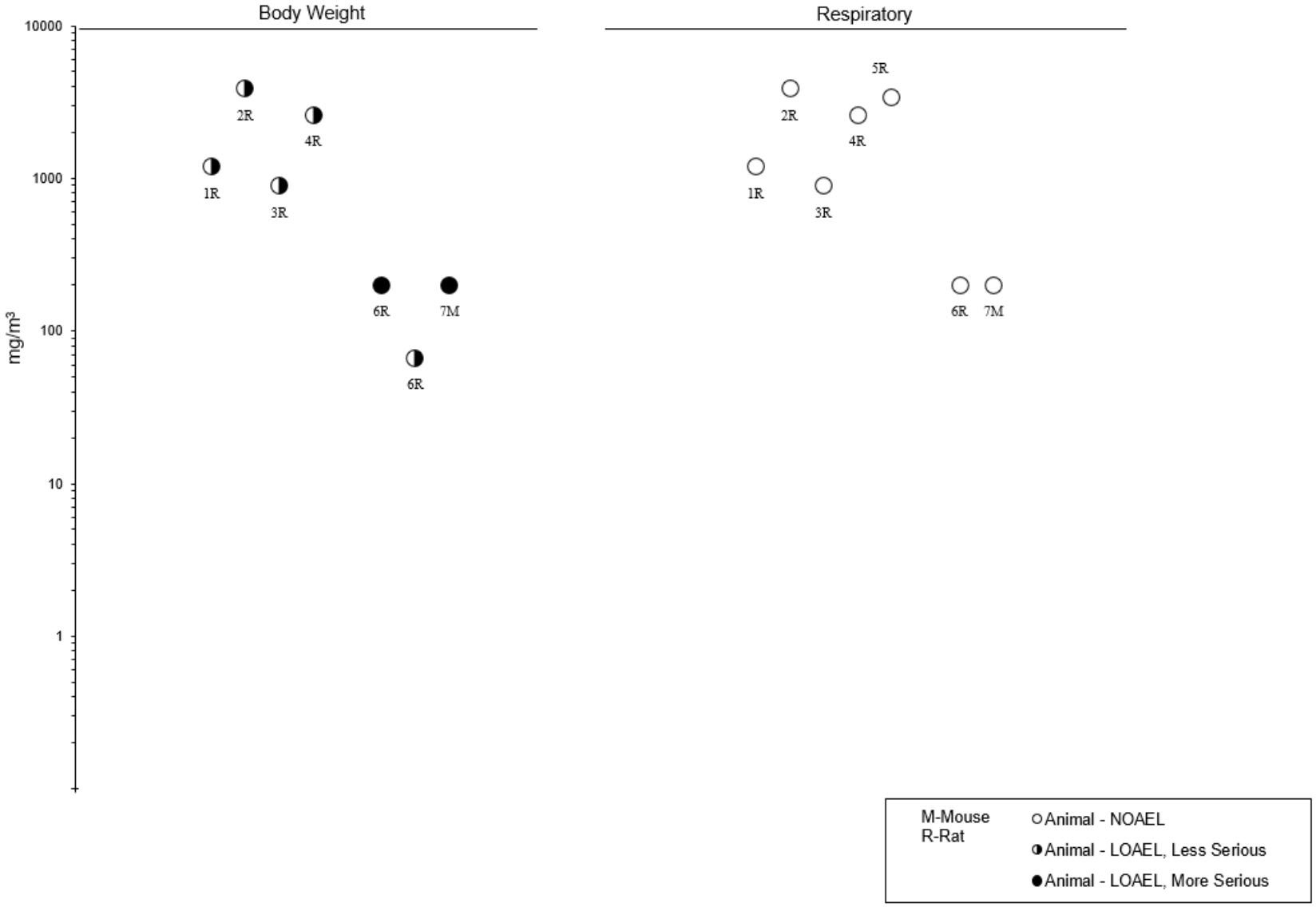


Figure 2-2. Levels of Significant Exposure to Molybdenum – Inhalation
Intermediate (15-364 days)

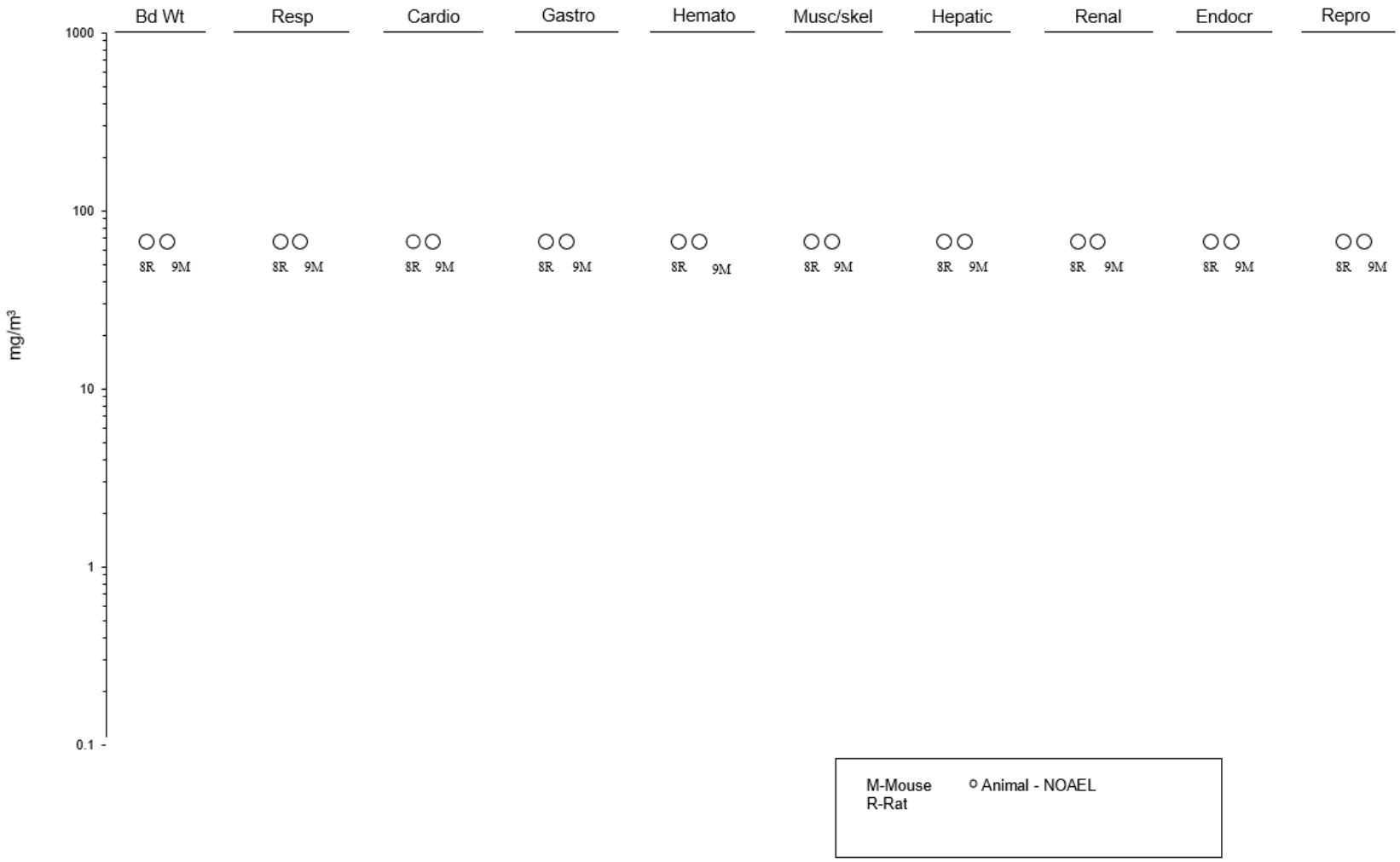


Figure 2-2. Levels of Significant Exposure to Molybdenum – Inhalation

Chronic (≥365 days)

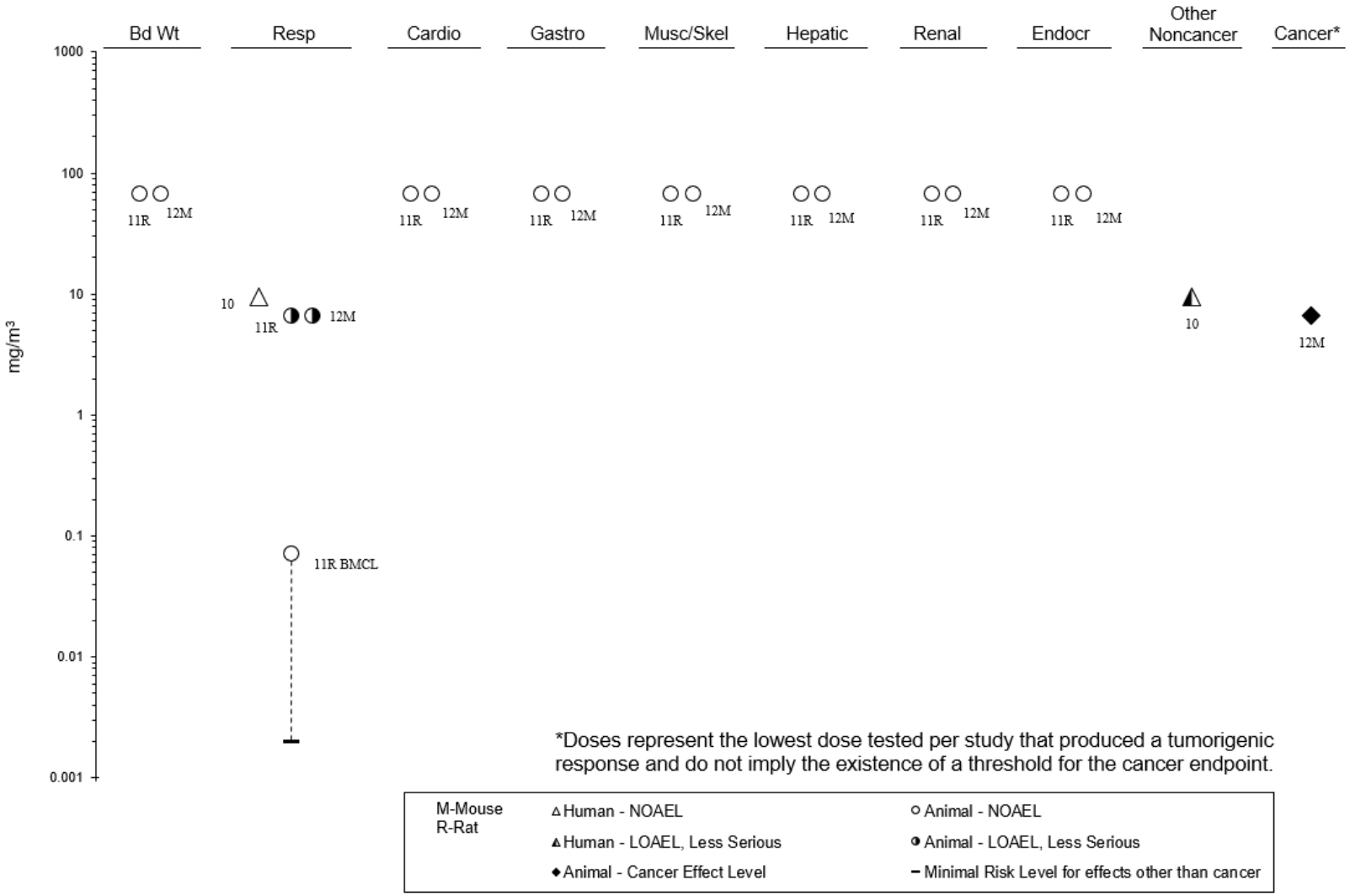


Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
ACUTE EXPOSURE									
1	Human 4 M	10 days (F)	0.00237, 0.00771, 0.022	UR	Other noncancer	0.022			No alterations in urinary uric acid levels
Ammonium molybdate Deosthale and Gopalan 1974									
2	Rat (Sprague-Dawley) 5 M, 5 F	Once (GO)	1,900, 2,400, 3,000	LE, CS, BW, GN	Death			2,291	LD_{50}
					Gastro	2,400	3,000		Thickening of the glandular stomach
Ammonium dimolybdate Baldrick and Healing 1990e									
3	Rat (Sprague-Dawley) 5 M, 5 F	Once (GO)	2,000, 2,500 (males only), 3,200, 4,000 (females only), 5,000	LE, CS, BW, GN	Death			2,566 F, 1,802 M	LD_{50}
Molybdenum trioxide Baldrick and Healing 1990f									
4	Rat (Sprague-Dawley) 5 M, 5 F	Once (GO)	1,500, 2,300, 3,000	LE, CS, BW, GN	Death			2,079 F, 1,912 M	LD_{50}
Sodium molybdate Baldrick and Healing 1990g									
5	Rat (Sprague-Dawley) 22 M	PNDs 4–17 (G)	0, 50	BW, HP	Bd wt	50			
					Musc/skel		50		Increased buccal and sulcal enamel lesions following pre-eruptive exposure to molybdenum and administration of a caries promoting diet
Sodium molybdate Hunt and Navia 1975									

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
6	Mouse (ICR) 25 F	14 days (W)	0, 1.3, 2.6, 5.3, 11	HP	Repro	2.6	5.3		Increase in the rate of abnormal MII oocytes and decrease in ovarian weights at 11 mg/kg/day; ovarian hyperemia at 5.3 and 11 mg/kg/day (incidence not reported)
Sodium molybdate Zhang et al. 2013									
7	Mouse (ICR) 10 M	14 days (W)	0, 3, 6, 12, 25, 49	RX	Repro	12	25		Decreases in relative epididymides weight, sperm concentration, and sperm motility and increase in rate of sperm abnormalities
Sodium molybdate Zhai et al. 2013									
8	Rabbit (New Zealand) 5 M	14 days (F)	0, 0.58	BW, HP	Bd wt	0.58			
					Hepatic	0.58			
					Renal	0.58			
Ammonium heptamolybdate Bersenyi et al. 2008									
INTERMEDIATE EXPOSURE									
9	Rat (Sprague-Dawley) 7 M	8 weeks (GW)	0, 40, 80	BW, OW, UR	Bd wt	40	80		Decrease in body weight gain; terminal body weight was 26% lower than in controls
					Renal	40	80		Increases in diuresis and creatinuria, decreases in creatinine clearance, increases in urinary kallikrein (distal tubule enzyme) levels, and increases in relative and absolute kidney weights
Ammonium heptamolybdate Bompart et al. 1990									

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
10	Rat (Sprague-Dawley) 6 F	8 weeks (W)	0, 0.76, 1.5, 7.6, 15	BW, WI, RX	Repro	0.76	1.5		Prolonged estrus phase (6–12 hours) of the estrous cycle at ≥1.5 mg/kg/day; no effects on fertility
Sodium molybdate Fungwe et al. 1990									
11	Rat (Sprague-Dawley) 3–6 M, 2–3 F	6 weeks (F)	0, 70	BW, HE	Hemato	70			
Sodium molybdate Gray and Daniel 1954									
12	Rat (Long-Evans) 4 M, 4 F	At least 8 weeks (F)	0, 7	BW, HE	Bd wt Hemato Repro Develop	7 7 7 7			
Sodium molybdate Jeter and Davis 1954									
13	Rat (Wistar) 4 M	5 weeks (F)	0, 74	BW, BI	Bd wt		74		36% decrease in body weight gain
Sodium molybdate Mills et al. 1958									
14	Rat (Sprague-Dawley) 10 M, 10 F	90 days (F)	0, 5, 17, 60	CS, BW, BC, HE, FI, GN, HP, OW	Bd wt Resp Cardio Gastro Hemato Hepatic Renal	17 M 60 60 60 60 60 17 F[b]	60 M 60 F		15.2% lower terminal body weight in males Slight diffuse hyperplasia in the renal proximal tubules were observed in 2/10 female rats

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
					Ocular	60			
					Endocr	60			
					Repro	60 F 60 M			
					Other noncancer	60			
Sodium molybdate Murray et al. 2014a									
15	Rat (Sprague-Dawley) 25 F	GDs 6–20 (F)	0, 3, 10, 20, 40	DX	Develop	40			
Sodium molybdate Murray et al. 2014b									
16	Rat (Sprague-Dawley) 24 M, 24 F	2 generations 10 weeks prior to mating, 10–17 days mating period, and gestation and lactation periods (W)	0, 5, 17, 40	CS, BW, OW, HP, RX, DX	Bd wt	40			
					Resp	40			
					Renal	40			
					Endocr	40			
					Repro	40			
					Develop	40			
Sodium molybdate Murray et al. 2019									
17	Rat (Sprague-Dawley) 24 M, 24 F	2 generations 10 weeks prior to mating, 10–17 days mating period, and gestation and lactation periods (F)	0, 40	CS, BW, OW, HP, RX, DX	Bd wt		40 F		Decreased maternal weight gain (22%) on GDs 0–7
					Resp	40			
					Renal	40			
					Endocr	40			
					Repro	40			
					Develop	40			

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
Sodium molybdate Murray et al. 2019									
18	Rat (Druckery) 10 M	5 days/week 60 days (GW)	0, 4.7, 14, 24	BW	Bd wt	24			
					Repro	4.7	14		Decreases in sperm count and sperm motility and increases in sperm abnormalities at ≥14 mg/kg/day; degeneration of seminiferous tubules in the testes at 24 mg/kg/day; it is unclear whether this was also observed at 14 mg/kg/day
Sodium molybdate Pandey and Singh 2002									
19	Rat (Druckery) 20 M	5 days/week 60 days (GW)	0, 14	DX, RX	Repro			14	Decrease in fertility (60% versus 80% in controls) and increased pre-implantation losses
					Develop			14	Increased post-implantation losses, increased resorptions, decreased number of live fetuses, and decreases in fetal weight and crown-rump length
Sodium molybdate Pandey and Singh 2002									
20	Rat (Wistar) 6 M	9 weeks (W)	0, 100	BW, BI, OW	Bd wt	100			
					Cardio	100			
					Other noncancer	100			No alterations in blood triglyceride, glucose, or insulin levels
Sodium molybdate Peredo et al. 2013									

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
21	Rat (Wistar) 10 M or 5 M, 5 F	4–5 weeks (F)	0, 110	BW, BI	Bd wt		110 M		46–48% decrease in body weight gain
Sodium molybdate Van Reem and Williams 1956									
22	Rat (Wistar) 8 NR	6 weeks (F)	0, 85	BW, BI	Bd wt	85			
Sodium molybdate Williams and Van Reem 1956									
23	Rat (Wistar) 8 NR	6 weeks (F)	0, 90, 144, 185	BW, BI	Bd wt		90		Decreases in body weight gain of 22, 44, and 60% in the 90, 144, and 185 mg/kg/day groups
Sodium molybdate Williams and Van Reem 1956									
24	Rat (Sprague-Dawley) 10 F	8 weeks (W)	0, 0.015, 0.076, 0.15, 0.30, 0.76, 1.5	BW, BI, OW	Bd wt	1.5			
Sodium molybdate Yang and Yang 1989									
25	Mouse (Kunming) 20 M	100 days (W)	0, 100	BW, BC, HP, RX	Bd wt	100			
					Repro		100		Decreased sperm density and motility; testicular atrophy (no incidence data reported)
Molybdenum Wang et al. 2016									

Table 2-2. Levels of Significant Exposure to Molybdenum – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
26	Rabbit (Dutch) 2–5 M, F	30–84 days (F)	0, 7.1, 25, 54, 120, 240	CS, LE, BW, HE	Death			120	4/5 and 2/2 died at 120 and 240 mg/kg/day; average survival was 44 and 30 days, respectively
					Bd wt	25		120	Weight loss at 120 and 240 mg/kg/day
					Hemato	25	54		Anemia in 2/5, 5/5, and 4/5 rabbits at 54, 120, and 240 mg/kg/day
					Musc/skel	25		54	Front leg abnormality described as weakness progressing to inability to "maintain weight and legs spread outward"
					Dermal	25	54		Alopecia
Sodium molybdate Arrington and Davis 1953									
CHRONIC EXPOSURE									
27	Human 262 M, F	NR (F)	0.21	BC	Other noncancer		0.21		Increased incidence of symptoms of gout and an increased blood uric acid levels
Molybdenum Koval'sky et al. 1961									

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.06 mg/kg/day based on a NOAEL of 17 mg molybdenum/kg/day, a total uncertainty factor of 100, and a modifying factor of 3.

BC = biochemistry; Bd wt or BW = body weight; BI = biochemical changes; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = minimal risk level; Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; NR = not reported; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive effects; UR = urinalysis; (W) = water; WI = water intake

Figure 2-3. Levels of Significant Exposure to Molybdenum – Oral
Acute (≤14 days)

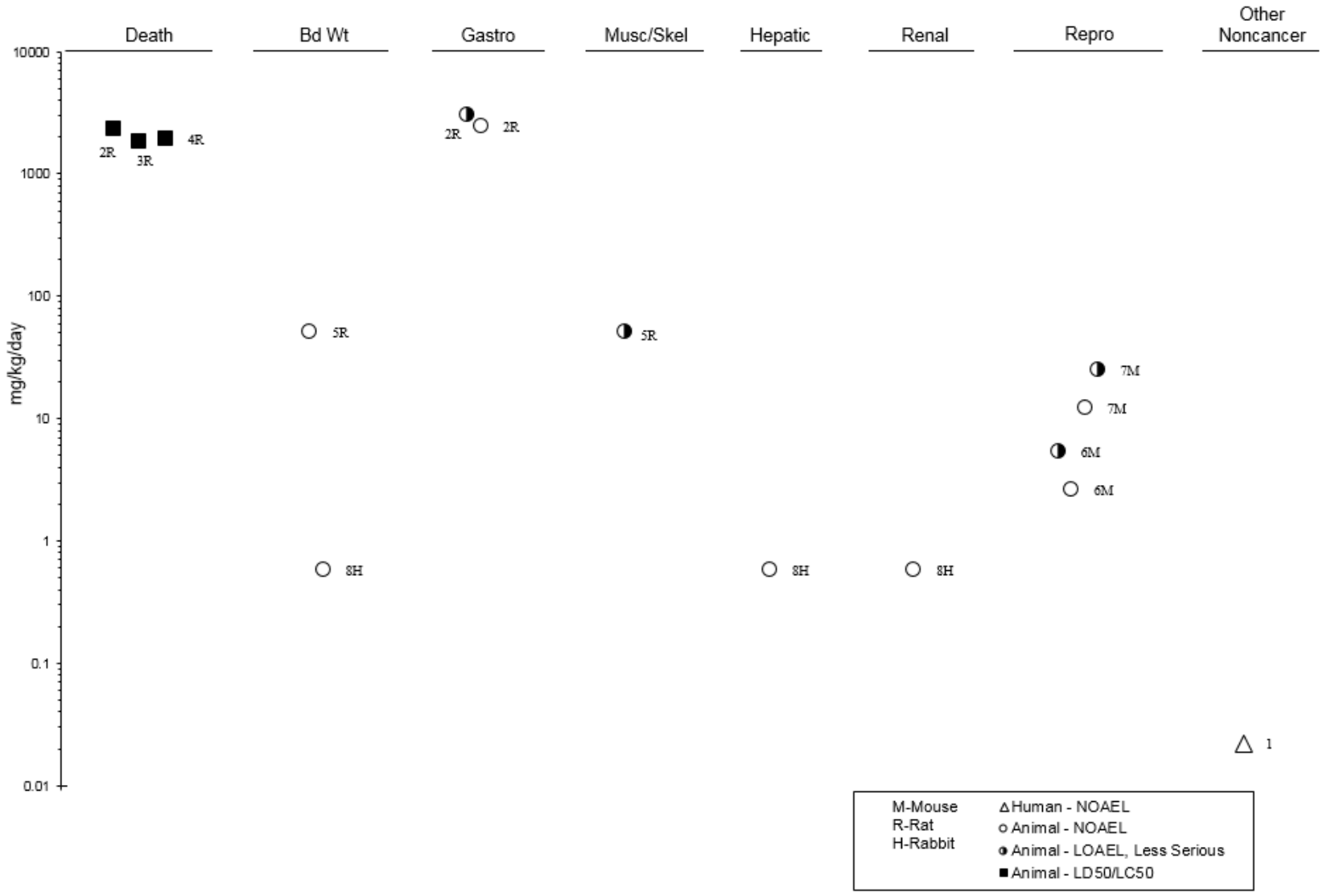


Figure 2-3. Levels of Significant Exposure to Molybdenum – Oral
Intermediate (15-364 days)

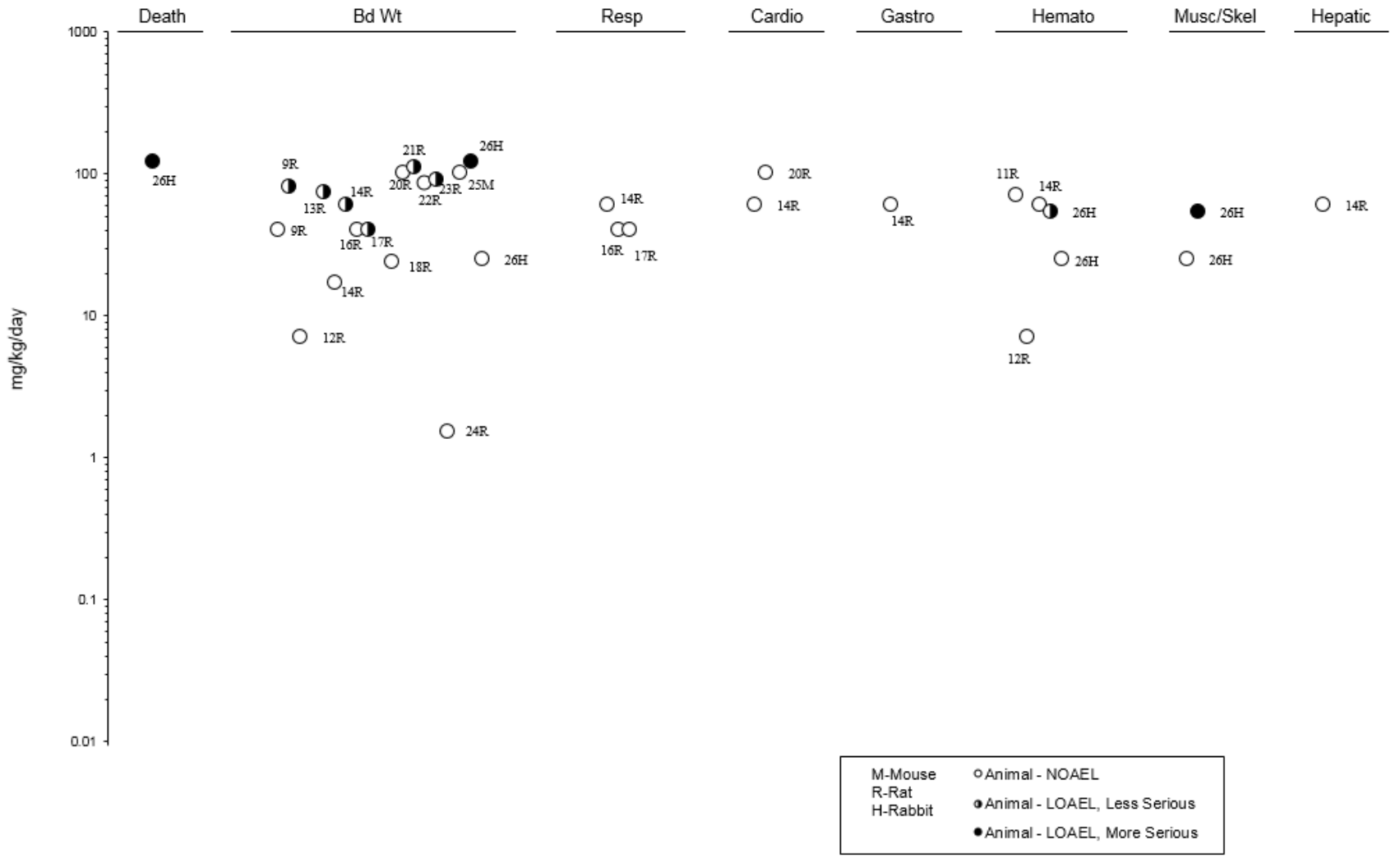


Figure 2-3. Levels of Significant Exposure to Molybdenum – Oral

Intermediate (15-364 days)

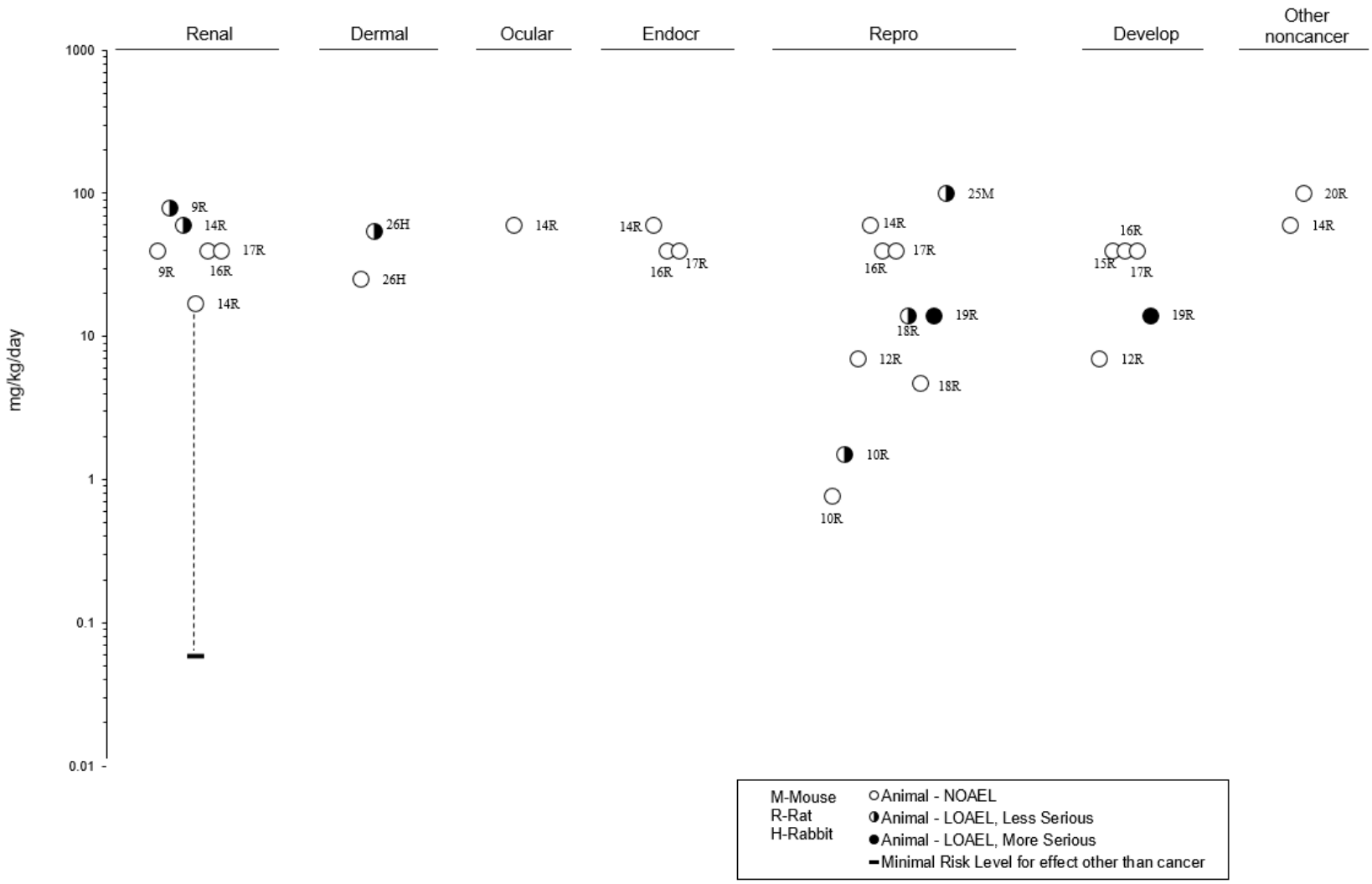


Figure 2-3. Levels of Significant Exposure to Molybdenum – Oral
Chronic (≥365 days)

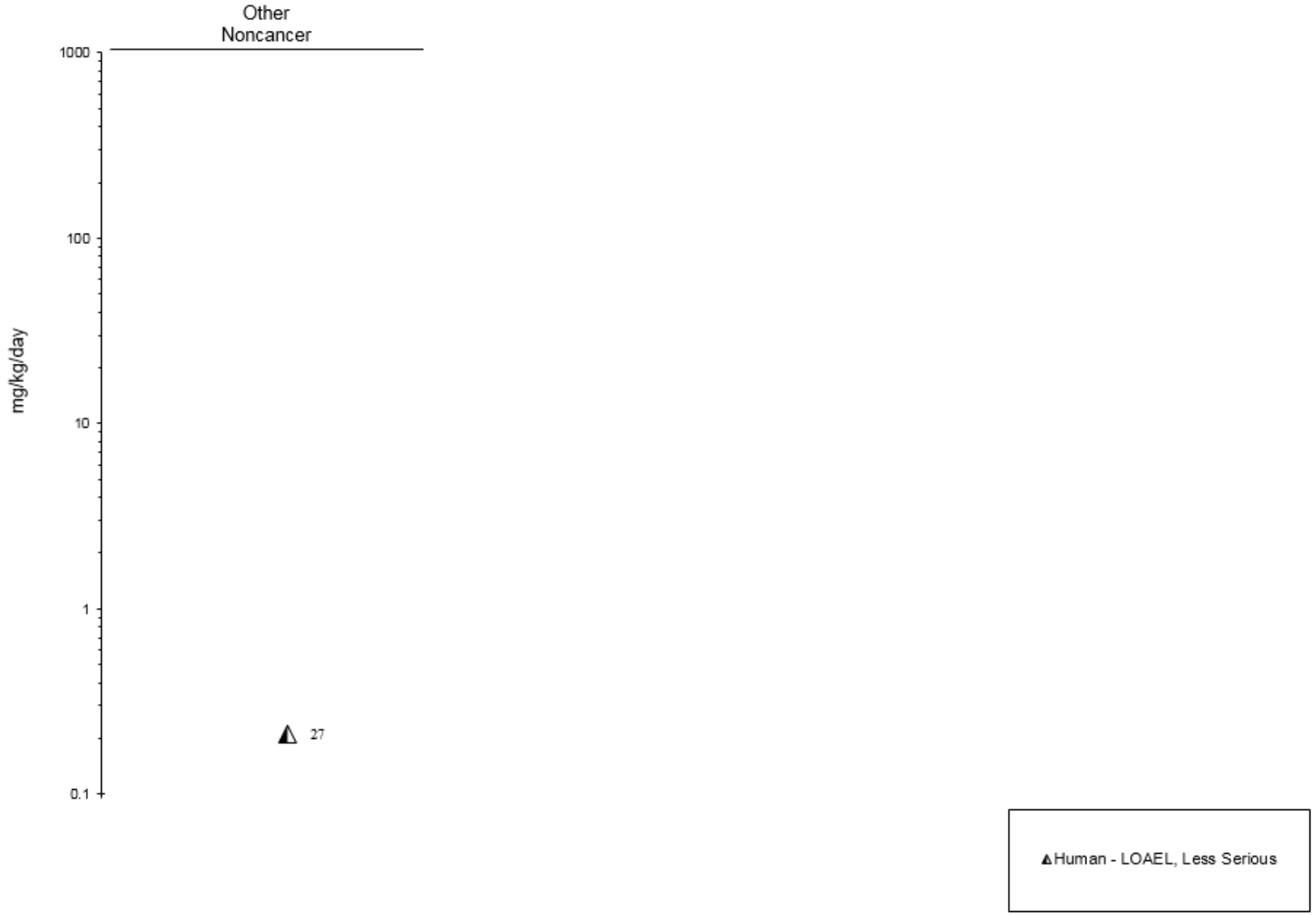


Table 2-3. Levels of Significant Exposure to Molybdenum – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
ACUTE EXPOSURE								
Guinea pig (Dunkin/Hartley) 20 F	Twice	90%	CS, BW, IX	Immuno	90			
Ammonium dimolybdate Allan 1996a								
Guinea pig (Dunkin/Hartley) 20 F	Twice	70%	CS, BW, IX	Immuno	70			
Molybdenum trioxide Allan 1996c								
Guinea pig (Dunkin/Hartley) 20 F	Twice	70%	CS, BW, IX	Immuno	70			
Sodium molybdate Allan 1996d								
Guinea pig (Dunkin/Hartley) 20 F	Twice	70%	CS, BW, IX	Immuno	70			
Molybdenum trioxide Allan 1996b								
Rat (CD) 5 M, 5 F	24 hours	0, 1,200 mg/kg	CS, BW, GN	Dermal	1,200			
Ammonium dimolybdate Baldrick and Healing 1990a								
Rat (CD) 5 M, 5 F	24 hours	0, 1,300 mg/kg	CS, BW, GN	Dermal	1,300			
Molybdenum trioxide Baldrick and Healing 1990b								
Rat (CD) 5 M, 5 F	24 hours	0, 930 mg/kg	CS, BW, GN	Dermal	930			
Sodium molybdate Baldrick and Healing 1990c								

Table 2-3. Levels of Significant Exposure to Molybdenum – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
Rat (CD) 5 M, 5 F	24 hours	0, 1,300 mg/kg	CS, BW, GN	Dermal	1,300			
Molybdenum trioxide Baldrick and Healing 1990d								
Rabbit (New Zealand) 6 M	Once	0, 56 mg	CS	Ocular		56		Mild conjunctival inflammation
Ammonium dimolybdate Liggett and McRae 1990a								
Rabbit (New Zealand) 6 M	Once	0, 67 mg	CS	Ocular		67		Mild conjunctival inflammation
Molybdenum trioxide Liggett and McRae 1990b								
Rabbit (New Zealand) 6 M	Once	0, 46 mg	CS	Ocular		46		Mild conjunctival inflammation
Sodium molybdate Liggett and McRae 1990c								
Rabbit (New Zealand) 6 M	Once	0, 67	CS	Ocular		67		Conjunctival inflammation
Molybdenum trioxide Liggett and McRae 1990d								
Rabbit (New Zealand) 6 F	4 hours	280 mg	CS	Dermal	280			
Ammonium dimolybdate Liggett and McRae 1990e								
Rabbit (New Zealand) 6 F	4 hours	340 mg	CS	Dermal	340			
Molybdenum trioxide Liggett and McRae 1990f								

Table 2-3. Levels of Significant Exposure to Molybdenum – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg Mo/kg/day)	Less serious LOAEL (mg Mo/kg/day)	Serious LOAEL (mg Mo/kg/day)	Effects
Rabbit (New Zealand) 6 F	4 hours	230 mg	CS	Dermal	230			
Sodium molybdate Liggett and McRae 1990g								

BW = body weight; CS = clinical signs; F = female(s); GN = gross necropsy; IX = immune function; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level

2.2 DEATH

The lethality of molybdenum compounds has been investigated in several inhalation and oral exposure studies in laboratory animals. In inhalation studies, no deaths were reported in rats or mice exposed to ≤200 mg molybdenum/m^3 for 14 days (NTP 1997) or ≤67 mg molybdenum/m^3 for 90 days or 2 years (NTP 1997).

Oral LD_{50} values have been estimated in rats exposed to several molybdenum compounds. The estimated LD_{50} values were 2,291 mg molybdenum/kg for ammonium dimolybdate (Baldrick and Healing 1990e), 1,802 and 2,566 mg molybdenum/kg for pure molybdenum trioxide for males and females, respectively (Baldrick and Healing 1990f), and 1,912 and 2,079 mg molybdenum/kg for sodium molybdate for males and females, respectively (Baldrick and Healing 1990g). A study of technical-grade molybdenum trioxide did not report deaths occurring in rats administered a single dose of 3,400 mg molybdenum/kg (Baldrick and Healing 1990h).

Several oral studies have reported deaths in rabbits repeatedly exposed to molybdenum. Mortality rates of 42–100% were observed in rabbits exposed to 59–120 mg molybdenum/kg/day for intermediate durations (Arrington and Davis 1953; Robinson et al. 1969; Valli et al. 1969; Widjajakusuma et al. 1973). Although the causes of death were not reported, anorexia, body weight loss, and anemia were observed in most of the studies at the lethal concentrations, suggesting that the deaths may be related to a functional copper deficiency. The copper content of the diet was adequate in the Arrington and Davis (1953) study and was not reported in the Widjajakusuma et al. (1973), Robinson et al. (1969), and Valli et al. (1969) studies. No deaths have been reported in rat studies (e.g., Lyubimov et al. 2004; Murray et al. 2014a, 2014; Pandey and Singh 2002).

2.3 BODY WEIGHT

There are limited epidemiological data evaluating possible associations between molybdenum and body weight. A cross-sectional study of National Health and Nutrition Examination Survey (NHANES) participants did not find an association between urinary molybdenum levels and the risk of being overweight (Mendy et al. 2012).

Several inhalation exposure studies have reported body weight effects in laboratory animals. Single 4-hour exposures to 1,200 mg molybdenum/m^3 as ammonium dimolybdate (Jackson et al. 1991a),

3,890 mg molybdenum/m^3 as molybdenum trioxide (Jackson et al. 1991b), 2,613 mg molybdenum/m^3 as molybdenum trioxide (Jackson et al. 1991d), or 899 mg molybdenum/m^3 as sodium molybdate (Jackson et al. 1991c) resulted in decreases in body weight gain or weight loss during the first 2–3 days post-exposure; thereafter, the body weight gain was similar to controls. Decreases in body weight gain and weight loss were observed in rats and mice exposed via inhalation to molybdenum trioxide for 14 days (NTP 1997). Terminal body weights were 10% lower in male rats exposed to 67 mg molybdenum/m^3 compared to controls, and weight loss was observed in male rats and mice exposed to 200 mg molybdenum/m^3. In female rats and mice exposed to 200 mg molybdenum/m^3, the terminal body weights were 13 and 10%, respectively, lower than the control groups. No significant alterations in body weight gain were observed in rats or mice exposed to airborne molybdenum trioxide concentrations as high as 67 mg molybdenum/m^3 for 13 weeks or 2 years (NTP 1997).

A large number of animal studies reported alterations in body weight following acute- or intermediate-duration oral exposure to molybdenum. Large differences in terminal body weights between controls and molybdenum-exposed groups and weight loss have been reported in many studies in which the basal diet did not provide adequate levels of copper (Brinkman and Miller 1961; Fell et al. 1979; Johnson and Miller 1961; Ostrom et al. 1961; Sasmal et al. 1968; Van Reen 1959). In one study, exposure to 500 mg molybdenum/kg/day as sodium molybdate resulted in weight loss in rats (Sasmal et al. 1968); no alterations in weight loss were observed at 50 or 100 mg molybdenum/kg/day. The weight loss began early in the study; the animals weighed about 35% less than at the start of the study after 1 week of exposure. In another study by this group (Sasmal et al. 1968), exposure to 50 mg molybdenum/kg/day as ammonium molybdate resulted in weight loss. Although the study suggests differences between the two molybdenum compounds, the very low copper content of the diet (no additional copper was added to the purified diet) precludes extrapolating these data to other conditions. In another study comparing molybdenum compounds, a 10-day dietary exposure to 0.6 mg molybdenum/kg/day as ammonium tetrathiomolybdate resulted in a 10% decrease in body weight in rats; however, no alterations in body weight gain were observed in rats exposed to 0.6 mg molybdenum/kg/day as ammonium heptamolybdate under the same exposure conditions (Parry et al. 1993). The copper content of the diet was 3 ppm, which is lower than the recommendation of 5 ppm in the diet (NAS 1995).

Decreases in body weight gain have been observed in studies in which the basal diet provided a nutritionally adequate level of copper (Arrington and Davis 1953; Bompart et al. 1990; Jeter and Davis 1954; Johnson et al. 1969; Lyubimov et al. 2004; Mills et al. 1958; Murray et al. 2014a; Van Reen and Williams 1956). Significant decreases in body weight gain were observed at 60–110 mg

molybdenum/kg/day as sodium molybdate or ammonium heptamolybdate in intermediate-duration studies (Bompart et al. 1990; Mills et al. 1958; Murray et al. 2014a; Van Reen and Williams 1956; Williams and Van Reen 1956). The magnitude of the decrease in body weight gain appeared to be related to the dose, with approximately 15% decreases observed at 60 mg molybdenum/kg/day and 48% decreases observed at 110 mg molybdenum/kg/day. Administration of ammonium tetrathiomolybdate resulted in a LOAEL of 4.4 mg molybdenum/kg/day for decreases in body weight gain (Lyubimov et al. 2004); the interaction between the ammonium tetrathiomolybdate and copper may have resulted in copper insufficiency and contributed to the body weight effect. Decreases in food intake have also been reported in dietary exposure studies (Murray et al. 2014a; Williams and Van Reen 1956) and a gavage study (Lyubimov et al. 2004). Williams and Van Reen (1956) found that when the control group food intake was matched to the molybdenum group, body weight was not adversely affected after 5 weeks of exposure to 85 mg molybdenum/kg/day as sodium molybdate. However, when the control group had *ad libitum* access to food, exposure to 90 mg molybdenum/kg/day as sodium molybdate resulted in a 22% decrease in body weight gain. In contrast, Murray et al. (2014a) found a decrease in food conversion efficiency suggesting that factors other than the reduction in feed intake resulted in the decreased body weight gain. Similarly, in a study by Johnson and Miller (1961) in which the basal diet contained 3.2 ppm copper, large differences (50–60% less) in food intake were observed between the control group and the group exposed to 20 ppm molybdenum/kg/day as sodium molybdate. However, when the control intake was matched to the molybdenum group's intake, significant decreases in body weight gain were still observed.

2.4 RESPIRATORY

Limited data are available on the toxicity of molybdenum to the respiratory tract of humans. A cohort study of workers exposed to molybdenum trioxide and other oxides at a molybdenite roasting plant reported normal lung function test results in 20/25 workers (Walravens et al. 1979). Some alterations in lung function (forced expiratory volume in 1 second, FEV_1) were observed in the remaining five workers; the decrease in FEV_1 was characterized as mild in three of the workers and "more marked" in two workers, which may be indicative of mild obstructive lung disease. The study did not provide lung function data for a reference group. The estimated 8-hour time-weighted average (TWA) molybdenum concentration in total dust was 9.46 mg molybdenum/m^3; the molybdenum content of the respirable dust ranged from 1.02 to 4.49 mg molybdenum/m^3. Another cohort study of workers exposed to fine and ultrafine molybdenum trioxide dust reported dyspnea and cough in symptomatic workers (Ott et al. 2004). Radiographic abnormalities were noted in the lungs of most of the symptomatic workers and in half of the asymptomatic workers, although none of the radiographs showed evidence of interstitial lung disease.

Significant differences in lung function (increased predicted FEV_1 and forced vital capacity) were also observed in the workers, as compared to a control group. In symptomatic workers, alterations in bronchioalveolar lavage cytology suggestive of subclinical alveolitis were noted. This study (Ott et al. 2004) has several limitations including the lack of monitoring data, minimal information on the control group, which does not appear to be comprised of workers at this facility, and differences in the mean and ranges of ages of the different groups (40.0 years [range of 24–58 years], 30.5 years [22–45 years], and 30.0 years [14–72 years] in the symptomatic workers, asymptomatic workers, and controls, respectively), which were not adjusted for in the statistical analyses.

The potential respiratory toxicity of molybdenum has been investigated in laboratory animals exposed to airborne molybdenum trioxide for acute, intermediate, and chronic durations and in intermediate-duration oral studies in rats. No histological alterations were observed in the lungs of rats exposed for 4 hours to 1,200 mg molybdenum/m^3 as ammonium dimolybdate (Jackson et al. 1991a), 2,613–3,890 mg molybdenum/m^3 as molybdenum trioxide (Jackson et al. 1991b, 1991d; Leuschner 2010), or 899 mg molybdenum/m^3 as sodium molybdate (Jackson et al. 1991c). In inhalation studies conducted by the National Toxicology Program (NTP 1997), no histological alterations were observed in the nasal cavity of rats and mice exposed to 200 mg molybdenum/m^3 as molybdenum trioxide for 14 days (NTP 1997); no other regions of the respiratory tract were examined. Similarly, no histological alterations were observed in the respiratory tract of rats or mice exposed to ≤67 mg molybdenum/m^3 as molybdenum trioxide for 13 weeks (NTP 1997). In contrast, chronic exposure resulted in lesions in the nose, larynx, and lungs in rats and mice exposed to molybdenum trioxide for 2 years (NTP 1997). In the nose, hyaline degeneration of the respiratory and olfactory epitheliums was observed in rats exposed to ≥6.7 mg molybdenum/m^3 and in mice exposed to 67 mg molybdenum/m^3; other nasal lesions observed in mice included suppurative inflammation at ≥20 mg molybdenum/m^3 and olfactory epithelial atrophy at 67 mg molybdenum/m^3. Squamous metaplasia of the epiglottis was observed in rats and mice exposed to ≥6.7 mg molybdenum/m^3. In the lungs, chronic inflammation was observed in rats exposed to ≥20 mg molybdenum/m^3, and alveolar epithelial metaplasia and histiocytic cellular infiltration were observed at ≥6.7 mg molybdenum/m^3.

Two laboratory animal studies examined the respiratory tract following oral exposure to molybdenum. No lesions were observed in the lungs of rats exposed to ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a) or ≤40 mg molybdenum/kg/day as sodium molybdate in the drinking water or diet for 147–158 days (Murray et al. 2019).

2.5 CARDIOVASCULAR

Using the dataset from the NHANES cross-sectional study (2009–2012), Shiue and Hristova (2014) found an association between urinary molybdenum levels and high blood pressure among adults after adjusting for potential confounders (adjusted odds ratio [OR] of 1.45; 95% confidence interval [CI] of 1.04–2.02). The investigators estimated that molybdenum accounted for 6.3% of the variance in the population risk and significant associations were also found for other metals including cesium, lead, platinum, antimony, arsenic, and tungsten and industrial pollutants including phthalates, bisphenol A, and parabens. In a cross-sectional study examining the possible association between municipal water constituents and cardiovascular mortality in residents of 94 large cities in the United States, Schroeder and Kraemer (1974) found a weak negative correlation between arteriosclerotic heart disease deaths and molybdenum levels among white males, but not white females or nonwhite males or females. The mean concentration of molybdenum in the municipal water samples was 1.25 µg/L (0.00003 mg molybdenum/kg/day, assuming a water intake of 2 L/day and body weight of 70 kg) with a range of 0–16 µg/L. These studies appear to provide conflicting results, with one study suggesting a beneficial effect of increased molybdenum (Schroeder and Kraemer 1974) and the other a detrimental effect (Shiue and Hristova 2014). However, a number of etiological factors contribute to the overall risk of both diseases and the contribution of molybdenum to the overall risk was low in both studies.

In the only laboratory animal study evaluating blood pressure, Peredo et al. (2013) reported a slight decrease (approximately 4%) in systolic blood pressure in rats exposed to 100 mg molybdenum/kg/day as sodium molybdate in drinking water for 9 weeks; this slight decrease in blood pressure was not considered biologically relevant. No histological alterations were observed in the hearts of rats or mice exposed to airborne molybdenum trioxide concentrations as high as 67 mg molybdenum/m^3 for 13 weeks or 2 years (NTP 1997) or in rats ingesting ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a).

2.6 GASTROINTESTINAL

Intermediate- or chronic-duration inhalation exposure to ≤67 mg molybdenum/m^3 as molybdenum trioxide did not result in histological alterations in the gastrointestinal tract (NTP 1997).

A single-dose oral lethality study reported thickening of the glandular stomach in rats receiving a gavage dose of 3,000 mg molybdenum/kg as ammonium dimolybdate (Baldrick and Healing 1990e). No

histological alterations were observed in the gastrointestinal tract of rats exposed to ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a). In contrast, Fell et al. (1979) reported soft feces and diarrhea and a number of histological alterations in the gastrointestinal tract of rats exposed for up to 21 days to 0.5 mg molybdenum/kg/day as ammonium tetrathiomolybdate in the diet (diet provided an inadequate amount of copper). The alterations included shortening of the gastric pits with a reduction in the amount of mucin in the stomach, an increase in the crypt to villus ratio in the small intestine due to a lengthening of the crypts, edema of the lamina propria in the ileum, and submucosal edema of the cecum resulting in a thickening of the cecum but no effect on the brush border. However, the investigators did not provide incidence data, which limits the assessment of these alterations.

2.7 HEMATOLOGICAL

No significant alterations in hematological parameters were observed in rats or mice following inhalation exposure to molybdenum trioxide at concentrations as high as 67 mg molybdenum/m^3 for 13 weeks (NTP 1997).

In general, the hematological system does not appear to be a target of molybdenum oral toxicity when the basal diet contains adequate levels of copper. In rats exposed to sodium molybdate or ammonium heptamolybdate, the highest NOAEL values for hematological alterations ranged from 3.35 to 150 mg molybdenum/kg/day for intermediate-duration exposure (Brinkman and Miller 1961; Franke and Moxon 1937; Gray and Daniel 1954; Hunt and Navia 1973; Jeter and Davis 1954; Johnson et al. 1969; Murray et al. 2014a). One study reported decreases in erythrocyte counts, hemoglobin, and hematocrit in rats exposed to 4.4 mg molybdenum/kg/day as ammonium tetrathiomolybdate administered via gavage for 59–61 days (Lyubimov et al. 2004). Although the basal diet contained the National Research Council's (NRC's) recommended amount of copper (NAS 1995), hematological effects were not observed in rats exposed to the same molybdenum dose receiving a diet containing additional copper (110 ppm), suggesting that the hematological effects may have been secondary to a molybdenum-induced copper deficiency (anemia is a sign of copper deficiency). In young rabbits, exposure to 54 mg molybdenum/kg/day as sodium molybdate in the diet resulted in anemia (Arrington and Davis 1953). Even though the reported copper concentration in the diet exceeded the more recently recommended standard of 3 ppm (NAS 1977), administration of additional copper resulted in increases in hemoglobin levels. In a similar study using mature rabbits, anemia was observed in one of two rabbits exposed to 30 mg molybdenum/kg/day as sodium molybdate in the diet (Arrington and Davis 1953). Decreases in

hemoglobin levels and packed cell volume were also observed in two other rabbit studies (Valli et al. 1969; Widjajakusuma et al. 1973) in which rabbits were exposed to 77 or 59 mg molybdenum/kg/day in the diet for approximately 4 weeks. Mortality was observed in both studies and neither study reported the copper levels of the basal diet; Valli et al. (1969) did note that the rabbits were fed a diet with a low copper content. In pigs, no hematological alterations were observed following dietary exposure to 20–100 ppm molybdenum as sodium molybdate or ammonium heptamolybdate in the diet for at least 8 weeks (Gipp et al. 1967; Kline et al. 1973); the studies did not provide sufficient information to allow for an estimation of the molybdenum dose.

2.8 MUSCULOSKELETAL

No histological alterations were observed in the bones of rats or mice exposed via inhalation to 6.7–67 mg molybdenum/m^3 as molybdenum trioxide for 13 weeks or 2 years (NTP 1997). Chronic molybdenum inhalation exposure also did not affect femoral bone density or curvature in groups of 10 rats exposed to concentrations as high as 67 mg molybdenum/m^3 (NTP 1997).

A number of oral exposure studies in laboratory animals have examined the effect of molybdenum on bone growth and strength and on the promotion of dental caries. Musculoskeletal effects were observed in two studies in which the diet contained at least the recommended level of copper. In a study by Johnson et al. (1969) in which rats were exposed to 150 mg molybdenum/kg/day as sodium molybdate in the diet for 6 weeks (the basal diet contained copper levels that were 3 times higher than the recommended amount), decreases in femur breaking strength (22% less than controls) and tail ring rupture strength (32% less than controls) were observed. Young rabbits exposed to ≥54 mg molybdenum/kg/day as sodium molybdate for 30–84 days exhibited a front limb abnormality characterized by weakness progressing to an inability to "maintain weight and legs spread outward" (Arrington and Davis 1953). This was not observed in mature rabbits exposed to ≤120 mg molybdenum/kg/day as sodium molybdate for at least 54 days (Arrington and Davis 1953). The investigators noted that in three of the seven affected animals, one or both feet bent inward at the carpus joint, the articular surface of the radius was exposed, and the tendon slipped out of normal position. It should also be noted that increases in mortality were also observed in the young rabbits exposed to 54 mg molybdenum/kg/day, and in two of the rabbits with limb abnormalities, administration of additionally copper did not reverse the skeletal effect, although there was improvement of other effects including anemia and body weight gain.

In an acute-duration study, femurs were significantly shorter in rats exposed to 0.6 mg molybdenum/kg/day as ammonium heptamolybdate or ammonium tetrathiomolybdate for 13 days (Parry et al. 1993). No alterations in the width of the growth plate or the bone composition (dry matter content, ash content, or percentage of calcium or phosphorus) were found. Similar findings were found in a 26-day study conducted by Parry et al. (1993); significant decreases in femur length were noted in rats exposed to 0.6 mg molybdenum/kg/day as ammonium heptamolybdate or ammonium tetrathiomolybdate in the diet. Although no direct comparisons were made between the two molybdenum groups, the magnitude of the decrease in femur length, as compared to the controls, was greater in the tetrathiomolybdate group. Increases in growth plate width were also observed in the rats exposed to ammonium tetrathiomolybdate, but not in rats exposed to ammonium heptamolybdate. In both experiments, the rats were fed a basal diet with inadequate copper levels (60% of the recommended concentration); in the ammonium tetrathiomolybdate study, plasma and liver copper levels indicated that the animals were extremely copper deficient. Spence et al. (1980) examined the development of widening of the epiphyseal growth plate over time in rats exposed to 1 mg molybdenum/kg/day as ammonium tetrathiomolybdate in the diet for 2–21 days. The study found cartilaginous dysplasia at the epiphyseal growth plate with impaired or arrested endochondral ossification, increases in periosteal osteogenesis and production of large amounts of disorganized bone, resorption of most trabecular bone, hemorrhaging within and tearing of tendons and ligaments, rotation and slipping of the distal epiphysis in the femur without fracture, and impaired fibrogenesis at ligamentous attachments to bone. Thickening and widening of the epiphyseal growth plate were observed in the distal femur and proximal and in the epiphyses of the humeral head, distal radius, and ulna; these effects were observed within the first 2 weeks of the study. Other morphological alterations in the bone were observed after 7 days of exposure; these included loss of alignment of hypertrophic cells at the periphery of the epiphyseal cartilage and localized increases in cell numbers. In rats allowed to recover for 39 days following the 21-day exposure period, osteogenesis and fibrogenesis returned to normal, and remodeling and growth returned (although some abnormal cartilage and bone were present). As with the Parry et al. (1993) study, the rats in the Spence et al. (1980) study were fed a basal diet containing an inadequate amount of copper (60% of the recommended level). Fejery et al. (1983) found an increase in femur breaking strength in rats exposed to 0.17 or 1.7 mg molybdenum/kg/day (copper content of the diet was not reported), which was considered a beneficial effect; at 17 mg molybdenum/kg/day, breaking strength was similar to controls. However, if the rats were maintained on a protein-deficient diet, decreases in breaking strength were observed at 1.7 and 17 mg molybdenum/kg/day. In rabbits exposed to a lethal concentration of sodium molybdate (77 mg molybdenum/kg/day) in the diet for 4 weeks, fractures of the humeral bone epiphyses were observed in 50% of the animals (Valli et al. 1969). Other effects included

longitudinal widening of the epiphyseal cartilage, marked reduction in trabecular bone, irregularly arranged spicules, and irregular metaphyseal calcification. In addition, the investigators noted that there was marked muscular degeneration in the pelvic limbs in 25% of the rabbits. The copper content of the basal diet was not reported in this study, although the investigators noted that the diet had a low copper content.

Alterations in tooth enamel and caries formation have also been observed in laboratory animals exposed to molybdenum. In rat pups administered 50 mg molybdenum/kg/day as sodium molybdate via gavage on postnatal days (PNDs) 4–17 (prior to tooth eruption) and fed a caries-promoting diet on PNDs 18–35, a 25% increase in buccal enamel lesion and 85 and 12.5% increases in lesions penetrating to the buccal and sulcal dentine-enamel junctions, respectively, were observed in the mandibular molars (Hunt and Navia 1975). Fejery et al. (1983) reported biphasic alterations in incisor tooth enamel microhardness in rats exposed to sodium molybdate in drinking water for 6 weeks (the copper content of the basal diet was not reported). At 1.7 mg molybdenum/kg/day, there were increases in microhardness (6–7% increases in surface and deep enamel microhardness), which was considered a beneficial effect. However, at 17 mg molybdenum/kg/day, tooth surface and deep enamel microhardness was decreased by 14.5 and 7.5%, respectively. The study also examined the possible effect of a low protein diet (3% in the low-protein groups compared to 18% in the protein-adequate groups) and found that the beneficial effect of 1.7 mg molybdenum/kg/day did not occur in the rats in the low-protein diet; a 4–5% reduction in microhardness was found at 1.7 mg/kg/day. Van Reen et al. (1962) did not find increases in dental caries in weanling NMRI-D rats (a caries susceptible strain) exposed to 8 mg molybdenum/kg/day as sodium molybdate for 5 weeks (the basal diet provided adequate copper levels).

2.9 HEPATIC

There are limited data on the hepatotoxicity of molybdenum in humans. Using the NHANES 2007–2008 cross-sectional study data, Mendy et al. (2012) found a significant association between urinary molybdenum levels and the risk of having a self-reported liver condition (OR 3.09; 95% CI 1.24–7.73). The geometric mean urinary molybdenum level of the population was 43.8 µg molybdenum/g creatinine (95% CI 42.61–45.19); the investigators did not report the urinary concentration associated with the increased risk of liver conditions. This study does not establish causality between molybdenum exposure and liver damage, and significant associations were also found between uranium and cesium levels and liver conditions.

The liver does not appear to be a sensitive target of molybdenum toxicity in laboratory animals, although some studies have reported biochemical alterations. No significant alterations in serum clinical chemistry parameters or liver weights were observed in rats or mice exposed to airborne molybdenum trioxide concentrations as high as 67 mg molybdenum/m^3 for 13 weeks (NTP 1997). No significant alterations in the incidence of hepatic lesions were observed following 13 weeks or 2 years of exposure (NTP 1997).

No histological alterations were observed in livers of rabbits exposed to 1.2 mg molybdenum/kg/day as ammonium heptamolybdate in the diet for 14 days (Bersenyi et al. 2008), rabbits exposed to 0.58 mg molybdenum/kg/day from carrots grown in ammonium heptamolybdate rich soil, or rats exposed to 60 mg molybdenum/kg/day in the diet for 90 days (Murray et al. 2014a); these are the only studies that included histological examination of the liver. The Bersenyi et al. (2008) female rabbit study did not find alterations in serum alanine or aspartate aminotransferases levels, γ-glutamyl transferase, alkaline phosphatase, or cholesterol levels; however, a 60% increase in serum triglyceride levels was found at 1.2 mg molybdenum/kg/day. In contrast, the Murray et al. (2014a) study examined similar serum clinical chemistry parameters (including triglyceride levels) and did not find any significant alterations.

A series of studies conducted by Rana and associates have also reported some liver alterations in rats exposed to 300–490 mg molybdenum/kg/day as ammonium molybdate. The reported alterations included increases in total lipid levels (Rana et al. 1980; Rana and Kumar 1980b, 1980c), decreases in "total carbohydrate" levels (Rana and Kumar 1980c), decreases in glycogen content (Rana et al. 1985), and increases in serum alanine aminotransferase and aspartate aminotransferase activities (Rana and Chauhan 2000). The addition of 100 mg/kg body weight/day copper to the basal diet (approximately 5 ppm) appeared to reverse the effects of molybdenum on hepatic lipid and carbohydrate levels (Rana and Kumar 1980c). There was low confidence in these studies due to the poor reporting of the study design (including route of oral administration, whether the dose was reported in terms of molybdenum or ammonium molybdate, and copper content of the diet), the lack of histological examination of the liver, and the reported body weight losses (Rana et al. 1980; Rana and Chauhan 2000).

2.10 RENAL

Intermediate- or chronic-duration inhalation exposure to molybdenum trioxide (highest concentration tested was 67 mg molybdenum/m^3) did not result in histological alterations in the kidney of rats or mice (NTP 1997).

The available data from laboratory animal studies suggest that the kidney may be a target of molybdenum toxicity following oral exposure. In the only available acute-duration study, no histological alterations were observed in the kidneys of female rabbits exposed to 1.2 mg molybdenum/kg/day as ammonium heptamolybdate in the diet for 14 days (Bersenyi et al. 2008) or male rabbits exposed to 0.58 mg molybdenum/kg/day from carrots grown in ammonium heptamolybdate-rich soil for 14 days (Bersenyi et al. 2008). Murray et al. (2014a) reported a slight diffuse hyperplasia in the renal proximal tubules in 2/10 female rats exposed to 60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days; no renal lesions were observed in females exposed to 60 mg molybdenum/kg/day for 90 days and allowed to recover for 60 days. No alterations were observed in the male rats. Although the incidence was low, the investigators considered it to be treatment-related because it is an uncommon finding in female rats of this age. In a subsequent 2-generation study by this group, no histological alterations were observed in male or female rats exposed to 40 mg molybdenum/kg/day as sodium molybdate in drinking water or diet for 147–158 days (Murray et al. 2019). Degenerative changes in the kidneys were noted in male rats exposed to 240 mg molybdenum/kg/day as ammonium molybdate (Bandyopadhyay et al. 1981). It should be noted that the food intake in the molybdenum group was paired to another group of rats fed a low-protein diet and exposed to molybdenum; the basal diet likely provided adequate copper levels. No other studies included histological examination of the kidneys.

Several studies reported alterations in serum and urinary parameters that could be suggestive of altered renal function. Diuresis and creatinuria and a decrease in creatinine clearance were observed in rats administered via gavage 80 mg molybdenum/kg/day as ammonium heptamolybdate for 8 weeks (Bompart et al. 1990). The study did not find significant alterations in urinary protein or glucose levels. Studies by Rana and associates have reported increases in total lipid levels in the kidneys (Rana et al. 1980; Rana and Kumar 1980c), decreases in “total carbohydrate” levels in the kidney (Rana and Kumar 1980c), increases in serum urea and urinary albumin levels (Rana and Kumar 1983), and increases in urine specific gravity (Rana and Kumar 1983) in rats exposed to high doses of ammonium molybdate (300–490 mg molybdenum/kg/day). The addition of copper (approximately 5 ppm) to the basal diet appeared to reverse the increased lipid and decreased carbohydrate levels (Rana and Kumar 1980c). As noted in the hepatic effects section, there is low confidence in these studies and the results should be interpreted cautiously.

2.11 DERMAL

Information on the dermal toxicity of molybdenum comes from a small number of oral exposure studies reporting skin and hair effects and acute-exposure dermal studies. In an oral exposure study of weanling rabbits (Arrington and Davis 1953), alopecia and slight dermatosis were observed in four of five rabbits exposed to 54 mg molybdenum/kg/day as sodium molybdate in the diet for 84 days; no dermal effects were observed at 25 mg molybdenum/kg/day. In another study by this group, alopecia and slight dermatosis were observed in one of two mature rabbits exposed to 30 mg molybdenum/kg/day as sodium molybdate. Anemia was also observed at these doses. In the study of weanling rabbits, administration of additional copper resulted in a return to a normal hair coat, suggesting that copper insufficiency, possibly molybdenum induced, was a contributing factor to the dermal toxicity. Johnson et al. (1969) reported decreases (25% lower than controls) in skin rupture strength in rats exposed to 150 mg molybdenum/kg/day as sodium molybdate in the diet for 6 weeks.

No dermal effects were observed in rats following a 24-hour dermal application of 280 or 1,200 mg molybdenum/kg as ammonium dimolybdate (Baldrick and Healing 1990a; Liggett and McRae 1990e), 340 or 1,300 mg molybdenum/kg as pure molybdenum trioxide (Baldrick and Healing 1990b; Liggett and McRae 1990f), 230 or 930 mg molybdenum/kg as sodium molybdate (Baldrick and Healing 1990c; Liggett and McRae 1990g), or 1,333 mg molybdenum/kg as technical-grade molybdenum trioxide (Baldrick and Healing 1990d).

2.12 OCULAR

No ocular lesions were observed in rats exposed to 60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a); no other oral or inhalation studies examined ocular endpoints.

Instillation of 56 mg molybdenum/kg as ammonium dimolybdate (Liggett and McRae 1990a), 67 mg molybdenum/kg as pure molybdenum trioxide (Liggett and McRae 1990b), 67 mg molybdenum/kg as technical grade molybdenum trioxide (Liggett and McRae 1990d), or 46 mg molybdenum/kg as sodium molybdate (Liggett and McRae 1990c) resulted in conjunctival inflammation in rabbits.

2.13 ENDOCRINE

The possible association between molybdenum and thyroid effects was investigated in adults (subjects did not report having thyroid disease, thyroid cancer, or taking thyroid medication on a medical questionnaire completed at the blood sampling) using the NHANES 2007–2008 cross-sectional study data set (Yorita Christensen 2013). Associations between decreased levels of triiodothyronine (free and total) and thyroxine (free) and higher urinary molybdenum levels were found. Although the study found associations, these data are inadequate for establishing causality. Another study of NHANES participants did not find an association between urinary molybdenum levels and thyroid problems (Mendy et al. 2012). A cross-sectional study of men at a fertility clinic found a significant inverse relationship between blood molybdenum levels and prolactin levels (Meeker et al. 2009). The men were categorized into three groups based on blood molybdenum levels (<70^{th}, 70^{th}–85^{th}, and >85^{th} percentile); the association was found in the men with blood molybdenum levels >85^{th} percentile, as compared to men with levels <70^{th} percentile. The study did not find a significant association with thyroid stimulating hormone and blood molybdenum levels.

Inhalation studies did not find histological alterations in the adrenal, pituitary, pancreas, parathyroid, or thyroid glands in rats and mice exposed to ≤67 mg molybdenum/m^3 as molybdenum trioxide for 13 weeks or 2 years (NTP 1997).

In oral exposure laboratory animal studies, increases in serum cortisol, prolactin, and follicle stimulating hormone levels were found in male rats administered 240 mg molybdenum/kg/day as ammonium molybdate for 4 weeks (Bandyopadhyay et al. 1981); as noted in the renal effects section, food intake was matched to a low-protein molybdenum group. No increases in the incidence of histological alterations in the adrenal glands, pituitary gland, or thyroid were observed in rats exposed to 60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a) or up to 40 mg molybdenum/kg/day as sodium molybdate in drinking water or diet for 147–158 days (Murray et al. 2019). Several thyroid effects were reported in rabbits exposed to 59 mg molybdenum/kg/day as sodium molybdate in the diet for 25–31 days (Widjajakusuma et al. 1973). The investigators did not report the copper content of the diet; it is likely to be low based on the severe decreases in body weight, hematological parameters, and increased mortality. The effects included decreases in thyroxine secretion rates; decreases in follicle size (height and diameter); atrophy of the follicular epithelium, colloids, and stroma; and degenerative alterations in the follicular epithelium and interfollicular connective tissue. With the exception of the degenerative changes, similar, but less prominent, thyroid effects were also

observed in pair-fed controls, suggesting that the decreases in food intake and body weight contributed to the thyroid toxicity.

2.14 IMMUNOLOGICAL

There are limited data on the immunotoxicity of molybdenum in humans. Studies of patients with stainless steel stents (which contain nickel, chromate, and molybdenum) or in patients prior to hip or knee replacements found a low rate of positive results in patch tests with molybdenum (Koster et al. 2000; Menezes et al. 2004; Zeng et al. 2014). In patients with stainless steel stents, 3% had a positive delayed-type contact hypersensitivity reaction to molybdenum chloride (Koster et al. 2000). In the other studies, exposure to an unspecified molybdenum compound did not result in any positive hypersensitivity results (Menezes et al. 2004; Zeng et al. 2014).

No studies have examined immune function following inhalation exposure to molybdenum. Intermediate- and chronic-duration studies in rats and mice did not report histological alterations in the thymus or spleen at molybdenum trioxide levels as high as 67 mg molybdenum/m^3 (NTP 1997). No studies were located regarding immune effects in laboratory animals following oral exposure to molybdenum.

Guinea pigs showed contact sensitization to a topical challenge with molybdenum pentachloride after induction via intradermal injection with 0.03% molybdenum and topical exposure to 5.2% molybdenum and an epicutaneous challenge with ≥0.35% molybdenum as molybdenum pentachloride (Boman et al. 1979). Similarly, guinea pigs were sensitized to 3.2% molybdenum as sodium molybdate following intradermal (3.2% molybdenum) or topical (8% molybdenum) induction (Boman et al. 1979). In contrast, other studies of skin sensitization in guinea pigs were negative for ammonium dimolybdate (Allan 1996a), pure and technical-grade molybdenum trioxide (Allan 1996b, 1996c), and sodium molybdate (Allan 1996d); these studies tested higher molybdenum concentrations (70–90% molybdenum) than the Boman et al. (1979) study.

2.15 NEUROLOGICAL

Information on the potential neurotoxicity of molybdenum comes from inhalation and oral exposure studies in laboratory animals evaluating brain histology or monitoring for overt signs of neurotoxicity. None of these studies included function testing. No overt signs of neurotoxicity were observed in

laboratory animal studies (e.g., Murray et al. 2014a; NTP 1997). No histological alterations were observed in the brain of rats and mice exposed via inhalation to ≤67 mg molybdenum/m^3 as molybdenum trioxide for 13 weeks or 2 years (NTP 1997) or rats exposed to ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a). In contrast, Helaly et al. (2018) reported dense inflammation and neurocyte degeneration in the cerebral cortex and hippocampus of rats receiving gavage doses of 30 mg molybdenum/kg/day as molybdenum dihydrate for 30 days; however, the study did not include incidence data.

2.16 REPRODUCTIVE

There are limited data on reproductive effects of molybdenum in humans. The available studies have evaluated correlations between ambient molybdate exposure and reproductive health measures, including semen quality (Meeker et al. 2008) and sex hormone levels (Meeker et al. 2010). A cross-sectional study by Meeker et al. (2008) reported an inverse association between higher molybdenum blood levels (>85th percentile, based on molybdenum levels in blood) and sperm concentration (adjusted OR 3.48; 95% CI 1.12–10.8) after adjustment for potential confounders and other metal exposures. No associations were found for sperm morphology (adjusted OR 2.61; 95% CI 0.97–7.0) or sperm motility (adjusted OR 2.24; 95% CI 0.77–6.49). In another cross-sectional study, Meeker et al. (2010) reported an inverse correlation between higher molybdenum blood levels (≥70th percentile) and testosterone and free androgen index (molar ratio of total testosterone sex hormone-binding globulin) levels. The men in these studies, who were recruited from Michigan infertility clinics and were not all considered to be infertile (i.e., their partners may have been infertile), were only exposed to molybdenum from their surroundings. An inverse association between a biomarker of molybdenum exposure (urinary levels) and serum testosterone levels was also observed in a cross-sectional study of males participating in NHANES (Lewis and Meeker 2015). The study found a 3.82% decrease in serum testosterone levels when urinary molybdenum levels doubled (after adjustment for age, body mass index [BMI], income, race, and smoking). Although these studies found associations, they do not establish causality and the alterations in reproductive parameters may be due to multiple factors rather than only to molybdenum exposure.

Studies in laboratory animals have evaluated potential alterations in male reproductive tissues, female reproductive tissue, and fertility following inhalation (no evaluation of fertility) or oral exposure. No studies have evaluated reproductive toxicity following dermal exposure.

Several studies have evaluated the reproductive toxicity in male laboratory animals. No alterations in sperm count or motility or histological alterations of male reproductive tissues were observed in rats or mice exposed via inhalation to molybdenum trioxide concentrations as high as 67 mg molybdenum/m^3 (NTP 1997). Murray et al. (2014a) did not find any alterations in spermatid, sperm counts, sperm motility, or sperm morphology in rats exposed to 60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days. Although the study found no alterations in the percentage of motile sperm, a slight, but statistically significant, decrease in the percentage of progressively motile sperm was observed at 60 mg molybdenum/kg/day (59.0% compared to 69.4% in controls). The investigators noted that the decrease was likely attributable to the control group having a value that approached the upper end of the range for historical controls (mean of 59.8±16.2%). No alterations in sperm parameters were observed in male rats exposed to ≤40 mg molybdenum/kg/day as sodium molybdate in drinking water in a 2-generation study (Murray et al. 2019). In parental-generation males exposed to 40 mg molybdenum/kg/day as sodium molybdate in the diet, an increase in the number of sperm with no head was found (Murray et al. 2019). However, the investigators did not consider this to be treatment-related since it was largely due to one male rat, was not observed in the F1 males, and the values were within the range of historical controls.

In contrast to these findings, other studies have reported male reproductive effects. Decreases in sperm motility and concentration and increases in sperm morphological changes were observed in rats administered via gavage 14 mg molybdenum/kg/day as sodium molybdate for 60 days (Pandey and Singh 2002), and in mice exposed to 25 mg molybdenum/kg/day as sodium molybdate in the drinking water for 14 days (Zhai et al. 2013). These studies also found decreases in epididymides, seminal vesicles, and/or prostate gland weights (Pandey and Singh 2002; Zhai et al. 2013). The Zhai et al. (2013) study also found increases in sperm motility and concentration and decreases in the occurrence of sperm morphological alterations in rats exposed to lower molybdenum doses (6 mg molybdenum/kg/day as sodium molybdate). A study in rabbits reported reductions in the number of germ cells and mature spermatocytes in the testes (Bersenyi et al. 2008); the investigators also noted a large number of syncytial giant cells and degenerated cells in the seminiferous tubules. Interpretation of these results are limited since incidence data or statistical analyses were not reported. Degeneration of the seminiferous tubules was found in rats at 7 mg molybdenum/kg/day as sodium molybdate, which was administered in the diet from weaning through sexual maturity (Jeter and Davis 1954); although this study provided an adequate amount of copper, there was evidence of copper deficiency (achromotrichia) at ≥7 mg molybdenum/kg/day. Degeneration of the seminiferous tubules was also reported by Pandey and Singh (2002) for intermediate-duration (60 days) exposures in rats administered molybdenum at doses up to 24 mg molybdenum/kg/day (sodium molybdate); however, the dose(s) producing the effects are unclear

and incidence data were not reported. The Pandey and Singh (2002) and Zhai et al. (2013) studies did not report the copper content of the basal diet, although both studies used commercial diets. Lyubimov et al. (2004) reported delayed spermiation, increased sperm and seminal fluid concentration, and increased sloughing of epididymal tail epithelial cells at 4.4 mg molybdenum/kg/day as ammonium tetrathiomolybdate. Although the basal diet in the Lyubimov et al. (2004) study provided 11 ppm of copper, which is above the National Academy of Sciences (NAS 1995) recommended amount for rats (5 ppm), dietary copper supplementation (110 ppm) prevented testicular toxicity. It is likely that the tetrathiomolybdate interfered with the absorption of dietary copper, resulting in a secondary effect of copper insufficiency.

As with the male reproductive effects, conflicting results have been reported for female reproductive effects. Murray et al. (2014a) did not find any alterations in vaginal cytology or estrus cycle in female rats exposed to ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days or in a 2-generation study in which rats were exposed to ≤40 mg molybdenum/kg/day as sodium molybdate in the drinking water or the diet (Murray et al. 2019). No histological alterations were observed in female reproductive tissues in rats or mice following inhalation exposure to ≤67 mg molybdenum/m^3 for 13 weeks or 2 years (NTP 1997), in rats exposed to ≤60 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days (Murray et al. 2014a), or in rabbits exposed to 1.2 mg molybdenum/kg/day as ammonium heptamolybdate in the diet for 14 day (Bersenyi et al. 2008). Zhang et al. (2013) reported an increase in the rate of MII oocyte morphological abnormalities and decreases in relative ovarian weights were observed in mice exposed to 11 mg molybdenum/kg/day as sodium molybdate in drinking water for 14 days. The investigators also reported ovarian hyperemia in mice exposed to 5.3 and 11 mg molybdenum/kg/day; however, the incidence and statistical significance were not reported. Irregularities in the estrous cycle were reported in rats administered 1.5 mg molybdenum/kg/day in the drinking water from weaning through sexual maturity (Fungwe et al. 1990).

Several intermediate-duration oral studies evaluated fertility. No alterations in fertility were observed in female rats exposed to ≤15 mg molybdenum/kg/day as sodium molybdate in drinking water (Fungwe et al. 1990), in a 2-generation study in rats exposed to ≤40 mg molybdenum/kg/day as sodium molybdate in drinking water or diet (Murray et al. 2019), or in male and female rats exposed to 7 mg molybdenum/kg/day as sodium molybdate in the diet when a high copper diet was administered (Jeter and Davis 1954). In contrast, Pandey and Singh (2002) reported decreases in fertility in males exposed to 14 mg molybdenum/kg/day as sodium molybdate and mated to unexposed females. Another study conducted by Jeter and Davis (1954) in which rats were exposed to 7 mg molybdenum/kg/day from

weaning to maturity also found impaired male fertility; in this study, there is some indication that the diet did not provide an adequate level of copper.

2.17 DEVELOPMENTAL

Information on the potential developmental toxicity of molybdenum is limited to two epidemiological studies and oral exposure studies in laboratory animals. Vazquez-Salas et al. (2014) found an association between third-trimester maternal urinary molybdenum levels (mean level of 54.0 µg/g creatinine) and infant psychomotor development indices, including gross and fine motor coordination, during the first 30 months of life in a cross-sectional study of women in Mexico participating in a prospective study of neurodevelopment in children. A doubling of creatinine corrected urinary molybdenum levels resulted in significant decreases in psychomotor development index scores. No association was found between maternal urinary molybdenum levels during pregnancy (mean levels ranged from 45.6 to 54.6 µg/g creatinine during the first, second, and third trimesters) and newborn body weight or infant mental development indices (sensory ability, memory, learning, problem solving, and verbal ability). Shirai et al. (2010) found no association between maternal urinary molybdenum levels and newborn body weight, length, or head circumference in a cross-sectional study of women in Japan with mean urinary molybdenum levels of 79.0 µg/g creatinine. As noted elsewhere in this document, these observational epidemiology studies do not establish causality between molybdenum and developmental effects, and other factors are likely to have contributed to the risk.

No developmental effects were reported in three studies of rats exposed to molybdenum in the presence of adequate copper concentrations in the basal diet (Jeter and Davis 1954; Murray et al. 2014b, 2019). In a 2-generation study, no alterations in pup survival, sex ratios, pup body weight, or developmental landmarks were observed in the F1 or F2 offspring of rats exposed to up to 40 mg molybdenum/kg/day as sodium molybdate in the drinking water or diet (Murray et al. 2019). In a single-generation study, Murray et al. (2014b) reported no effects on litter size, embryofetal survival, sex ratio, fetal body weight, or fetal malformations and variations in rats exposed to 40 mg molybdenum/kg/day as sodium molybdate in the diet on gestation days (GDs) 6–20. No alterations in birth weights were observed in the offspring of male and female rats exposed to 7 mg molybdenum/kg/day as sodium molybdate for at least 14 weeks (Jeter and Davis 1954). In contrast to these findings, one study found decreases in the number of live fetuses, fetal crown-rump length, and fetal body weight in the offspring of male rats administered 14 mg molybdenum/kg as sodium molybdate via gavage for 60 days prior to mating to untreated females

(Pandey and Singh 2002). The copper content of the commercial diet was not reported but was assumed to be adequate.

Developmental effects have also been reported in studies in which the copper content of the diets was lower than the NAS-recommended standard of 8 ppm for pregnant rats (NAS 1995). Fungwe et al. (1990) reported increases in fetal resorptions and decreases in litter weights in female rats exposed to 1.3 mg molybdenum/kg/day as sodium molybdate in the drinking water for 8 weeks prior to mating through GD 21; the copper content in the basal diet was 6.3 ppm. Decreased maternal body weight gain was also observed at doses resulting in developmental toxicity. Decreased weaning weights were observed in the offspring of rats exposed to ≥2 mg molybdenum/kg/day as sodium molybdate; the copper content of the diet was 5 ppm (Jeter and Davis 1954). Lyubimov et al. (2004) found no effects on litter size or fetal survival in rats administered molybdenum daily via gavage at 4.4 mg molybdenum/kg/day as ammonium tetrathiomolybdate for 59–61 days (for 29 days prior to mating, during mating, and thereafter until sacrifice) in males or for 22–35 days (for 15 days prior to mating, during mating, and during GDs 0–6) in females. Two studies only available as abstracts provide additional information on the potential developmental toxicity of molybdenum. Lyubimov et al. (2002) found no developmental effects in rats exposed to 6 mg/kg/day as tetrathiomolybdate on GDs 6–17. Exposure on GDs 7–20 resulted in an increase in carpal/tarsal flexure in the offspring of dams exposed to 20 mg/kg/day ammonium tetrathiomolybdate (Lyubimov et al. 2003). Although neither study provided information on the copper content of the diet, it is assumed to be adequate based on Lyubimov et al. (2004).

2.18 OTHER NONCANCER

Several studies have evaluated the possible associations between molybdenum and uric acid levels. Slight, but significant increases in serum uric acid levels were observed in molybdenite roasting facility workers exposed to a TWA concentration of 9.47 mg molybdenum/m^3 as molybdenum trioxide and other oxides (Walravens et al. 1979). The serum uric acid levels were 5.90 mg/dL in the exposed workers and 5.01 mg/dL in the controls; these levels are within the normal range. No significant associations between serum molybdenum levels and serum uric acid levels were found, and none of the workers reported gout-like symptoms.

Koval'skiy et al. (1961) reported a significant increase in blood uric acid levels and symptoms of gout in a cross-sectional study of residents living in an area of Armenia with high levels of molybdenum in the soil and food, as compared to residents living outside of this area. The mean uric acid levels in a subset

of the examined population (n=52) was 6.2 mg/dL, as compared to levels in five control subjects who had a mean level of 3.8 mg/dL; the mean uric acid levels were 8.1 mg/dL among the subjects with gout symptoms and 5.3 mg/dL among the exposed subjects without symptoms. The investigators reported that copper intakes (5–10 mg/day) were lower in the high molybdenum area as compared to copper intake for residents outside of this area (10–15 mg/day). It was also noted that gout-like symptoms have not been observed in other high molybdenum areas that have higher copper intakes (Koval'skiy et al. 1961). Interpretation of the result of this study is limited by the small control group, as compared to the exposed group; lack of information on the selection of controls, particularly if they were matched to the exposed group; and lack of information on diet and alcohol exposure, which could influence uric acid levels. Additionally, NAS (2001) noted potential analytical problems with the serum and urine copper measurements. Based on the levels of molybdenum in the foodstuff, the investigators estimated a daily dose of 10–15 mg (0.14–0.21 mg/kg/day assuming a 70-kg body weight). Deosthale and Gopalan (1974) did not find significant increases in urinary uric acid levels in four subjects exposed to a low molybdenum diet for 10 days followed by a high molybdenum diet with an ammonium molybdate supplement for 7 days (TWA molybdenum intake was 0.014 mg molybdenum/kg/day), as compared to uric acid levels when the subjects were fed a low molybdenum diet. A series of studies in Colorado investigated uric acid levels in communities with high molybdenum levels in the drinking water from mine tailings pollution (EPA 1979). Comparisons between subjects living in areas with high molybdenum in the drinking water (80–200 µg/L; approximately 0.002–0.006 mg/kg/day) to those living in areas with lower levels (<40 µg/L; <0.001 mg/kg/day) did not result in any significant differences in serum uric acid levels or urinary molybdenum levels. Another study (EPA 1979) noted that serum uric acid levels were within the normal range in students with an estimated molybdenum intake of 500 µg/day (0.007 mg/kg/day) (EPA 1979). A third study found significant increases in uric acid levels in residents with low molybdenum (20 µg/L; 0.0006 mg/kg/day) levels in the water and in residents with high molybdenum levels (150–200 µg/L; 0.004–0.006 mg/kg/day) in the drinking water; as compared to residents with drinking water levels of 0–50 µg/L (0–0.001 mg/kg/day). The inconsistencies in the results could be explained by the lack of control of several variables including age, sex, alcohol intake, dietary habits, and altitude.

Murray et al. (2014s) found a statistically significant decrease in serum uric acid levels in female rats exposed to ≥5 mg molybdenum/kg/day as sodium molybdate in the diet for 90 days; no alterations were observed in male rats exposed to up to 60 mg molybdenum/kg/day. Other statistically significant alterations in serum clinical chemistry parameters noted in the Murray et al. (2014a) study include decreases in total protein and calcium at 60 mg molybdenum/kg/day in males and decreases in serum creatinine at ≥5 mg molybdenum/kg/day in females. The investigators noted that the changes in serum

clinical chemistry (including uric acid levels) were not considered treatment-related because the alterations were of small magnitude, not dose-related, due to outliers in the controls, and/or consistent with normal variability. Quantitative data for the serum clinical chemistry parameters were not provided in the published paper.

Possible associations between molybdenum and diabetes and related outcomes have also been investigated in a limited number of epidemiological and laboratory animal studies. In a cross-sectional study of 9,447 NHANES participants, Menke et al. (2016) found an association between urinary molybdenum levels and diabetes. The ORs and 95% CIs for subjects with urinary molybdenum levels in the second, third, and fourth quartiles, as compared to the first quartile were 1.46 (1.09–1.97), 1.89 (1.35–2.66), and 1.76 (1.24–2.50), respectively. Associations were also found for Homeostatic Model Assessment (HOMA) insulin resistance levels for all subjects and in subjects without diabetes.

Two studies in rats did not find significant alterations in serum glucose levels following intermediate-duration exposure to 60 or 100 mg molybdenum/kg/day (Murray et al. 2014a; Peredo et al. 2013); additionally, serum insulin levels were not altered by exposure to 100 mg molybdenum/kg/day (Peredo et al. 2013). Prakash (1989) reported decreases in glycogen levels in the hindlimb muscles of rats administered 490 mg molybdenum/kg/day as ammonium molybdate via gavage for 30 days. The significance of this effect is difficult to determine since the study did not provide information on body weight gain.

2.19 CANCER

The potential carcinogenicity of molybdenum compounds has been evaluated in an occupational exposure study and in a rat and mouse inhalation study. In a case-control study examining the potential association between lung cancer and exposure to 16 potential carcinogens, Droste et al. (1999) did not find a significant increase in lung cancer among workers who self-reported exposure to molybdenum. However, an increased risk of lung cancer was found in workers who self-reported working in industries that could involve exposure to molybdenum (OR 2.1; 95% CI 1.2–3.7); the job most often related to molybdenum exposure was processing of stainless steel in the manufacture of metal goods, which could also involve exposure to other carcinogens including chromium, nickel, and arsenic. Limitations of this study, including self-reported exposure and the potential exposure to other lung carcinogens, preclude its use in assessing the potential carcinogenicity of molybdenum.

In the 2-year NTP rat study (NTP 1997), an increase in the combined incidence of alveolar/bronchiolar adenoma or carcinoma was observed in male rats exposed to 67 mg molybdenum/m^3 as molybdenum trioxide; however, the incidence was within the range of historical controls and NTP considered this to be equivocal evidence of carcinogenic activity of molybdenum trioxide. No other concentration-related increases in neoplastic lesions were observed in the rats. In mice, there were significant increases in the incidences of alveolar/bronchiolar carcinoma in males at ≥6.7 mg molybdenum/m^3, alveolar/bronchiolar adenoma or carcinoma in males at 6.7 and 20 mg molybdenum/m^3, alveolar/bronchiolar adenoma in females at 20 and 67 mg molybdenum/m^3, and alveolar/bronchiolar adenoma or carcinoma in females at 67 mg molybdenum/m^3 (NTP 1997). The incidences of alveolar/bronchiolar adenoma and carcinoma were highest in the 6.7 mg molybdenum/m^3 groups and lowest in the 67 mg molybdenum/m^3 groups. NTP (1997) concluded that the male and female mouse data provided some evidence of carcinogenic activity of molybdenum trioxide.

The Department of Health and Human Services (NTP 2016) and EPA have not evaluated the carcinogenic potential of molybdenum. IARC has categorized molybdenum trioxide as possibly carcinogenic to human (Group 2B).

2.20 GENOTOXICITY

No studies were available regarding genotoxic effects of molybdenum compounds in humans following environmental or occupational exposure to these compounds. The genotoxicity of molybdenum compounds has been studied mostly in *in vitro* assays utilizing prokaryotic organisms and in mammalian cells. Limited information is available regarding the *in vivo* genotoxicity of molybdenum.

As shown in Table 2-4, sodium molybdate induced a modest, but statistically significant, increase in micronucleated bone marrow cells (polychromatic erythrocytes [PCEs]) from male C57BL/6J mice following two intraperitoneal injections of 200 or 400 mg/kg sodium molybdate on 2 consecutive days (Titenko-Holland et al. 1998). The increase in micronucleated cells per 1,000 PCE or in micronuclei per 1,000 PCE were about half of those produced by colchicine, the positive control. The same group of investigators reported that sodium molybdate induced a positive response in the dominant lethal assay in mice. In these experiments, male C57BL/6J mice were treated with 200 or 400 mg/kg sodium molybdate and were mated with non-treated female C3H/J mice at various times after dosing. Sodium molybdate did not significantly affect pregnancy rate, but induced a significant dose-related increase in post-implantation loss.

Table 2-4. Genotoxicity of Molybdenum Compounds *In Vivo*

Species	Compound	Endpoint	Results	Reference
Mouse (male C57BL/6J)	Sodium molybdate	Micronuclei in bone marrow cells	(+)	Titenko-Holland et al. 1998
Mouse (male C57BL/6J)	Sodium molybdate	Dominant lethal assay	(+)	Titenko-Holland et al. 1998
Drosophila melanogaster wing spot test	Molybdenum chloride	Gene mutation	+	Ogawa et al. 1994

+ = positive result; (+) = weakly positive result

Assessment of the activity of molybdenum chloride in the *Drosophila melanogaster* wing spot test showed that the compound induced spots with one or two mutant hairs (small spots) (Ogawa et al. 1994). Almost all of the spots detected were mutant clones expressing the *mwh* phenotype which, according to the investigators, suggested a nonlethal genetic change such as gene mutation or mitotic recombination occurring at a late developmental stage, or a semi-lethal change such as partial aneuploidy for a chromosomal region containing the *mwh* locus.

Table 2-5 summarizes studies of genotoxic effects of molybdenum compounds in *in vitro* systems. Results of gene mutation and DNA tests performed in prokaryotic organisms, almost all conducted without metabolic activation, were mixed, but negative results outnumbered positive results. It is worth noting the positive results reported for potassium molybdate and ammonium molybdate in the DNA repair assay (Nishioka 1975).

Table 2-5. Genotoxicity of Molybdenum Compounds *In Vitro*

			Results		
Species (test system)	Compound	Endpoint	With activation	Without activation	Reference
Prokaryotic organisms:					
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, 1538	Ammonium molybdate	Gene mutation	No data	–	Arlauskas et al. 1985
S. typhimurium, TA97, TA98, TA100, TA 1535, TA1537	Molybdenum trioxide	Gene mutation	–	–	NTP 1997; Zeiger et al. 1992
S. *typhimurium*, TA98, TA100, TA1535, TA1537	Molybdenum trioxide	Gene mutation	–	–	Jones 2004

Table 2-5. Genotoxicity of Molybdenum Compounds *In Vitro*

Species (test system)	Compound	Endpoint	Results		Reference
			With activation	Without activation	
S. *typhimurium*, TA98, TA100, TA1535, TA1537, TA102	Sodium molybdate	Gene mutation	–	–	Beevers 2009
S. *typhimurium*, TA98, TA100, TA1535, TA1537, TA102	Sodium molybdate	Gene mutation	–	–	Burzlaff et al. 2017
S. *typhimurium*, TA98, TA100, TA1535, TA1537, TA102	Sodium molybdate	Gene mutation	–	–	Burzlaff et al. 2017
Saccharomyces cerevisiae D3	Sodium molybdate	Gene conversion and mutation	No data	–	Singh 1983
Escherichia coli, WP2uvrA$^-$	Ammonium molybdate	Reverse gene mutation	No data	–	Arlauskas et al. 1985
E. coli, WP2uvrA	Molybdenum trioxide	Gene mutation	–	–	Jones 2004
E. coli, 2 WP2 strains	Ammonium heptamolybdate	Reverse gene mutation	No data	+	Nishioka 1975
E. coli, CM571	Ammonium heptamolybdate	Reverse gene mutation	No data	–	Nishioka 1975
E. coli PQ37	Molybdenum chloride	DNA damage	No data	–	Olivier and Marzin 1987
E. coli WP2$_s$(ƛ)	Sodium molybdate	DNA damage	No data	(+)	Rossman et al. 1984
E. coli WP2$_s$(ƛ)	Sodium molybdate	DNA damage	No data	(+)	Rossman et al. 1991
Bacillus subtilis, H17 and M45	Molybdic acid	DNA repair assay	No data	–	Kanematsu et al. 1980
B. subtilis H17 and M45	Molybdenum disulfide	DNA repair assay	No data	–	Kanematsu et al. 1980
B. subtilis H17 and M45	Molybdenum pentachloride	DNA repair assay	No data	–	Nishioka 1975
B. subtilis H17 and M45	Potassium molybdate	DNA repair assay	No data	(+)	Nishioka 1975
B. subtilis H17 and M45	Ammonium heptamolybdate	DNA repair assay	No data	+	Nishioka 1975
Photobacterium fischeri	Sodium molybdate	Direct mutation	No data	–	Ulitzur and Barak 1988

Table 2-5. Genotoxicity of Molybdenum Compounds *In Vitro*

Species (test system)	Compound	Endpoint	Results		Reference
			With activation	Without activation	
Mammalian cells:					
Mouse lymphoma (L5178Y) cells	Sodium molybdate	Gene mutation	–	–	Lloyd 2009
Mouse lymphoma L5178Y tk (+/-) cells	Sodium molybdate dihydrate	Gene mutation	–	–	Burzlaff et al. 2017
Human peripheral lymphocytes	Sodium molybdate	Micronucleus assay	No data	(+)	Titenko-Holland et al. 1998
Human peripheral lymphocytes	Sodium molybdate	Micronucleus assay	–	–	Taylor 2009
Human peripheral blood lymphocytes	Sodium molybdate dihydrate	Micronucleus assay	–	–	Burzlaff et al. 2017
Human peripheral lymphocytes	Ammonium molybdate	Micronucleus assay	No data	+	Titenko-Holland et al. 1998
Human peripheral lymphocytes	Molybdenum Trioxide	Micronucleus assay	–	–	Fox 2005
Syrian hamster embryo (SHE) cells	Molybdenum trioxide	Micronucleus assay	No data	+	Gibson et al. 1997
Chinese hamster ovary (CHO) cells	Molybdenum trioxide	Chromosomal aberrations	–	–	NTP 1997
CHO cells	Molybdenum trioxide	Sister chromatid exchanges	–	–	NTP 1997

+ = positive result; (+) = weakly positive result; – = negative result; ± = equivocal result

The few studies that tested molybdenum compounds in mammalian cells provided mixed results (Table 2-4). For molybdenum trioxide, NTP (1997) reported negative results for chromosomal aberrations; Fox (2005) and Gibson et al. (1997) reported negative and positive results, respectively, for micronuclei formation, with both studies evaluating overlapping dose ranges. Titenko-Holland et al. (1998) reported positive results for micronuclei formation in human peripheral lymphocytes incubated with sodium or ammonium molybdate. However, cell viability was affected by treatment, and blood was collected from only two donors; therefore, the results from this study should be interpreted with caution. More recent studies with human peripheral lymphocytes did not find increases in micronuclei formation for molybdenum trioxide (Fox 2005) or sodium molybdate (Burzlaff et al. 2017; Taylor 2009).

In summary, the limited information regarding effects *in vivo* of molybdenum compounds is insufficient to infer possible outcomes of exposure in human populations. *In vitro* studies in prokaryotic organisms mostly found no alterations in gene mutations and mixed results for DNA damage and repair. *In vitro* studies in mammalian cells primarily found no alterations in the occurrence of clastogenic effects.

2.21 MECHANISMS OF ACTION

The mechanism of molybdenum toxicity has not been well-established. There are some indications that the mode of action may involve altered copper utilization; however, it is likely that other mechanisms, including direct molybdenum alterations, are involved. Support of the mode of action involving impaired copper utilization comes from toxicology studies demonstrating more severe effects when animals are maintained on a copper-deficient diet; molybdenum induced increases in copper levels in the plasma, liver, and kidneys; and apparent reversal of adverse effects following administration of high doses of copper. A number of the effects observed in animals orally exposed to molybdenum, particularly decreases in body weight and anemia (Arrington and Davis 1953; Brinkman and Miller 1961; Franke and Moxon 1937; Gray and Daniel 1954; Johnson et al. 1969), are similar to those observed in copper-deficient animals. Administration of high levels of copper results in a fairly rapid improvement or prevents the effect from occurring (Arrington and Davis 1953; Lyubimov et al. 2004). In rats fed a copper-adequate diet, exposure to high levels of molybdenum in the diet resulted in significant increases in plasma copper levels (Nederbragt 1980, 1982), most of which were in a "tightly bound form" that did not appear to be associated with ceruloplasmin (major copper-carrying protein in the blood), as evidenced by the lack of an increase in ceruloplasmin levels (Nederbragt 1980). Significant increases in liver and kidney copper levels were also observed in rats exposed to molybdenum in the diet and maintained on a copper-adequate diet.

In ruminants, which appear to be very sensitive to molybdenum toxicity, it is believed that molybdenum reacts with sulfate generated in the rumen to form thiomolybdates; copper can bind to these thiomolybdates, which impairs its absorption. There is also some indication that cupric thiomolybdates can form in the blood if dietary copper levels are inadequate (Telfer et al. 2004). The copper in these cupric thiomolybdates is unavailable to ceruloplasmin and other copper-containing proteins, resulting in a functional copper deficiency (Vyskocil and Viau 1999). In monogastric animals exposed to sodium molybdate, administration of sulfate decreases the toxicity of molybdenum (Miller et al. 1956; Van Reen 1959). However, when rats were fed diets containing molybdate and sulfide, there was a substantial increase in plasma molybdenum and copper levels and liver molybdenum levels and a decrease in

ceruloplasmin activity. In the plasma, there was a shift in the fraction of copper associated with albumin and ceruloplasmin (Mills et al. 1981a). Similar findings were observed in rats administered tetrathiomolybdates, but not in rats exposed to molybdates in the absence of sulfide (Mills et al. 1981a). In rats, exposure to tetrathiomolybdates resulted in effects similar to those observed in ruminants including signs of copper deficiency, such as loss of pigmentation in hair and a similar distribution of copper between the plasma proteins (Mills et al. 1981b). However, these interactions between tetrathiomolybdate and copper only occurred when both were present in the gastrointestinal tract (Mills et al. 1981b). It is not known if the interactions between copper and molybdenum only occur at higher molybdenum doses. As discussed by Brewer et al. (2000), tetrathiomolybdate can form a tripartite complex with copper and protein, which can prevent copper absorption through the gastrointestinal tract. When tetrathiomolybdate is not administered with food, it can complex with copper and serum albumin, which prevents cellular uptake of copper. Due to these mechanisms, tetrathiomolybdate is used to treat individuals with Wilson's disease, which is a metabolic defect that limits the excretion of copper. Other molybdenum compounds may also interfere with copper balance in humans. Significant increases in serum and urine copper levels were observed in men exposed to 0.022 mg molybdenum/kg/day (the source of molybdenum was high molybdenum sorghum supplemented with ammonium molybdate) for 10 days, as compared to exposure to 0.00771 mg molybdenum/kg/day for 10 days (Deosthale and Gopalan 1974). However, there was no difference in fecal excretion of copper, suggesting that copper absorption was not affected. In contrast, another study (Turnlund and Keys 2000) did not find any significant alterations in serum copper levels in humans exposed to molybdenum levels of 22–1,490 µg/day (0.0003–0.02 mg/kg/day) for 24 days (subjects were fed diets containing naturally high or low levels of molybdenum).

A number of studies have reported that molybdenum induces oxidative stress. An *in vitro* study in mouse fibroblasts and liver cancer cells found that trivalent molybdenum induced oxidative stress as indicated by increases in reactive oxygen species generation and increases in malondialdehyde concentration (Terpilowska and Siwicki 2019). This possible mechanism of action is supported by several *in vivo* studies. A general population study found an association between urinary molybdenum levels and ratio of oxidized glutathione to reduced glutathione in the general population suggestive of a relationship between molybdenum and oxidative stress (Domingo-Relloso et al. 2019). Zhai et al. (2013) showed that the levels of two enzymatic antioxidants (superoxide dismutase and glutathione peroxidase) in the testes of mice paralleled the molybdenum-induced sperm effects. Increases in antioxidant levels and improvements in sperm parameters were observed at lower molybdenum doses. However, at higher molybdenum doses, there were significant decreases in antioxidant levels and significant decreases in

sperm motility and concentration and an increase in the rate of sperm abnormalities. Zhang et al. (2013) reported a similar finding for superoxide dismutase and glutathione peroxidase levels in the ovaries of mice and the rate of MII oocyte abnormalities. Molybdenum-induced hepatocyte apoptosis was observed in goats orally exposed to ammonium molybdate for 50 days (Zhuang et al. 2016). Molybdenum exposure resulted in down-regulation of superoxide dismutase and catalase expression in liver cells and an up-regulation of malondialdehyde, nitric oxide, and total nitric oxide synthase expression. The investigators suggested that the observed effect may be due to a disruption of the mitochondrial antioxidant defense system resulting in apoptosis via activation of the mitochondrial signaling pathways.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- A number of factors can influence the oral absorption of molybdenum; absorption can range between 40 and 100%. The amount absorbed decreased with increasing doses and was lower when the molybdenum was ingested with a meal. There is evidence for absorption of airborne molybdenum, but no data on the amount absorbed. Molybdenum is poorly absorbed (approximately 0.2%) through the skin.
- Absorbed molybdenum is widely distributed throughout the body, with the highest concentrations found in the kidneys and liver.
- Molybdenum is not metabolized; however, it can undergo oxidation and reduction.
- Molybdenum is primarily excreted in the urine, with lesser amounts excreted in feces.

3.1.1 Absorption

Inhaled molybdenum particles that deposit in the respiratory tract are subject to three general distribution processes: (1) bronchial and tracheal mucociliary transport to the gastrointestinal tract; (2) transport to thoracic lymph nodes (e.g., lung, tracheobronchial, mediastinal); or (3) absorption into blood and/or lymph and transfer to other tissues (e.g., peripheral lymph tissues, liver, kidney). The above processes apply to all forms of deposited molybdenum, although the relative contributions of each pathway and rates associated with each pathway vary with the physical characteristics (e.g., particle size, solubility). Particles having diameters >5 μm are deposited primarily in the upper airways (extrathoracic, tracheobronchial regions) and are cleared from the respiratory tract primarily by mucociliary transport to the gastrointestinal tract (Bailey et al. 2007; ICRP 1994). Smaller particles (≤5 μm) are deposited primarily in the pulmonary region (terminal bronchioles and alveoli). Particles are cleared from the pulmonary region primarily by absorption, lymph drainage, macrophage phagocytosis and migration, and upward mucociliary flow. Dissolved molybdenum is absorbed into blood. The rate of absorption will depend on solubility. ICRP (2012) assigns molybdenum sulfide, oxides, and hydroxides to a "slow" classification in their absorption, which equates to an expected terminal absorption half-time of approximately 19 years (Bailey et al. 2007; ICRP 1994). More soluble forms of molybdenum, such as molybdenum trioxide ($Mo^{VI}O_3$), would be expected to undergo more rapid dissolution and absorption.

Quantitative estimates of absorption following inhalation exposure to molybdenum in humans or animals were not identified. Evidence for absorption of molybdenum trioxide is available from inhalation studies

on molybdenum trioxide conducted in rodents (Fairhall et al. 1945; NTP 1997). Fairhall et al. (1945) showed distribution to several tissues following inhalation exposure of guinea pigs to molybdenum trioxide. In rats and mice exposed to inhaled molybdenum trioxide (6.7–67 mg molybdenum/m^3, 6 hours/day, 5 days/week for 2 years), exposure-dependent increases in blood molybdenum were observed (NTP 1997). The respective blood molybdenum levels in the 0, 6.7, 20, and 67 mg molybdenum/m^3 groups were 0.221, 0.800, 1.774, and 6.036 μg/g in male rats, 0.059, 0.355, 0.655, and 2.411 μg/g in female rats, 0.102, 0.208, and 0.770 μg/g in male mice (no data were available for controls), and 0.043, 0.066, 0.198, and 0.523 μg/g for female mice.

Absorption of ingested molybdenum has been studied in human adults and infants (Cantone et al. 1993, 1997; Engel et al. 1967; Giussani et al. 1998, 2006, 2007; Novotny and Turnlund 2006, 2007; Robinson et al. 1973; Sievers et al. 2001a, 2001b; Turnlund et al. 1995a, 1995b; Werner et al. 1998; Yoshida et al. 2006). These studies fall into two general categories: mass balance studies and bioavailability studies. Mass balance studies estimate the absorption fraction from measurements of the difference between the ingested dose of molybdenum and fecal excretion (the difference being net absorption). Bioavailability studies estimate the absorption fraction from measurements of the plasma concentration of molybdenum following the oral dose. These methods provide estimates of net absorption in that absorbed molybdenum that is excreted into the gastrointestinal tract (e.g., biliary excretion) may not be accurately quantified from mass balance or bioavailability estimates. However, both approaches have been facilitated by the use of stable isotopes of molybdenum (^{95}Mo, ^{96}Mo), which allow measurement of plasma and excretion kinetics following concurrent intravenous and oral dosing. The use of stable isotopes also allows measurement of the administered molybdenum in plasma and excreta, distinct from background sources of molybdenum derived from other sources and preexisting body stores. By incorporating elimination kinetics data into mathematical models that include parameters representing absorption and fecal excretion of absorbed molybdenum, the absorption fraction can be estimated. In most reported stable isotope studies, the exact form of molybdenum administered was not reported. However, the isotope dosing material was typically prepared from an acid dissolution of metallic molybdenum (Mo^0). The resulting material dissolved in water most likely was a mixture of soluble molybdate anion ($Mo^{VI}O_4^{2-}$) and other soluble molybdenum oxide hydrates.

Balance and bioavailability studies conducted in humans have shown that the fraction of ingested molybdenum that is absorbed depends on numerous factors, including molybdenum dose level, fasting, diet, and nutritional status. Absorption was estimated to be 80–100% in replete fasted adults who ingested molybdenum dissolved in water or in a beverage (Giussani et al. 2006; Novotny and Turnlund

2006, 2007; Turnlund et al. 1995a). Absorption was 80–100% following a single dose of 20–40 µg molybdenum/kg dissolved in water and decreased with increasing dose level; absorption was 60% after a dose of 60 µg molybdenum /kg (Giussani et al. 2006). Absorption was lower when molybdenum was ingested with a meal (40–60%), when dissolved in black tea (20%), or when incorporated into vegetables cultivated with ^{96}Mo (30–60%), compared to when ingested without a meal (80–100%) (Giussani et al. 2006; Werner et al. 1998). Absorption was lower when molybdenum was incorporated into the diet (83%) compared to when it was administered in a beverage (90–94%) (Novotny and Turnlund 2007). Absorption appears to be affected by dietary molybdenum intake and molybdenum nutritional status. The absorption fraction was 90% in adults fed a diet containing 22 µg/day (approximately 0.3 µg molybdenum/kg/day), compared to 94% when fed a diet containing 467 µg molybdenum/day (approximately 7 µg molybdenum/kg/day) (Novotny and Turnlund 2007). Absorption in infants (gestational age 30–39 weeks) was 96–99% when a stable isotope of molybdenum was mixed with breast milk or infant formula (Sievers et al. 2001a, 2001b).

Long-term diet mass balance studies, without the aid of stable isotopes, have been conducted in adults and children (Engel et al. 1967; Robinson et al. 1973; Tipton et al. 1966). Because these studies cannot distinguish between the ingested dose of molybdenum and molybdenum excreted from body stores, these studies will underestimate the absorption fraction. A 50-week balance study conducted in two adult males (age 23 and 25 years) found absorption to range from 60 to 80% (Tipton et al. 1966). A 3-week balance study conducted in women (age 19–21 years) found absorption to range from 40 to 70% (Robinson et al. 1973). An 8-day balance study conducted in women (age 18–23 years) found absorption to range from 72 to 84% (Yoshida et al. 2006). Balance studies (18–30 days) conducted in female children (age 6–10 years) estimated the absorption fraction from diet to range from 67 to 85% (Engel et al. 1967).

Measurements of the time course of plasma molybdenum following oral doses of molybdenum indicate that absorption is relatively rapid, with peak concentrations in plasma attained within 100 minutes of dosing (Giusanni et al. 2006; Novotny and Turnlund 2007).

Studies of absorption and elimination kinetics conducted in swine provide estimates of absorption of ingested molybdenum. Based on cumulative urinary and fecal excretion measurements in swine dosed with a stable isotope of molybdenum, absorption was estimated to be between 80 and 90% (Bell et al. 1964). Studies conducted in rats have shown that tetrathiomolybdate ($Mo^{VI}S_4^{2-}$) is more poorly absorbed

when ingested in the diet; approximately 21% was absorbed when the copper content of the diet was 8 ppm (Mills et al. 1981b).

Roper (2009) evaluated the *in vitro* percutaneous absorption of sodium molybdate through human skin. Following an 8-hour application of 3.97 or 19.83 mg molybdenum/mL, the potentially absorbable doses were 0.21 and 0.16%, respectively.

Mechanisms that participate in absorptive transport of molybdenum in the gastrointestinal tract have not been characterized. Molybdate (MoO_4^{2-}) and sulfate (SO_4^{2-}) show mutually competitive inhibition for absorptive transport in rat small intestine, suggesting involvement of a common transporter for both anions (Cardin and Mason 1975, 1976). This transporter may be the Na^+/SO_4^{2-} symporter (NaS1 or SLC13A1) expressed in rodent small intestine and renal proximal tubule (Markovich and Aronson 2007; Murer et al. 1994). In humans, NaS1 is expressed in kidney but not small intestine, suggesting that mechanisms of absorptive transport in humans may be different from that of rodents (Lee at al. 2000).

3.1.2 Distribution

Very little information on the distribution on molybdenum following inhalation exposure is available. Following exposure of guinea pigs to inhaled molybdenum trioxide (150–300 mg/m^3, 1 hour/day, 5 days/week for 5 weeks), molybdenum was distributed to the lungs, liver, kidneys, and bone (Fairhall et al. 1945). Tissue levels decreased by approximately 20% in the 2-week postexposure period.

Absorbed molybdenum distributes to various tissues. Human autopsy studies have found that the kidney and liver have the highest amounts of molybdenum (Iyengar et al. 1978; Schroeder et al. 1970; Sorensen and Archambault 1963; Sumino et al. 1975; Tipton and Cook 1963; Tipton et al. 1965; Yoo et al. 2002; Zeisler et al. 1988). Based on a review of these data, Giussani (2008) estimated liver and kidney molybdenum concentrations to be approximately 0.5–1.5 µg molybdenum/g wet in liver (700–2,700 µg) and 0.2–0.4 µg molybdenum/g wet in kidney (55–120 µg). Coughtrey and Thorne (1983) reported relatively high concentrations (56 µg molybdenum/g) in bone, based on their recalculation of measurements of molybdenum in bone ash reported in Nusbaum et al. (1965) and Iyengar et al. (1978). However, these results are not supported by other studies (previously cited) and have been attributed to overestimation of tissue concentrations measured by arc emission spectrometry in the Nusbaum et al. (1965) and Iyengar et al. (1978) studies (Giussani 2008).

Results of studies in rats and guinea pigs exposed to oral molybdenum show that molybdenum is widely distributed (Bibr et al. 1977; Howell et al. 1993; Murray et al. 2014b; Pandey et al. 2002). Generally, the highest molybdenum tissue concentration is observed in the kidney. Molybdenum also is distributed to liver, spleen, brain, lungs, heart, bone, muscles, testes, epididymides, seminal vesicles, prostate, blood cells, and plasma.

Maternal-Fetal Transfer. Results of studies in humans and animals show that molybdenum is distributed to the fetus. In humans, maternal and fetal cord blood levels obtained from 33 maternal-fetal pairs at parturition show similar molybdenum levels (maternal: 1.44±0.75 µg/L, mean±standard deviation [SD]; fetal: 1.44±0.89 µg/L) (Bougle et al. 1989). Molybdenum concentrations in venous cord blood (flowing from the placenta to the fetus; 0.7±0.2 µg/L, mean±SD) were slightly higher than in arterial cord blood (flowing from the fetus to the placenta; 0.6±0.2 µg/L), indicating fetal retention of molybdenum (Krachler et al. 1999); the study did not evaluate whether there was a statistical difference between the molybdenum concentrations in venous and arterial blood.

Gestational exposure of rats to ammonium molybdate and thiomolybdate shows distribution of molybdenum to fetal liver, kidney, bone, and brain (Howell et al. 1993). Levels in liver, kidney, and bone were similar, with lower levels in brain. In rats, dose-dependent increases in placental and maternal liver content of molybdenum were observed following gestational exposure to molybdenum (sodium molybdate) in drinking water (5–100 mg molybdenum/L; equivalent to approximately 0.76–15 mg/kg/day, based on intermediate exposure to nonpregnant female rats) over the full dose range (Fungwe et al. 1989). However, neonatal whole-body levels of molybdenum reached a plateau at drinking water concentrations ≥50 mg/L (Fungwe et al. 1989). Results suggest that molybdenum levels in the fetus reach steady state more rapidly than in dams.

Maternal-Infant Transfer. Several studies have measured molybdenum in breast milk (Anderson 1992; Aquilio et al. 1996; Biego et al. 1998; Bougle et al. 1988; Casey and Neville 1987; Dang et al. 1984; Friel et al. 1999a; Krachler et al. 1998; Wappelhorst et al. 2002); the mean concentrations ranged from 0.02 to 24 µg/L. Breast milk concentrations are highest at the start of breast feeding and then decline (EFSA 2013). In the only study comparing maternal intake to breast milk levels, Wappelhorst et al. (2002) did not find a correlation between breast milk concentrations of molybdenum and maternal molybdenum intake. The mean concentration in breast milk was 72 µg/L and the mean maternal intake was 132 µg/day.

Bacteria and eukaryotes express cell membrane molybdate transporters, one of which (MoT_2) also appears to be expressed in humans (Tejada-Jimenez et al. 2007, 2011). In eukaryotes, this transporter participates in the delivery of molybdate into cells for incorporation into molybdopterin-cofactor (Moco), the biologically active prosthetic group in molybdenum-dependent enzymes (Schwarz et al. 2009). MoT_2 transport of molybdate is inhibited by sulfate, suggesting a common carrier for molybdate and sulfate. A sulfate-insensitive oxalate-sensitive molybdate transporter has been described in mammalian MEK-293T cells grown in culture (Nakanishi et al. 2013). Uptake of molybdate into human red blood cells involves participation of the Cl^{-1}/HCO_3^{-1} anion exchanger (Gimenez et al. 1993).

3.1.3 Metabolism

Molybdenum exists in several valence states and may undergo oxidation and reduction. The primary form of molybdenum that interacts with enzyme systems is Mo^{VI}, as the molybdate anion ($Mo^{VI}O_2^{2-}$) (Nakanishi et al. 2013). After molybdate is taken into a cell, it is incorporated into a molybdopterin to form molybdenum cofactor (Moco). Moco is a sulfur-molybdate complex that forms the prosthetic group in molybdenum-dependent enzymes (Mendel and Kruse 2012; Schwarz et al. 2009). Given that Moco is extremely sensitive to oxidation, it is believed that it is bound to a Moco-binding protein in the cell (Mendel and Kruse 2012). This stored Moco would be readily available to meet the cell's demand for molybdenum enzymes. Molybdate forms complexes with copper and binds to plasma proteins as a copper-molybdenum-sulfur (Cu-Mo-S) complex (Nederbragt 1980, 1982).

3.1.4 Excretion

Studies investigating the elimination and excretion of molybdenum following inhalation exposure were not identified.

Absorbed molybdenum is excreted in urine and feces in humans. Urine is the dominant excretion route, accounting for the excretion of approximately 75–90% of the absorbed dose (Giussani 2008; Novotny and Turnlund 2007). The fraction excreted in urine increases with increasing dietary intake (Novotny and Turnlund 2007). Urine also is the dominant excretory route for absorbed molybdenum in swine. Following an oral dose, approximately 90% of the dose was excreted in urine (Bell et al. 1964). To measure urinary and fecal excretion of molybdenum, Turnlund et al. (1995a, 1995b) exposed four healthy adult males to various doses of a radioactive isotope of molybdenum (24–1,378 µg ^{100}Mo/day) and administered intravenous doses of stable isotope of molybdenum (33 µg ^{97}Mo). Six days after exposure

to ^{100}Mo in the diet, 17.8% of the ^{100}Mo label was excreted in the urine at the lowest dose tested (total molybdenum dose of 24 µg/day). As the molybdenum dose increased, the amount excreted in the urine also increased; at the highest dose (1488 µg/day), 82.1% of the ^{100}Mo was excreted in the urine. A similar pattern of urinary excretion was found when ^{97}Mo was measured: 32.7% of the label at 24 µg/day and 86.7% at 1,488 µg/day. The percentage of the molybdenum dose excreted in the feces decreased with increasing doses. At the lowest dose tested, 9.4% of the ^{100}Mo dose was excreted in the feces; at the highest dose, 7.5% of the ^{100}Mo dose was excreted in the feces. In contrast, no consistent pattern of fecal ^{97}Mo excretion was found. When total molybdenum excretion was measured, the study found that 41% was excreted in feces and 59% was excreted in urine at the lowest dose tested and 6% was excreted in feces and 94% was excreted in urine at the highest dose tested. Fecal excretion of absorbed molybdenum is thought to result from biliary secretion. Studies conducted in bile-duct cannulated rats have shown that, following an intravenous dose of $Mo^{[V]}$ or $Mo^{[VI]}$, approximately 1% of the molybdenum dose was secreted into bile in a period of 4 hours (Lener and Bibr 1979).

The rate of elimination of molybdenum from plasma has been studied in human clinical studies (Cantone et al. 1997; Rosoff and Spencer 1964; Thompson et al. 1996; Werner et al. 2000). Elimination is approximately biphasic, with mean half-times of 30 minutes and 6.6 hours (Giussani 2008).

The whole-body elimination rate in rats is dose-dependent (Bibr and Lener 1973). Following oral administration of $Mo^{[VI]}$ at doses <3 µg molybdenum/kg, elimination was mono-phasic with a half-time of approximately 47 hours. Following doses >3 µg molybdenum/kg, the rate of elimination increased, with an increasing proportion of elimination contributed by a fast phase having a half-time of 6 hours.

Mechanisms that participate in the renal excretion of molybdenum have not been characterized. In sheep, reabsorption of filtered molybdate (MoO_4^{2-}) is saturable, which results in an increase in the fraction of filtered molybdate excreted as the plasma molybdate concentration increases and approaches or exceeds the tubular maximum (Ryan et al. 1987). In sheep and rat kidney, sodium-dependent reabsorptive transport of molybdate (MoO_4^{2-}) and sulfate (SO_4^{-2}) exhibit mutual inhibition (Ryan et al. 1987). This is consistent with participation of the Na^+/SO_4^{2-} symporter (NaS1 or SLC13A1) in the reabsorption of molybdate. This symporter is also expressed in the human renal proximal tubule (Markovich and Aronson 2007; Murer et al. 1994).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Several multi-compartmental models of the kinetics of molybdenum in humans have been developed (Giussani 2008; Giussani et al. 1998, 2000; Novotny and Turnlund 2007; Thompson et al. 1996). The latest of these are the Giussani (2008) and Novotny and Turnlund (2007) models. Both models yield similar predictions when applied to the same dosing scenarios (Giusanni 2008). The Giussani (2008) model has been adopted for use by the International Commission on Radiological Protection (ICRP) and is described in this section.

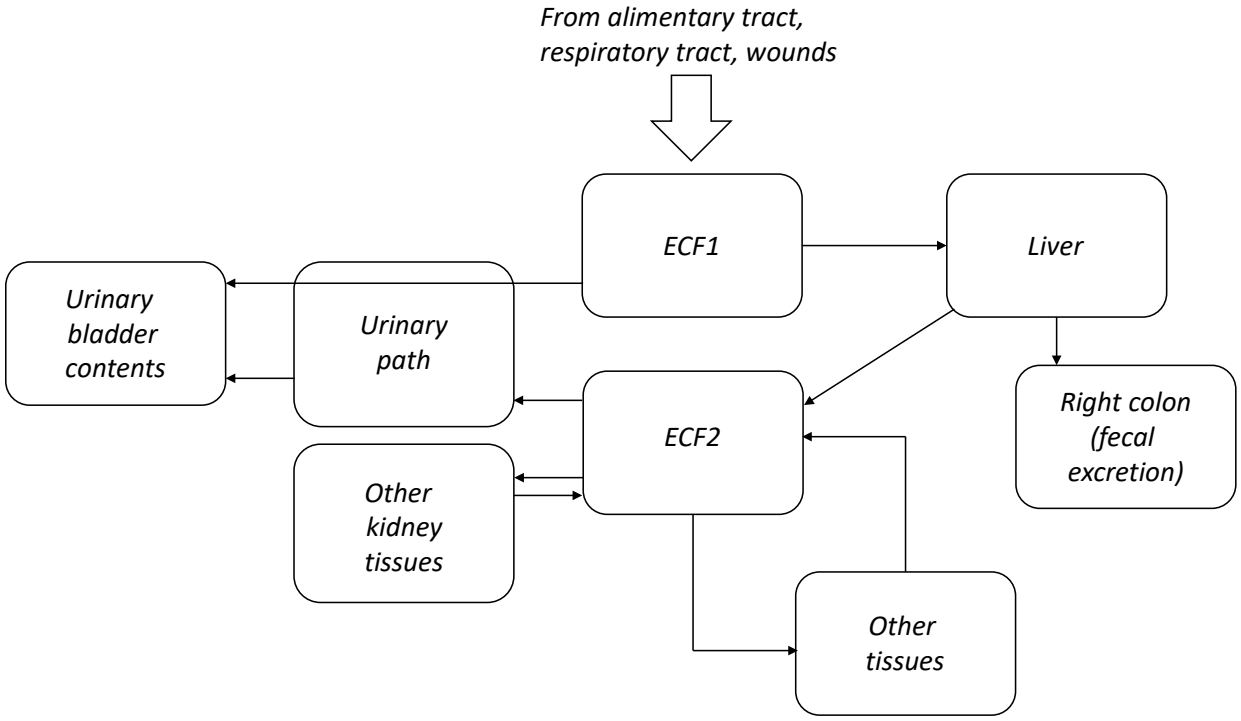
Giussani (2008) Model

Giussani (2008) developed a model of molybdenum kinetics in humans. The structure of the model is shown in Figure 3-1 and parameter values are presented in Table 3-1. Data used to derive and evaluate the model are described in Giusanni (2008) and included human clinical studies in which subjects were administered intravenous or oral doses of stable isotopes of molybdenum (Giusanni et al. 2006, 2007; Novotny and Turnlund 2006, 2007; Turnlund et al. 1995a; Werner et al. 1998, 2000). The Giussani (2008) model has been adopted for use by the ICRP and is described in this section.

The model consists of two central compartments representing extracellular fluids (ECF) and compartments representing liver, kidney (two subcompartments), and a lumped compartment representing all other tissues. All transfers of molybdenum between compartments are first order and governed by first-order rate coefficients (day^{-1}). The two ECF compartments represent fast and slow transfer pathways out of the ECF and were based on studies conducted in humans, which provide evidence for multi-phasic clearance of molybdenum from plasma (Giussani et al. 2007; Werner et al. 2000). The half-times for the two ECF compartments are approximately 30 minutes for ECF1 and 280 minutes for ECF2. Transfers

from the fast compartment (ECF1) are to liver, kidney, and urine. Transfers from the slow compartment (ECF2) are to urine, kidney, and other tissues; the slow compartment also receives molybdenum from the liver. Retention half-times in tissues are 41 days for liver, 14.5 days for kidney, and 21.5 days for the other tissue compartment. Excretion of absorbed molybdenum occurs in urine (88%) and transfer from liver to the gastrointestinal tract (12%).

Figure 3-1. The Proposed Systemic Model for Molybdenum Radionuclides



ECF = extracellular fluid

Source: Reprinted from Giussani (2008) with permission from Elsevier.

Table 3-1. Transfer Rates (Day^{-1}) for the Molybdenum Model

Transfer rate	Value (day^{-1})
ECF1 to ECF2	12.5
ECF1 to liver	14.2
ECF1 to urinary bladder contents	6.5
ECF2 to urinary path	1.7
ECF2 to other kidney tissues	0.115
ECF2 to other tissues	1.73
Liver to alimentary tract	0.0048

Table 3-1. Transfer Rates (Day^{-1}) for the Molybdenum Model

Transfer rate	Value (day^{-1})
Liver to ECF2	0.0122
Other kidney tissues to ECF2	0.0474
Other tissues to ECF2	0.0323
Urinary path to urinary bladder contents	1.40
Urinary bladder contents to urine	12
Modified parameters of the alimentary tract	
Stomach to small intestine (liquid form)	100
Stomach to small intestine (diet)	40
Small intestine to right colon (liquid form)	10
Small intestine to right colon (diet)	16
f_A (liquid form)[a]	0.9
f_A (diet)[a]	0.6

Source: Reprinted from Giussani (2008) with permission from Elsevier.

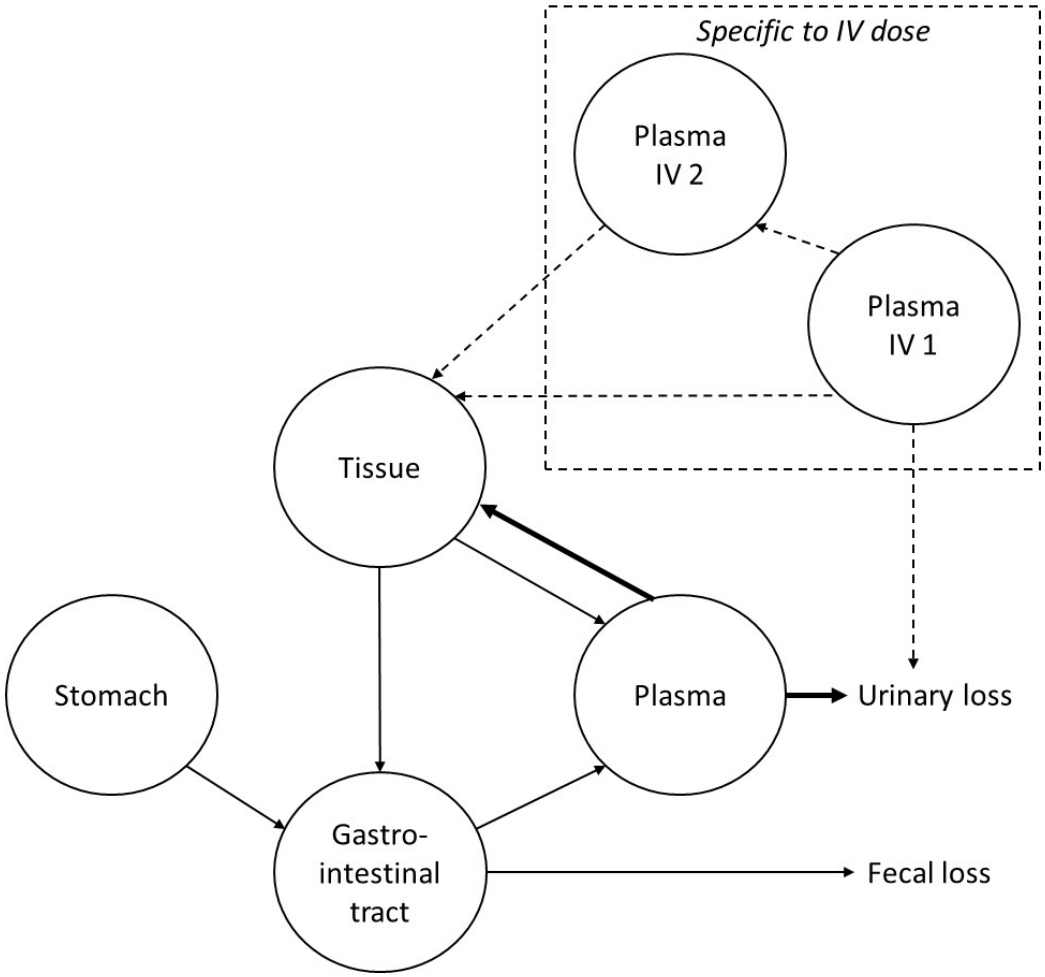
The model can simulate absorption from the gastrointestinal tract and respiratory tract. The absorption fraction for the gastrointestinal pathway uses an absorption fraction of 0.9 for molybdenum ingested in liquids and 0.6 for molybdenum ingested in the diet. The model predicts a steady state for constant dietary intake of molybdenum in adults, in which approximately 52% of the molybdenum body burden is in liver, 3% is in kidney, 45% is in other tissues, 53% of the daily dose is excreted in urine, and 47% of the daily dose is excreted in feces (Giussani 2008). The model is constructed to be able to interface with output from the ICRP Human Respiratory Tract Model (HRTM) (Bailey et al. 2007; ICRP 1994). The inputs to the Giussani (2008) model from the HRTM would be simulated transfers of molybdenum to the gastrointestinal tract (mucociliary transfer) and to blood (absorption from the respiratory tract), depending on the particle size and solubility of the inhaled molybdenum and other physiological factors (e.g., age, activity).

Novotny and Turnlund (2007) Model

The major difference between the structures of the Giussani (2008) and Novotny and Turnlund (2007) models is that the Novotny and Turnlund (2007) model has a single lumped compartment representing all tissues outside of the vasculature. The Novotny and Turnlund (2007) model illustrated in Figure 3-2 has two configurations: an intravenous configuration, which has two plasma compartments, representing fast

and slower clearance, and an oral configuration, which has a single plasma compartment. Molybdenum exchanges between plasma and a lumped tissue compartment. Urinary excretion is represented as a direct transfer from plasma. Absorbed molybdenum is also transferred to the gastrointestinal tract.

Figure 3-2. Diagram of the Compartment Molybdenum Model*



*Circles show distinct kinetic compartments and arrows show pathways of flow between compartments. Dashed lines show structures that are specific to the intravenous (IV) dosing. Bold arrows show paths that appear to be involved in molybdenum regulation as suggested by kinetic modeling.

Source: Novotny and Turnlund (2007), by permission of the American Society for Nutrition (via Oxford University Press)

Novotny and Turnlund (2006, 2007) conducted mass balance studies with subjects who ingested stable isotopes of molybdenum in the context of varying dietary intakes of molybdenum (22–1,490 µg molybdenum/day) and found that certain model parameters were dependent on dietary intake. These included, in association with increasing dietary intake, increases in the first-order rate coefficients for gastrointestinal absorption and urinary excretion and a decrease in the rate coefficients for transfer from

plasma to tissues. The largest adjustments were needed to simulate molybdenum kinetics in subjects who consumed >121 µg molybdenum/day and included a 70% decrease in the coefficient for transfer of molybdenum from plasma to tissues and a 660% increase in the rate coefficient for transfer from plasma to urine. These results suggest that high molybdenum intakes (>121 µg molybdenum/day) result in physiological adaptations that increase excretion of absorbed molybdenum (Novotny and Turnlund 2007).

3.1.6 Animal-to-Human Extrapolations

There are limited data to evaluate potential differences in the toxicity of molybdenum between laboratory animals and humans. Most of the available oral exposure studies were conducted in rats, and human data are mostly limited to a small number of cross-sectional studies. Within laboratory animal species, some differences have been observed between rats and rabbits, with rabbits appearing to be more sensitive than rats. However, the studies are not directly comparable due to differences in the copper content and other dietary constituents. In the absence of data to the contrary, it is assumed that the toxicity of molybdenum will be similar across species (excluding ruminants).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to molybdenum are discussed in Section 5.7, Populations with Potentially High Exposures.

There are limited data on the toxicity of molybdenum in children. In studies in rat pups maintained on a caries-promoting diet, administration of 50 mg molybdenum/kg/day as sodium molybdate resulted in an increase in buccal enamel lesions (Hunt and Navia 1975), but exposure to 8 mg molybdenum/kg/day did not result in increases in dental caries (Van Reen et al. 1962). Arrington and Davis (1953) exposed young (6 weeks of age at the start of the study) and mature rabbits to sodium molybdate in the diet for 30–84 days. Marked muscular/skeletal effects were observed in the young rabbits, but not in the mature rabbits. Since the investigators did not provide information on dietary intake, it is difficult to make direct comparisons across the studies.

An observational study did not find an association between maternal urinary molybdenum levels and newborn body weight or infant mental development (Shirai et al. 2010). However, another study did find an association between third-trimester maternal urinary molybdenum levels and infant psychomotor development indices (Vazquez-Salas et al. 2014). Two rat studies in which the copper content of the diet was adequate did not find significant alterations in fetal growth, survival, or malformations at maternal doses of 40 mg molybdenum/kg/day (Murray et al. 2014b, 2019). However, a third study reported decreases in growth and number of live fetuses in the offspring of male rats administered 14 mg molybdenum/kg as sodium molybdate 5 days/week for 60 days prior to mating with unexposed females (Pandey and Singh 2002).

Studies in laboratory animals have found that maintenance on a copper-deficient diet enhances the toxicity of molybdenum (Brinkman and Miller 1961; Franke and Moxon 1937; Johnson and Miller 1961; Sasmal et al. 1968; Valli et al. 1969; Van Reen 1959; Widjajakuma et al. 1973). Administration of additional copper results in a reversal of the adverse effect (Arrington and Davis 1953). Thus, individuals with low copper intakes may be unusually susceptible to the toxicity of molybdenum. Additionally, individuals with high dietary molybdenum intake, including individuals taking supplements containing high levels of molybdenum, may be at an increased risk from exposure to high levels of molybdenum in the environment.

Studies in rats suggest that the toxicity of molybdenum may be increased in animals maintained on a low protein diet. The magnitudes of the decrease in body weight gain (Bandyopadhyay et al. 1981; Cox et al. 1960) and decreases in femur breaking strength (Fejery et al. 1983) were greater in rats exposed to a low protein diet, as compared to those maintained on a diet with sufficient protein.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to molybdenum are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for molybdenum from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by molybdenum are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Molybdenum levels can readily be measured in tissues, body fluids, and excreta. Dose-related increases in serum molybdenum levels were observed in rats and mice exposed via inhalation to molybdenum trioxide for 2 years (NTP 1997). In a study examining the relationship between plasma molybdenum levels and dietary intake, Turnland and Keyes (2004) reported a baseline plasma molybdenum level of 8.2±0.5 nmol/L; 25 days after the subjects were maintained on a low molybdenum diet (22 µg/day), the plasma molybdenum level was 5.1±0.5 nmol/L. Although a significant correlation between plasma molybdenum and dietary molybdenum was observed, comparison between plasma molybdenum levels at different dietary intakes showed that a significant increase in plasma molybdenum was not observed until the dietary intake exceeded 460 µg/day (6.6 mg/kg/day) and that tripling the intake resulted in a doubling of the plasma molybdenum levels. Urinary molybdenum levels were also significantly correlated to dietary intakes (Turnland and Keyes 2004) and appeared to be more responsive to changes in dietary intake. At all dietary concentrations, the urinary molybdenum levels were slightly lower than the dietary intakes (Turnland and Keyes 2004). The investigators concluded that plasma molybdenum levels are an indicator of dietary intake, but urinary levels had a more direct relationship with dietary intake.

Molybdenum levels were measured in urine samples collected during the NHANES study. The geometric mean urinary molybdenum levels in the United States in 2011–2012 was 37.1 µg/L and the creatinine-corrected value was 42.0 µg/g creatinine (CDC 2015); see Section 5.6 for additional information.

Although several studies have reported molybdenum levels in hair samples (DiPietro et al. 1989; Nagra et al. 1992; Paschal et al. 1989), no relationship between molybdenum exposure and hair levels has been established. Furthermore, Miekeley et al. (1998) demonstrated large interlaboratory differences in the molybdenum levels measured in hair.

3.3.2 Biomarkers of Effect

No biomarkers to characterize effects caused by molybdenum have been identified.

3.4 INTERACTIONS WITH OTHER CHEMICALS

The interaction between copper and molybdenum has been well-established in animals. The levels of copper in the diet have been shown to influence the toxicity of molybdenum. Marked toxicity has been

reported in studies in which the copper content of the diet was inadequate. Observed effects included mortality in rabbits (Valli et al. 1969; Widjajakuma et al. 1973), marked decreases in body weight gain and weight loss in rats and rabbits (Brinkman and Miller 1961; Johnson and Miller 1961; Sasmal et al. 1968; Valli et al. 1969; Van Reen 1959), and anemia in rats and rabbits (Brinkman and Miller 1961; Franke and Moxon 1937; Gray and Daniel 1954; Johnson et al. 1969; Valli et al. 1969). In general, these effects (or the severity of the effects) have not been observed when the diet contains an adequate level of copper (Mills et al. 1958; Murray et al. 2014a; Pandey and Singh 2002; Peredo et al. 2013). Exposure to high levels of copper has been shown to reduce the toxicity of molybdenum. Administration of high doses of copper to moribund rabbits resulted in a return to normal body weight gain and increases in hemoglobin levels within 2–3 weeks (Arrington and Davis 1953). Lyubimov et al. (2004) showed that administration of a high dose of copper prevented the molybdenum-induced testicular toxicity observed in rats fed a copper-adequate diet. Similarly, in an environmental exposure study of men at infertility clinics, Meeker et al. (2008) found a greater decline in sperm concentration in men with high molybdenum blood levels and copper blood levels below the median, as compared to when the men were not stratified by blood copper levels.

Kinetic studies have demonstrated differences in plasma, liver, and kidney copper and molybdenum concentrations in rats fed copper-deficient, copper-adequate, and copper-excessive diets (Nederbragt 1980). Administration of molybdenum results in increases in plasma, liver, and kidney copper levels in rats fed a copper-adequate diet (Nederbragt 1980); the increases in copper appear to be molybdenum-dose-related. Most of the rise in plasma copper levels was in the tightly-bound fraction, which is likely to be poorly available for copper metabolism. Excess copper in the diet resulted in a smaller increase in copper concentrations in plasma, liver, and kidneys and molybdenum concentrations in the liver and kidney, as compared to levels in rats fed a copper-adequate diet. Similarly, lower rises in liver copper and molybdenum and kidney molybdenum levels were observed in rats fed a copper-deficient and high-molybdenum diet, as compared to the copper-adequate diet. At the lowest molybdenum dose, kidney molybdenum levels were higher in the copper-deficient groups. In another study (Nederbragt 1982), kidney levels of copper and molybdenum were 5 and 3 times higher, respectively, in the copper-adequate groups as compared to the copper-deficient group. Two human studies have also evaluated the effect of molybdenum on copper levels. In one study, increases in serum and urine copper levels were found following a 10-day exposure to 0.022 mg molybdenum/kg/day (Deosthale and Gopalan 1974). Another study found no significant alterations in serum copper levels in humans exposed to 0.0003–0.02 mg molybdenum/kg/day for 24 days (Turnlund and Keys 2000).

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Molybdenum (Mo) is a naturally occurring metallic trace element found in natural minerals, but not as the free metal. Biologically, it is an important micronutrient in plants and animals, including humans. It is used widely in industry for metallurgical applications (EPA 1979).

Molybdenum metal is a dark-gray or black powder with a metallic luster (NLM 2020a). It is a transition element in Group 6 of the Periodic Table. It has oxidation states from -2 to +6. Commonly encountered compounds are those of molybdenum in oxidation state +6 (Mo(VI), MoO_3, molybdates) and +4 (Mo(IV), MoS_2). It does not occur naturally in the pure metallic form; it more commonly occurs in the mineral, molybdenite (Sebenik et al. 2012). Other naturally occurring molybdenum-containing minerals are powellite, wulfenite, ferrimolybdite, and ilsemannite; however, molybdenite is the primary commercial source of molybdenum. Molybdenum (VI) anions include molybdate (MoO_4^{-2}) with molybdenum at the center of a tetrahedron of four oxygen atoms, and polyberic anions ('isopolymolybdates') of which the most common are heptamolybdate ($Mo_7O_{24}^{6-}$) and octamolybdate ($Mo_8O_{26}^{4-}$) (EPA 1979). These anions occur in sodium and ammonium salts, often hydrated, which are the common sources of molybdenum in commerce and industrial applications.

There are 33 known isotopes of molybdenum. Seven isotopes occur naturally: mass numbers 92, 94, 95, 96, 97, 98, and 100. ^{98}Mo is the most abundant isomer, comprising approximately 24.3% (Rumble et al. 2018). Radioisotopes of masses 83–91, 93, 99, and 101–115 have been reported. The only one of major worldwide importance is Mo-99 (^{99}Mo), a 100% beta-emitting isotope with a 65.976-hour radioactive half-life that is used to produce technetium-99m (Tc-99^m or ^{99m}Tc) for medical scans (Doll et al. 2014; Parma 2009; Richards 1989).

Under physiological conditions (pH >6.5), the molybdate anion, $[MoO_4]^{2-}$, is the sole molybdenum species in aqueous media (Cruywagen 2000; Cruywagen et al. 2002). Molybdenum compounds (e.g., molybdenum trioxide and polymolybdates) transform rapidly to the $[MoO_4]^{2-}$ ion under environmentally relevant test conditions (Deltombe et al. 1974; Greenwood and Earnshaw 1997). Protonated forms, such as $[HMoO_4]^-$ and H_2MoO_4, are found at pH <5 (Smedley and Kinniburgh 2017). Molybdenum tends to be more mobile under alkaline conditions, but adsorption increases with decreasing pH (Goldberg et al. 2002).

Information regarding the chemical identity of molybdenum and molybdenum compounds is provided in Table 4-1.

Table 4-1. Chemical Identity of Molybdenum and Compounds

Characteristic	Information		
Chemical name	Molybdenum[a]	Molybdenum disulfide[b]	Molybdenum trioxide[c]
Synonym(s) and registered trade names[d]	MChVL; TsM1; Amperit 105.054; Amperit 106.2; Metco 63	Molybdenite (natural mineral); molybdenum(IV) sulfide; DAG 325; Molykote	Molybdenum(VI) oxide; molybdic acid anhydride; molybdic anhydride; molybdic oxide
Chemical formula	Mo	MoS_2	MoO_3
CAS Registry Number	7439-98-7	1309-56-4/1317-33-5 (natural mineral form)[e]; 12612-50-9 (synthetic form)	1313-27-5
Chemical name	Sodium molybdate[f]	Ammonium dimolybdate	Ammonium heptamolybdate tetrahydrate[g]
Synonym(s) and registered trade names[d]	Disodium molybdate; molybdic acid, disodium salt[e]	Ammonium molybdenum oxide[e]	Ammonium paramolybdate tetrahydrate; hexammonium molybdate
Chemical formula	Na_2MoO_4	$(NH_4)_2Mo_2O_7$	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$
CAS Registry Number	7631-95-0	27546-07-2	12054-85-2/12027-67-7 (anhydrous)[h]
Chemical name	Diammonium molybdate	Ammonium tetrathiomolybdate	
Synonym(s) and registered trade names[d]	Ammonium molybdate; molybdic acid, diammonium salt[i]	Tiomolibdate diammonium; ammonium molybdenum sulfide; ammonium tetrathiomolybdate; thiomolybdic acid, diammonium salt; Coprexa; TM; ATTM[j]	
Chemical formula	$(NH_4)_2MoO_4$	$(NH_4)_2MoS_4$	
CAS Registry Number	13106-76-8	15060-55-6	

[a]All information in this column obtained from NLM (2020a), unless otherwise noted.
[b]All information in this column obtained from NLM (2020b), unless otherwise noted.
[c]All information in this column obtained from NLM (2020c), unless otherwise noted.
[d]Additional synonyms and trade names may be queried using the Common Chemistry service from Chemical Abstracts Service (http://www.commonchemistry.org/).
[e]EPA 2019a.
[f]All information in this column obtained from NLM (2020d), unless otherwise noted.
[g]All information in this column obtained from NLM (2020e), unless otherwise noted.
[h]OECD 2013.
[i]EPA 2019b.
[j]NLM 2019.

CAS = Chemical Abstracts Service

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Metallic molybdenum, in the form of fine molybdenum powder, is considered nonflammable.

Information regarding the physical and chemical properties of molybdenum and molybdenum compounds is provided in Table 4-2. Much of the information presented was obtained from the chapter, Molybdenum and Molybdenum Compounds, in the Ullmann's Encyclopedia of Industrial Chemistry (Sebenik et al. 2012), or handbooks such as the CRC Handbook of Chemistry and Physics or the European Chemicals Agency (ECHA) registration dossiers.

Table 4-2. Physical and Chemical Properties of Molybdenum and Compounds[a]

Property	Information		
Chemical name	Molybdenum	Molybdenite (natural mineral)/molybdenum disulfide	Molybdenum trioxide
Molecular weight	95.94	160.07	143.95
Color	Dull gray	Black	White, turns slightly blue in light
Physical state	Powder	Crystalline solid	Crystalline solid
Melting point	2,617°C	>1,600°C (rhombohedral crystal); did not melt at 1,800°C[b]	801°C
Boiling point	4,612°C	No data	1,155°C
Density/specific gravity	10.22 g/cm^3	5.05 g/cm^3	4.692 g/cm^3 (21°C)
Odor	No data	Odorless	Odorless[c]
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water at 25°C	Insoluble; 5.5–12 mg/L at 20°C and pH 3.5–4.3[d]	Insoluble	490 mg/L (28°C)
Organic solvents	No data	Insoluble	Insoluble
Inorganic solvents	Dissolved by a mixture of concentrated nitric and concentrated hydrofluoric acids	Dissolves only in strongly oxidizing acids (e.g., aqua regia)	Soluble in aqueous alkali and ammonia; 14,000 mg/L in nitric acid (4 mol/L, 20°C)
Partition coefficients:			
Log K_{ow}	NA	NA	NA
Log K_{oc}	NA	NA	NA
Vapor pressure[e]:			
at 20°C	No data	No data	No data
at 2,469°C	7.5x10^{-3} mm Hg		
at 2,721°C	7.5x10^{-2} mm Hg		
at 3,039°C	0.75 mm Hg		
at 3,434°C	7.5 mm Hg		
at 3,939°C	75 mm Hg		
at 4,606°C	750 mm Hg		
Henry's law constant at 25°C	NA	NA	NA
Autoignition temperature	NA	NA	NA
Flashpoint	NA	NA	NA
Flammability limits	Not flammable	Not flammable	Not flammable[c]
Explosive limits	NA	NA	NA
Conversion factors	NA	NA	NA

Table 4-2. Physical and Chemical Properties of Molybdenum and Compounds[a]

Property	Information		
Chemical name	Sodium molybdate	Ammonium dimolybdate	Ammonium heptamolybdate tetrahydrate
Molecular weight	205.92	339.95	1,235.8
Color	White		White
Physical state	Crystalline powder[f]	Solid, powder[g]	Crystalline solid[f]
Melting point	687°C[f]	Decomposes from ca. 150°C[g]	Decomposition at 90°C[h]
Boiling point	Not applicable	Decomposes from ca. 150°C[f]	Decomposition at 90°C[h]
Density/specific gravity	3.5 g/cm[h]	2.97 at 20°C[g]	2.86 (20°C)[f]
Odor	Odorless[f]	Odorless[f]	Odorless[f]
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	40 wt% (anhydrous salt in 100 g saturated solution, 25°C)	228.4 g/L (20°C, pH 6.8)[g]	206.5 g/L (20°C, tetrahydrate)[f]
Organic solvents	No data	No data	No data
Inorganic solvents	No data	No data	No data
Partition coefficients:			
Log K_{ow}	NA	NA	NA
Log K_{oc}	NA	NA	NA
Vapor pressure at 20°C	No data	No data	No data
Henry's law constant at 25°C	NA	NA	NA
Autoignition temperature	NA	No data	NA
Flashpoint	No data	No data	No data
Flammability limits	No data	Non flammable[g]	No data
Explosive limits	NA	NA	NA
Conversion factors	NA	NA	NA

Table 4-2. Physical and Chemical Properties of Molybdenum and Compounds[a]

Property	Information	
Chemical name	Diammonium molybdate	Ammonium tetrathiomolybdate
Molecular weight	196.01	260.28
Color	Colorless, white, or slightly greenish-yellowish[i]	Deep red[h]
Physical state	Crystalline solid[h]	Crystalline solid[h]
Melting point	No data	>300°C[j]
Boiling point	No data	No data
Density	1.4[i]	No data
Odor	Odorless[i]	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water	>10,000 mg/L[k]; 39 wt% (in 100 g saturated solution, 25°C)	Insoluble (hygroscopic)[l]
Organic solvents	No data	No data
Inorganic solvents	No data	No data
Partition coefficients:		
Log K_{ow}	NA	No data
Log K_{oc}	No data	No data
Vapor pressure at 25°C	No data	NA
Henry's law constant at 25°C	NA	No data
Autoignition temperature	NA	NA
Flashpoint	NA	NA
Flammability limits	Not flammable[i]	No data
Explosive limits	NA	NA
Conversion factors	NA	NA

[a]All information was obtained from Sebenik et al. (2012) unless otherwise noted.
[b]Cannon 1959.
[c]NOAA 2015.
[d]ECHA 2019a.
[e]Lide 2005.
[f]OECD 2013.
[g]ECHA 2019b.
[h]Haynes et al. 2014.
[i]NJDOH 2009.
[j]Sigma-Aldrich 2015.
[k]ECHA 2019c.
[l]Alfa Aesar 2015.

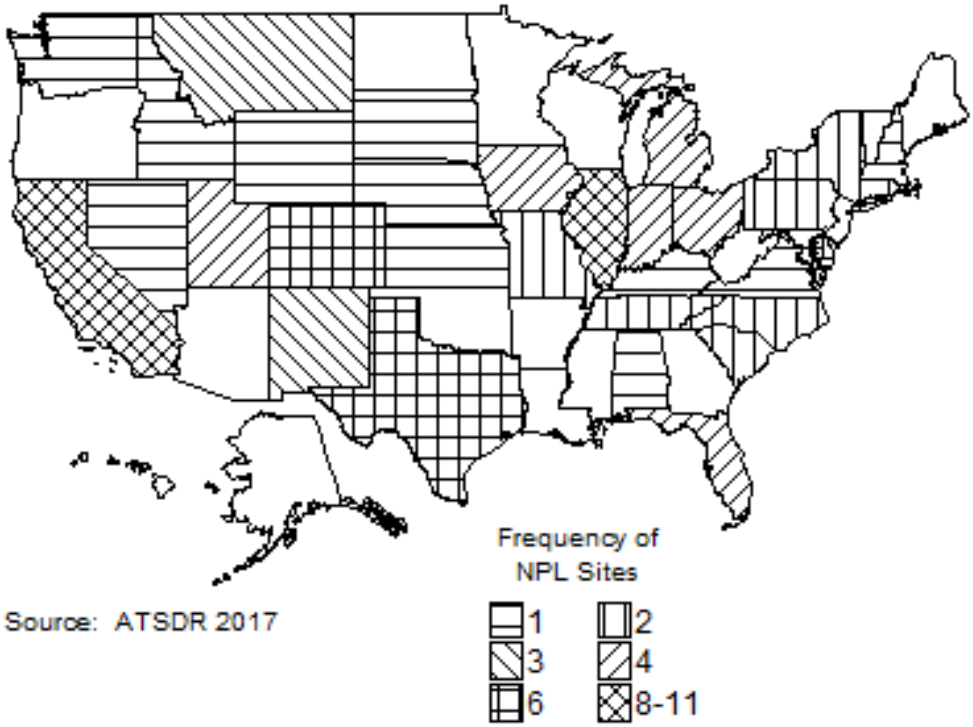
NA = not applicable

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Molybdenum has been identified in at least 86 of the 1,854 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2017). However, the number of sites in which molybdenum has been evaluated is not known. The number of sites in each state is shown in Figure 5-1. Of these sites, all are located within the United States.

Figure 5-1. Number of NPL Sites with Molybdenum Contamination



- The general population is primarily exposed to molybdenum through dietary intake.
- Inhalation exposure and ingestion of molybdenum from drinking water is typically low for the general population; however, water levels near mining operations may be higher and exposure may be greater for populations near these activities.
- Molybdenum compounds (e.g., molybdenum trioxide and polymolybdates) transform to the $[MoO_4]^{2-}$ ion under neutral or alkaline conditions; however, protonated forms, such as $[HMoO_4]^-$ and H_2MoO_4, are found at pH <5.

Molybdenum is a naturally occurring trace element found extensively in nature. Biologically, molybdenum plays an essential role as a micronutrient in plants and animals, including humans. It is also used widely in industry for metallurgical applications (EPA 1979). A radioactive isotope of molybdenum (^{99}Mo) is used as a source for producing metastable technetium-99 (^{99m}Tc), which is an important radiopharmaceutical that is used in the vast majority of high resolution medical imaging tests (Parma 2009). Important, naturally occurring molybdenum compounds are the minerals molybdenite, powellite, wulfenite, ferrimolybdite, and ilsemannite. When in the form of molybdate, a tetrahedral polyatomic anion, or other isopolyanions, it can be processed into salts used in industrial applications. The molybdate ion is the most common form of molybdenum found in the aqueous environment (EPA 1979).

If released to the atmosphere, molybdenum will be returned to earth by wet and dry deposition. In water, pH levels and oxidation/reduction conditions of the sediment govern the speciation of molybdenum and adsorption potential in natural aquifers. In the pH range of 3–5, molybdenum tends to exist as hydrogen molybdate and is adsorbed to sediment composed of clay and other oxic minerals (Fitzgerald et al. 2008). The adsorption and mobility of molybdenum in soils is also inversely correlated with pH. Adsorption of molybdenum to 36 surface and subsurface soils was maximized under acidic conditions (pH 2–5) and decreased rapidly at pH 5–8 (Goldberg et al. 2002). The availability of molybdenum to plants and vegetation is also affected by pH and soil properties. Since adsorption to soil decreases with increasing pH, it becomes more bioavailable for uptake to vegetation under nonacidic conditions.

Molybdenum is infrequently detected in ambient air, but is a natural constituent of water and soils. The earth's crust contains an average of 0.0001% (1 ppm) of molybdenum, and surface waters usually have molybdenum concentrations of <5 µg/L (EPA 1979). A decade-long study conducted by the U.S. Geological Survey (USGS) of >5,000 monitoring and drinking water wells from over 40 major aquifers in the United States reported a median molybdenum concentration of 1 µg/L (USGS 2011).

Anthropogenic activities such as mining operations may result in localized areas where molybdenum levels greatly exceed background levels.

The primary route of exposure for the general population to molybdenum is through the ingestion of food. NAS has estimated that the average dietary intakes (AVDIs) of molybdenum by adult men and women are 109 and 76 µg/day, respectively (NAS 2001). Other routes of exposure, such as inhalation of ambient air, ingestion of drinking water, and dermal exposure, are negligible for the general population; however, they may be important routes of exposure in certain occupational settings such as mining activities and

metallurgical applications where molybdenum is used. For example, molybdenum levels in air samples of two plants that produce molybdenum salts were 0.5–200 and 0.2–30 mg/m^3, depending upon the location of the sample and operation being performed (EPA 1979). Respirable dust samples contained molybdenum at levels of 0.471, 1.318, 0.142, and 0.318 mg/m^3 during mining, crushing, milling, and open pit operations, respectively, at a Colorado mine (EPA 1979).

The extensive nationwide use of radioactive ^{99}Mo in generators that produce ^{99m}Tc for nuclear medicine imaging scans can expose medical staff and the public in medical facilities to low levels of ionizing radiation. The extent of those exposures is limited by U.S. Nuclear Regulatory Commission (USNRC) and agreement state regulations (USNRC 2016a, 2016b).

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Molybdenum is a naturally occurring trace element that can be found extensively in nature. Biologically, it plays an important role as a micronutrient in plants and animals, including humans. It is also used widely in industry for metallurgical applications (USGS 2015a).

Molybdenum does not occur naturally in the pure metallic form, but is in minerals, principally as oxide or sulfide compounds (Barceloux 1999; EPA 1979). Important naturally occurring molybdenum compounds are the minerals molybdenite (MoS_2, the predominant source), powellite, wulfenite, ferrimolybdite, and ilsemannite. Molybdenum may also form molybdate, a tetrahedral poly atomic anion, or other isopolyanions, which can form salts used in industrial applications. The earth's crust contains an average of 0.0004% (4 ppm) of molybdenum (Sebenik et al. 2012). Deposits that are economically feasible for mining contain ≥200 ppm of molybdenum, with lower concentrations obtained as a byproduct of copper mining (EPA 1979).

Molybdenite (MoS_2) is the principal mineral from which molybdenum is obtained. Mining and milling of crude ore produce molybdenite concentrate containing ≥90% of MoS_2, almost all of which is converted to technical-grade molybdenum trioxide. Molybdenum trioxide is the base material for the production of a variety of chemical compounds, ferromolybdenum, and purified molybdenum (EPA 1979).

Roasting molybdenite concentrate in a multiple hearth furnace at temperatures up to 600°C produces technical-grade molybdenum trioxide. This can be further purified by sublimation or selective recrystallization at about 1,000–1,100°C (Sebenik et al. 2012).

Worldwide mine production of molybdenum was estimated to be 258,000 metric tons in 2013, with approximately 92% produced, in descending order, by China, the United States, Chile, Peru, Mexico, and Canada. The United States accounted for 24% of world production with 60,700 metric tons in 2013, down slightly from 61,500 metric tons in 2012. Primary molybdenum operations accounted for 53% of total U.S. molybdenum production, while byproduct production made up 47% of the total in 2013. All U.S. molybdenum concentrates and products are from the mining of ore (USGS 2015a). U.S. production of molybdenum increased roughly 8% in 2014 to 65,500 metric tons (USGS 2015b). U.S. production of molybdenum for 2018 was 41,900 metric tons (USGS 2019). The USGS Mineral Industry Survey for molybdenum reported that domestic production for the first 3 months of 2019 was 3,620 metric tons (January), 3,420 metric tons (February) and 3,650 metric tons (March) (USGS 2019).

Table 5-1 contains a list the number of facilities per state that produced, processed, or used molybdenum trioxide in 2017, as well as information on the amount of molybdenum trioxide on site and related activities and uses (TRI17 2018).

Table 5-1. Facilities that Produce, Process, or Use Molybdenum Trioxide

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	5	100	99,999	7, 10, 12
AR	2	100,000	999,999	7
AZ	3	100,000	9,999,999	1, 4, 7, 9
CA	16	1,000	9,999,999	1, 2, 3, 7, 9, 10, 12, 13
CO	1	100,000	999,999	1, 6, 12, 13
DE	1	0	99	12
GA	1	0	0	0
IA	3	1,000,000	9,999,999	1, 3, 4, 6, 9
ID	1	10,000	99,999	12
IL	8	10,000	999,999	1, 5, 6, 7, 10
IN	7	1,000	999,999	1, 5, 6, 7, 8, 9, 10
KS	4	0	999,999	2, 3, 8, 10
KY	5	10,000	999,999	1, 2, 3, 4, 6, 7, 10
LA	27	0	49,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13

Table 5-1. Facilities that Produce, Process, or Use Molybdenum Trioxide

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
MD	1	100,000	999,999	7
ME	1	10,000	99,999	1, 5, 6
MI	4	0	99,999	1, 5, 6, 7, 10
MN	3	10,000	9,999,999	1, 7, 9, 10, 11, 13
MS	2	10,000	999,999	1, 5, 7, 10
MT	3	10,000	999,999	1, 2, 3, 5, 10, 12, 13
ND	2	10,000	99,999	10
NE	1	10,000	99,999	10
NJ	2	100,000	999,999	10
NM	2	10,000	999,999	10
NV	1	10,000	99,999	12
OH	8	1,000	999,999	1, 6, 7, 8, 9, 11, 13
OK	4	10,000	999,999	1, 4, 5, 7, 10, 13
OR	1	10,000	99,999	7
PA	17	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12
TX	40	0	99,999,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12
UT	3	100	999,999	10, 11
WA	3	1,000	999,999	7, 10, 11, 12
WI	1	10,000	99,999	10, 11
WV	2	10,000	9,999,999	2, 3, 7, 10
WY	3	10,000	999,999	10

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI17 2018 (Data are from 2017)

Molybdenum-99 (^{99}Mo) is a radioactive form of molybdenum and the only molybdenum radioisotope of commercial importance. It is produced in nuclear reactors, and then processed, packaged, and shipped to medical facilities throughout the world, where the ^{99}Tc progeny into which it transforms is eluted and injected into patients for imaging purposes (e.g., cardiac stress tests).

^{99}Mo was produced in one of eight nuclear reactors (mainly at the Chalk River complex in Canada) using highly enriched uranium, and then commercialized at five processing facilities and six generator manufacturing facilities. The availability of those reactors was reduced by the closure of the Chalk River facility, and this impacted the supply stream. The United States has established a high national priority on assuring an adequate supply of ^{99}Mo and urged manufacturers to switch from using highly enriched uranium (HEU) to low enriched uranium (LEU) to reduce the use of HEU for civilian applications (Ballinger 2010; The White House 2012; USNRC 2015; Van Noorden 2013). At a NAS symposium in 2017, several companies discussed their plans to produce ^{99}Mo in the United States (NAS 2018).

Currently, ^{99}Mo can be produced by placing HEU or LEU targets in an operating nuclear reactor and allowing the neutron flux to produce ^{99}Mo and its radioactive precursors. The quantity of ^{99}Mo peaks after approximately 6 days, at which time, the target is removed, processed, and prepared for shipment. New facilities for producing ^{99}Mo from LEU in the United States are being planned (Welsh et al. 2015).

5.2.2 Import/Export

Molybdenum-containing exports rose from 49,900 metric tons in 2010 to 55,300 metric tons in 2014, while imports for consumption rose from 19,700 metric tons in 2010 to 23,600 metric tons in 2014 (USGS 2015b). Imports of molybdenum (excluding ore) were 22,190 metric tons in 2018 and exports totaled 44,700 metric tons (USGS 2019). These data, along with U.S. production volumes, are summarized in Table 5-2.

Table 5-2. Molybdenum U.S. Production, Import, and Export Data from 2010 to 2014 and 2018 in Metric Tons

	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2018[b]
Total U.S. production	59,400	63,700	61,500	60,700	65,500	41,900
U.S. imports for consumption	19,700	21,100	19,800	20,200	23,600	22,219[c]
U.S. exports for consumption	49,900	56,700	48,900	53,100	55,300	44,700[d]

[a]USGS 2015b.
[b]USGS 2019.
[c]Excludes imports of ore and concentrate.
[d]Includes ores and concentrates.

5.2.3 Use

Molybdenum is used primarily in metallurgical applications, including as an alloying agent in cast iron, steel, and superalloys to enhance properties such as hardenability, strength, toughness, and wear- and corrosion-resistance. Molybdenum is commonly used in combination with other alloy metals like chromium, cobalt, manganese, nickel, niobium, and tungsten. The leading form of molybdenum used by industry, particularly in stainless steel production, is molybdenum trioxide (USGS 2015a).

Molybdenum is used significantly as a refractory metal and molybdenum compounds in a variety of non-metallurgical chemical applications, such as catalysts, lubricants, and pigments. For example, MoS_2 is used along with cobalt during the desulfurization process of petroleum (Sebenik et al. 2012). Most molybdenum nitride catalysts are nitrogen deficient due to thermodynamically unfavorable conditions at atmospheric pressure; however, molybdenum nitride was recently produced in a high temperature and pressure environment by solid state ion exchange. Testing found its catalytic activity to be 3 times that of MoS_2 and its selectivity to hydrogenation to be 3 times that of MoS_2 for hydrodesulfurizing dibenzo-thiophene (Wang et al. 2015). As green technology is becoming more popular, molybdenum has become increasingly important in areas like biofuels, catalysts, ethanol, solar panels, and wind power (USGS 2015a).

A radioactive isotope of molybdenum, ^{99}Mo, is used as a source to produce the metastable radioisotope technetium-99m (^{99m}Tc), which is used in the vast majority of medical imaging tests performed today (Doll et al. 2014; Parma 2009; Richards 1989). It was estimated that 85% of all medical radioisotope procedures use ^{99m}Tc and that about 50,000 ^{99m}Tc-based diagnostic procedures are performed in the United States each day, resulting in about 13 million procedures annually (Parma 2009).

Molybdenum concentrate produced by U.S. mines is roasted, exported for conversion, or purified to lubricant-grade molybdenum disulfide. Purified MoS_2 is used directly as a solid or in coatings that are bonded onto the metal surface by burnishing, vapor deposition, or bonding processes that use binders, solvents, and mechanochemical procedures (Stiefel 2011).

Metallurgical applications accounted for about 87% of total molybdenum use in 2013. The principle non-metallurgical use was in catalysts, primarily catalysts used in petroleum refining. Molybdenum compounds are also used to produce pigments (USGS 2015a).

5.2.4 Disposal

Recycling is the most environmentally acceptable means of disposal for stable molybdenum (USGS 2015b). Because molybdenum is difficult to remove from waste water, it often is adsorbed to biosolids in municipal waste water treatment facilities. Biosolids are beneficial and are often used as fertilizer or compost for agricultural applications. In the United States, the land application ceiling limit for molybdenum in biosolids is 75 mg/kg (EPA 2018b).

A ^{99m}Tc generator containing a depleted uranium shield or sufficient residual ^{99}Mo radioactivity to be considered radioactive can be disposed of by shipping to an authorized licensee following USNRC agreement state requirements along with those of the Department of Transportation (USNRC 2015). If the ^{99}Mo is allowed to decay sufficiently (typically ≥10 half-lives) and the internal shield is lead or tungsten, then disposal should follow state and local requirements.

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

Molybdenum mining, milling, and smelting, along with its association with uranium mining and milling, copper mining and milling, shale oil production, oil refining, and coal-fired power plants, have resulted in major releases to the environment (EPA 1979).

5.3.1 Air

Estimated releases of 83,484 pounds (~37.87 metric tons) of molybdenum trioxide to the atmosphere from 188 domestic manufacturing and processing facilities in 2017, accounted for about 4.23% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2018). These releases are summarized in Table 5-3.

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use Molybdenum Trioxide[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	5	59	0	0	8,295	0	4,539	3,815	8,354
AR	2	105	0	0	0	4,398	105	4,398	4,503
AZ	3	7,400	0	0	14,011	5	7,400	14,016	21,416
CA	16	638	43	0	11,305	70,649	10,152	72,484	82,635
CO	1	26	0	0	0	0	26	0	26
DE	1	14	0	0	2	0	14	2	15
GA	1	0	0	0	0	0	0	0	0
IA	3	8,650	6,100	0	5,705	0	15,500	4,955	20,455
ID	1	3	0	0	22,265	0	22,268	0	22,268
IL	8	18,361	2,685	0	16,464	2,449	21,046	18,913	39,959
IN	7	394	12,002	0	420,655	3,091	73,394	362,748	436,142
KS	4	250	0	0	5	0	255	0	255
KY	5	297	0	0	1,071	21	307	1,082	1,390
LA	27	6,761	1,733	81,533	279,468	9,012	258,237	120,270	378,507
MD	1	500	6,500	0	250	0	7,250	0	7,250
ME	1	147	0	0	0	0	147	0	147
MI	4	5	44	0	0	0	49	0	49
MN	3	124	5	0	227	0	129	227	356
MS	2	85	740	0	3,100	0	825	3,100	3,925
MT	3	129	0	0	24	0	129	24	153
ND	2	3	0	0	46	0	3	46	49
NE	1	0	0	0	0	0	0	0	0
NJ	2	0	0	0	13,620	0	0	13,620	13,620
NM	2	3	0	0	0	0	3	0	3
NV	1	1	0	0	42,143	0	42,144	0	42,144
OH	8	281	2,029	50,320	547	83	50,606	2,654	53,259
OK	4	3,501	20	0	0	46,900	3,521	46,900	50,421

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use Molybdenum Trioxide[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
OR	1	17	0	0	1,266	0	1,153	130	1,283
PA	17	25,837	743	0	25,890	340	46,536	6,274	52,810
TX	40	9,616	6,224	54,360	659,999	0	593,414	136,784	730,199
UT	3	12	0	0	60	0	12	60	72
WA	3	260	0	0	7	0	260	7	267
WI	1	0	0	0	0	0	0	0	0
WV	2	0	0	0	0	0	0	0	0
WY	3	5	0	0	0	0	5	0	5
Total	188	83,484	38,868	186,213	1,526,424	136,948	1,159,428	812,509	1,971,937

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI17 2018 (Data are from 2017)

The primary source of molybdenum emissions to the atmosphere is coal combustion. In 1970, it was estimated that 550 metric tons of molybdenum were released via coal combustion in the United States, in comparison to 900 metric tons estimated from all air pollution sources (EPA 1979). A total of 909 metric tons of molybdenum can be emitted from a single 1,000 megawatt power plant per year (EPA 1979). Historical concentrations of molybdenum in fly ash from coal combustion were reported to range from 7 to 160 mg/kg (Barceloux 1999). Advances in sorbent and air pollution control technology such as fabric filters and electrostatic precipitators in power plants have resulted in a reduction of atmospheric emissions of molybdenum and other metals as compared to emissions from decades ago (Cho and Wu 2004; EPA 2009a). A report from the EPA, which compiled data on 73 coal combustion residues (CCR),

typically found molybdenum levels of 8–30 µg/g (mg/kg) in fly ash and scrubber sludges and about 1–10 µg/g (mg/kg) in gypsum (EPA 2009a). The study reported that no correlation was observed in molybdenum content and coal type or air pollution control system employed.

5.3.2 Water

Estimated releases of 38,868 pounds (~17.63 metric tons) of molybdenum trioxide to surface water from 188 domestic manufacturing and processing facilities in 2017, accounted for about 1.97% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2018). This estimate includes releases to waste water treatment and publicly owned treatment works (POTWs) (TRI17 2018). These releases are summarized in Table 5-3.

Per year, it has been estimated that natural processes result in the release of $3.6x10^{10}$ g of molybdenum into surface waters (EPA 1979).

Aqueous effluents from industries with a high presence of molybdenum, including molybdenum mining, milling, and smelting; uranium mining and milling; copper mining and milling; shale oil production; oil refining; and coal-fired power plants, contain molybdenum at concentrations ranging from 100 to 800,000 µg/L (EPA 1979). Molybdenum levels in leachate samples obtained from a landfill located in Caledonia, Wisconsin ranged from 1.28 to 16 µg/L (WDNR 2013).

Effluent concentrations of molybdenum from three molybdenum mining and milling operations (two in Colorado, one in New Mexico) ranged on the order of 1,000–10,000 µg/L. In 1972, a mine in Colorado released approximately 100,000 kg of molybdenum into a receiving stream. Releases of molybdenum from coal power plants to surface waters in the United States average about 1,800 metric tons/year. A uranium mill in Colorado reported leaking of the tailings ponds containing 860,000 µg/L molybdenum in 1965. Some uranium operations in New Mexico reported as much as 1,000 µg/L molybdenum in aqueous effluents. Copper milling operations have reported molybdenum effluent concentrations as high as 30,000 µg/L (EPA 1979).

Frasacoli and Hudson-Edwards (2018) compiled monitoring data on molybdenum levels in mining-affected areas in different parts of the world, which included groundwaters, nearby rivers, and tailing pore water. The largest levels of molybdenum were observed in mine waste from a coal mine located in Poland (2,332,000 µg/L). Groundwater from an area near 13 nonactive mines in Mexico ranged from

<5 to 150 µg/L. Tailing pore water from an inactive mining operation in Manitoba, Canada had molybdenum levels of <5–1,100 µg/L. Molybdenum concentrations from tailings channel water from an active copper mining facility in Chile ranged from 2,670 to 3,900 µg/L. Mine drainage samples obtained from an operational mine in Peru had molybdenum levels of 0.001–13.9 ppm (1–13,900 µg/L) (Skierszkan et al. 2016).

5.3.3 Soil

Estimated releases of 1,526,424 pounds (~692.37 metric tons) of molybdenum trioxide to soil from 188 domestic manufacturing and processing facilities in 2017, accounted for about 77.41% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2018). An additional 186,213 pounds (~84.46 metric tons), accounted for about 9.44% of the total environmental emissions, were released via underground injection (TRI17 2018). These releases are summarized in Table 5-3.

Metals, such as molybdenum, may leach into soil via municipal solid waste incineration bottom ash (IMOA 2015).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Molybdenum released to the air by industrial processes will be subject to atmospheric deposition (IMOA 2015). Deposition from the atmosphere is only a minor source to terrestrial and aquatic environments (Fitzgerald et al. 2008).

Water. Molybdenum can be leached into the aquatic environment near industrial use areas via direct release or atmospheric wet deposition by rain (IMOA 2015). The pH of water, along with the composition and redox conditions of the sediment, greatly affect the speciation and adsorption behavior of molybdenum in natural waterbodies. Molybdenum accumulation in the sediment phase is favored under conditions of low pH and in sediments with low redox potential and high iron and organic matter content (Fitzgerald et al. 2008). In more favorable reducing geochemical conditions, solid-phase iron and manganese oxyhydroxides tend to undergo dissolution, and sorbed molybdenum may be released back into the water phase.

Sediment and Soil. In a seasonally anoxic basin, the distribution of molybdenum in the pore water of sediments was relatively uniform. In a perennially oxic basin, however, there was a redistribution of molybdenum in the sediment-water interface subsequent to deposition. This was determined to be a consequence of adsorption of molybdenum to iron oxyhydroxides at a rate of 36 cm^3/molecule-second in the first 1–2 cm depth (IMOA 2015).

Geological uplift and atmospheric deposition result in the molybdenum enrichment of surface soils (IMOA 2015). Molybdenum concentrations are found to be the highest in the topsoil layer, due to strong binding to natural organic matter. Goldberg et al. (2002) studied the adsorption potential of molybdenum as a function of pH on 36 surface and subsurface soil samples from 27 soil series belonging to six different soil orders, which provided a wide range of soil physical-chemical characteristics such as organic carbon content, cation exchange capacity, and iron content. In general, maximum adsorption occurred under acidic pH conditions (pH 2–5) in which molybdenum adsorbed to oxyhydroxide mineral surfaces and sorption decreased rapidly from pH 5 to 8 and was minimal in all soils at pH >9. Skierszkan et al. (2016) studied the stable isotopic composition of molybdenum and zinc in mine wastes and noted a large variation in δ^{98}Mo (a measure of how the isotopic composition in the liquid or solid waste differed from a National Institute of Standards and Technology [NIST] standard solution) as a function of adsorption. At lower pH, adsorption of molybdenum is greatest, and the molybdenum isotope profile shifts toward heavier isotopic composition, as the adsorption process preferentially removes lighter isotopes. In contrast, zinc has the opposite behavior as it is more mobile under acidic conditions and adsorption is enhanced under alkaline conditions with lighter zinc isotopes more prominent. These results suggest the possibility of using isotopic composition as a method to understand attenuation mechanisms such as adsorption and molybdate precipitation during the weathering process.

Other Media. As reviewed by Regoli et al. (2012), the bioaccumulation factor (BAF) ranged from 30.1 to 71.6 (average of 49) in fish exposed to molybdenum levels <65 ug/L. At higher molybdenum levels (up to 766 µg/L), the BAF ranged from 0.4 to 9.9 (average 1.4). A laboratory study in rainbow trout found a similar inverse relationship between molybdenum concentration in the water and bioconcentration factor (BCF) (Regoli et al. 2012). A 60-day exposure to 880 µg/L resulted in tissue levels below the limit of detection. Exposure to 11,100 µg/L for 28 days resulted in whole-body molybdenum levels of 0.53 mg/kg fish; the calculated average BCF was 0.05. In another study, fish in a creek near a molybdenum tailings pile had measured BCFs of <100 after a 2-week exposure (CCME 1999). The accumulation data show that the BAF decreases with increasing molybdenum levels. At low

molybdenum concentrations, there is an active accumulation of essential metals in organisms (and often non-essential metals via the same uptake mechanisms) to ensure that metabolic requirements are met. This active uptake process decreases when organisms are exposed to higher metal concentrations. At higher concentrations, organisms with active regulation mechanisms are even limiting their uptake by excretion of excess metals. EPA published a framework for metals risk assessment that discusses the difference in interpreting BCF and BAF values for organic versus inorganic compounds (EPA 2017a). It was generally concluded that the most recent scientific data on bioaccumulation do not currently support the use of BCF and BAF values when applied as generic threshold criteria when assessing the hazardous potential of metals. Moreover, single-value BCF/BAF data are most applicable to site-specific assessments; for more general regional or national assessments, the media chemistry and metal concentrations for a particular species should be considered for BCF/BAF studies.

5.4.2 Transformation and Degradation

As a naturally occurring trace element, molybdenum can be found extensively in nature. The predominant form of molybdenum in natural waters is as the molybdate anion, $[MoO_4]^{2-}$ (Barceloux 1999), while naturally occurring molybdenum salts are the dominant form in dry environments (EPA 1979).

Air. No information regarding the chemical forms of molybdenum in the atmosphere and their transformations could be located. It is generally assumed that metals, especially those from combustion sources, exist in the atmosphere as oxides since metallic species are readily attacked by atmospheric oxidants.

Water. The speciation of molybdenum in aqueous media as a function of pH and molybdenum concentration, has been thoroughly investigated and reported upon in open literature. As discussed in Chapter 4, at pH >6.5, the sole molybdenum species is the molybdate anion, $[MoO_4]^{2-}$ (Cruywagen 2000; Cruywagen et al. 2002). Molybdenum compounds transform rapidly into the $[MoO_4]^{2-}$ ion under environmentally relevant conditions (Greenwood and Earnshaw 1997). In low redox environments, the molybdate anion can be reduced to molybdenum disulfide or molybdenite (Fitzgerald et al. 2008).

Sediment and Soil. Molybdenum is found naturally in soil as the minerals molybdenite, powellite, wulfenite, ferrimolybdite, and ilsemannite (EPA 1979; Fitzgerald et al. 2008).

The predominant form of molybdenum in wet soil is as the molybdate anion, $[MoO_4]^{2-}$ (Barceloux 1999).

Other Media. No data for the degradation of molybdenum in other media were located.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to molybdenum depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of molybdenum in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on molybdenum levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-4 shows the typical limits of detection that are achieved by commonly employed analytical methods in environmental media. Smedley and Kinniburgh (2017) compiled a list of ranges for molybdenum in environmental matrices from primary references, including analytical detection limits. The American Public Health Association publishes analytical methods for molybdenum and other metals in aqueous samples and the EPA publishes laboratory analytical methods and procedures to test for analytes in air, water, solids, and hazardous waste. An overview summary of the range of concentrations detected in environmental media is also presented in Table 5-5.

Table 5-4. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.48 ng/m^3	EPA 1999 (Method IO-3.3)
Drinking water	0.3 µg/L	EPA 1994 (Method 200.8)
Surface water and groundwater	0.3 µg/L; 8 µg/L	APHA 1989 (Method 3120B); EPA 1994 (Method 200.8)
Soil	0.000090–0.0023 mg/kg	Campillo et al 2002
Sediment	0.000090–0.0023 mg/kg	Campillo et al. 2002
Whole blood	~0.1 ng/mL (µg/L)	Keyes and Turnland (2002)

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-5. Summary of Environmental Levels of Molybdenum

Media	Low	High	For more information
Outdoor air (µg/m^3)	0.2	8.05	Section 5.5.1
Surface water (µg/L)	<1	157	Section 5.5.2
Groundwater (µg/L)	1	4,700	Section 5.5.2
Drinking water (µg/L)	<1	>40	Section 5.5.2
Food (ppb)	<1	1,800	Section 5.6
Soil (mg/kg)	<0.05	94.7	Section 5.5.3

Detections of molybdenum in air, water, and soil at NPL sites are summarized in Table 5-6.

Table 5-6. Molybdenum Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (µg/L)	340	229	14.4	16	10
Soil (mg/kg)	57	56.2	0.00794	7	6
Air (µg/m$^{3)}$	0.0655	0.0515	2.30	4	2

[a]Concentrations found in ATSDR site documents from 1981 to 2017 for 1,854 NPL sites (ATSDR 2017). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

Molybdenum concentrations in ambient air have been reported to range from below detection limits to 0.03 mg/m^3 (EPA 1979). Concentrations of molybdenum in ambient air of urban areas, 0.01–0.03 µg/m^3, are higher than those found in rural areas, 0.001–0.0032 µg/m^3 (Barceloux 1999). Data from the EPA Air Quality System Database reported 24-hour concentrations of molybdenum at several locations in the United States for 2018 (EPA 2018c). These data are summarized in Table 5-7.

Table 5-7. 24-Hour Molybdenum Concentrations (µg/m^3) in Air Samples (2018 Data)

State (sample type)	Arithmetic mean	99th percentile	75th percentile	50th Percentile	10th Percentile
California (TSP)	0.0007	0.0015	0.0007	0.0007	0.0007
California (TSP)	0.0008	0.0019	0.0007	0.0007	0.0007
California (TSP)	0.0009	0.0016	0.0015	0.0007	0.0007
California (TSP)	0.0015	0.0053	0.0019	0.0007	0.0007
California (TSP)	0.0010	0.0023	0.0015	0.0007	0.0007
California (TSP)	0.0030	0.0063	0.0042	0.0028	0.0007
California (TSP)	0.0037	0.0068	0.0047	0.0038	0.0007
California (TSP)	0.0008	0.0016	0.0007	0.0007	0.0007
California (TSP)	0.0009	0.0023	0.0007	0.0007	0.0007
California (TSP)	0.0008	0.0019	0.0007	0.0007	0.0007
California (TSP)	0.0008	0.0021	0.0007	0.0007	0.0007
California (TSP)	0.0013	0.0042	0.0015	0.0007	0.0007
California (TSP)	0.0010	0.0026	0.0015	0.0007	0.0007
California (TSP)	0.0010	0.0025	0.0015	0.0007	0.0007
California (TSP)	0.0015	0.0046	0.0019	0.0007	0.0007
California (TSP)	0.0007	0.0014	0.0007	0.0007	0.0007
California (PM_{10})	2.0000	2.0000	2.0000	2.0000	2.0000
California (PM_{10})	2.0000	2.0000	2.0000	2.0000	2.0000
California (PM_{10})	0.0002	0.0020	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0003	0.0030	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0001	0.0030	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0003	0.0030	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0003	0.0030	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0003	0.0020	0.0000	0.0000	0.0000
California ($PM_{2.5}$)	0.0013	0.0043	0.0017	0.0010	0.0004
Michigan (TSP)	0.0011	0.0026	0.0016	0.0010	0.0002
Michigan (TSP)	3.0787	49.5000	1.4000	0.9000	0.5000
Michigan (PM_{10})	0.9935	2.2000	1.5000	0.9000	0.4000
Michigan (PM_{10})	1.0057	2.6000	1.4000	0.8000	0.4000
Michigan (PM_{10})	1.1778	2.4000	1.5000	1.1000	0.4000
Texas ($PM_{2.5}$)	0.0005	0.0040	0.0010	0.0000	0.0000

Table 5-7. 24-Hour Molybdenum Concentrations (µg/m^3) in Air Samples (2018 Data)

State (sample type)	Arithmetic mean	99th percentile	75th percentile	50th Percentile	10th Percentile
Texas ($PM_{2.5}$)	0.0006	0.0030	0.0010	0.0000	0.0000
Texas ($PM_{2.5}$)	0.0006	0.0020	0.0010	0.0000	0.0000
Vermont ($PM_{2.5}$)	0.0726	0.4700	0.1100	0.0400	0.0100
Vermont (PM_{10})	0.0655	0.2700	0.1100	0.0400	0.0000
Vermont (PM_{10})	0.1955	0.3800	0.2700	0.1500	0.0900
Vermont (PM_{10})	0.1787	0.5900	0.3000	0.1000	0.0400

PM_{10} = particulate matter ≤10 µm in diameter; $PM_{2.5}$ = particulate matter ≤2.5 µm in diameter; TSP = total suspended particulate

Source: EPA 2018c

5.5.2 Water

It has been reported that concentrations of molybdenum are generally <1.0 µg/L in surface waters (USGS 2006) and 1.0 µg/L in drinking water (USGS 2011). Groundwaters contain about 1.0 µg/L (USGS 2011). Smedley and Kinniburgh (2017) compiled ranges of molybdenum levels in rain water, stream water, rivers, lakes, estuaries, and oceans. Most surface water levels were <1 µg/L; however, there was wide variability, with levels tending to be higher with increasing salinity of the body of water (for example, molybdenum levels in the Salton Sea, California were reported as high as 37 µg/L).

A USGS study of surface water from 51 of the nation's major river basins was conducted from 1991 to 2002 (USGS 2006). The median concentration of molybdenum in 2,773 surface water samples was <1.0 µg/L, with a maximum concentration of 157 µg/L. There were eight samples (approximately 0.29% of the total) that exceeded the health-based screening level of 40 µg/L for molybdenum.

In a study of surface waters collected from 197 sampling stations in Colorado, molybdenum was found at concentrations <10 µg/L in 87% of the 299 samples. Samples that contained concentrations >5 µg/L were concluded to be the result of proximity to mineralization or mining and milling operations (EPA 1979). However, another study comparing surface waters draining highly mineralized areas to those with baseline molybdenum areas found that molybdenum mineralization did not contribute significantly to

concentrations in surface waters. The waters from streams draining the highly mineralized areas rarely had molybdenum concentrations above 1–2 µg/L (EPA 1979).

Huang et al. (2010) discussed surface water concentrations of metals including molybdenum in the Gyama Valley, an area impacted by four metal mining operations. Molybdenum concentrations ranged from <0.6 (detection limit) to 10.4 µg/L in the Gyamaxung-chu stream/river.

DOI (1967) collected river and lake water samples from 100 sampling stations around the United States from 1962 to 1967. The samples were taken from areas susceptible to contamination, including highly populated areas, industrial areas, recreational use areas, and state and national boundaries. Molybdenum was detected in the water samples at maximum concentrations >100 µg/L at 38 of the sample sites, while 26 sites had mean molybdenum concentrations >50 µg/L.

Molybdenum levels of 9.3–10.4 µg/L for open oxic seawater and 0.67–3.74 µg/L in euxinic waters of the Black Sea were reported (Smedley and Kinniburgh 2017). Kulathilake and Chatt (1980) reported the molybdenum concentration in the Atlantic Ocean as 7.2–7.9 µg/L. Another study reported that the molybdenum concentration in the North Atlantic ranged from 0.5 to 1.0 µg/L (Chan and Riley 1966). In the Pacific Ocean, measured molybdenum concentrations included 8.8 µg/L in the Eastern Pacific (Kiriyama and Kuroda 1984) and 1.5 µg/L in the Western Pacific (Nakata et al. 1983). Kawabuchi and Kuroda (1969) reported a mean molybdenum concentration of 7.7 µg/L in Tokyo Bay. Molybdenum concentrations measured in the English Channel ranged from 12 to 16 µg/L (Chan and Riley 1966), while the Irish Sea was reported to have a mean molybdenum concentration of 8.4 µg/L (Riley and Taylor 1968).

A comprehensive groundwater monitoring study conducted from 1992 to 2003 by the USGS of 5,183 monitoring and drinking-water wells representative of over 40 principal aquifers in humid and dry regions and in various land-use settings reported that the median concentration of molybdenum in 3,063 samples was 1.0 µg/L, with a maximum value of 4,700 µg/L (USGS 2011). Approximately 1.5% of the groundwater samples had molybdenum levels exceeding the health-based screening level of 40 µg/L (USGS 2011). Levels of molybdenum tended to be greatest in glacial unconsolidated sand and gravel aquifers as compared to other major aquifer groups in the study.

A report issued by the Wisconsin Department of Natural Resources found elevated levels of molybdenum in private supply wells and groundwater monitoring wells near the We Energies Oak Creek power plant

located in Caledonia, Wisconsin (WDNR 2013). Molybdenum levels in 21 private well samples exceeded the state of Wisconsin groundwater enforcement standard of 40 µg/L. It was not determined whether the elevated levels of molybdenum were naturally occurring or were a consequence of the activities of the power plant and the coal ash fill areas located nearby the plant.

In January of 2017, the EPA published the final results of the third Unregulated Contaminant Monitoring Rule (UCMR 3) program. Molybdenum levels >1 µg/L were measured in 25,377 out of 62,981 analyzed drinking water samples, and 151 samples had levels greater than the health-based screening level of 40 µg/L. In 40 of the 4,922 public water systems tested, at least one measurable level above 40 µg/L was found (EPA 2017b). Concentrations as high as 1,400 µg/L have been detected in drinking waters in areas impacted by mining and milling operations (USGS 2011).

In a study of finished drinking water supplies from the 100 largest cities in the United States in 1964, median and maximum molybdenum concentrations of 1.4 and 68 µg/L, respectively, were reported (USGS 1964). Another study reported a mean molybdenum concentration of 8 µg/L in samples collected from 161 drinking water sources from 44 states in the United States (Hadjimarkos 1967). Molybdenum levels measured onsite at 12 public water facilities across England and Wales ranged from below the detection limit (0.03 µg/L) to 1.51 µg/L over an 18-month collection period (Smedley et al. 2014). Corresponding molybdenum levels in tap water from 24 residences in three towns (North Wales, the English Midlands, and South East England) served by these public water facilities ranged from <0.03 to 1.00 µg/L. The study indicated that there was little variability in molybdenum concentrations when comparing levels in tap water versus respective water supply facilities, construction ages of the residences (i.e., new homes versus older homes), and pre-flush versus post-flush tap samples, suggesting that water distribution pipework has a negligible effect on supplied tap water levels of molybdenum.

Drinking water may also be affected by industrial contamination, as water treatment facilities are ineffective at removing molybdenum from source waters. In tap waters samples collected in 1971 from Golden, Colorado, a community that derives its water supply from a stream draining a molybdenum mine and mill, the mean molybdenum concentration was reported to be 440 µg/L. However, after the mine closed in 1974, the mean concentration in drinking water samples decreased to 150 µg/L by January 1975, 60 µg/L by June 1975, and 30 µg/L by 1977 (EPA 1979).

5.5.3 Sediment and Soil

Globally, most soils contain molybdenum at concentrations between 0.6 and 3.5 mg/kg, although total concentrations in soils can vary widely depending on geological composition, soil horizon, or industrial contamination. Statistical analysis of 4,841 samples of soil collected from a depth of 0–5 cm in the conterminous United States showed molybdenum levels ranging from <0.05 to 75.7 mg/kg (USGS 2014). The 5th, 25th, 50th, 75th, and 95th percentile concentrations were 0.24, 0.51, 0.78, 1.14, and 2.27 mg/kg, respectively (USGS 2014). From 4,780 samples of C horizon (substratum) soils in the United States, the molybdenum levels were reported as ranging from <0.05 to 94.7 mg/kg and the 5th, 25th, 50th, 75th, and 95th percentile concentrations were 0.20, 0.51, 0.83, 1.27, and 2.88 mg/kg, respectively (USGS 2014). A review of 25,673 deep soil samples from the British Geological Survey reported molybdenum concentrations of <0.6–885 mg/kg, with a median value of 1.4 mg/kg (Smedley and Kinniburgh 2017). The Forum of European Geological Surveys (FOREGS), under the International Union of Geological Sciences/International Association of Geochemistry (IUGS/IAGC) Global Geochemical Baselines Programme, collected 840 topsoil samples from 26 European countries and reported molybdenum concentrations ranging from <0.1 to 21.3 mg/kg (mean 0.943 mg/kg) (FOREGS 2005).

Above average molybdenum soil concentrations may occur in areas containing molybdenum-rich rock formations or in areas of industrial contamination. Natural sources sampled, including soils covering a mineralized area, soil derived from a marine black shale, alluvial soils on the eastern footslopes of Sierra Nevada, and soils formed from volcanic ash in Kauai, Hawaii, contained mean molybdenum concentrations of 76, 12, 17.4, and 14.9 mg/kg, respectively. Soils sampled near industrial contamination, such as soils downstream from a molybdenum mine and mill in Colorado, soil irrigated with water contaminated by a uranium mill, and soils 2 miles from a molybdenum smelter in Pennsylvania, had mean molybdenum concentrations of 59, 61, and 29 mg/kg, respectively (EPA 1979).

Typical molybdenum concentrations found in stream sediments were reported to range from 1 to 5 mg/kg (EPA 1979). Sediments in streams that drain water from natural deposits of molybdenum in the United States have been reported to have molybdenum concentrations ranging from 10 to 200 ppm (10–200 mg/kg). Another study reported molybdenum levels of up to 300 mg/kg in sediments derived from black marine shales in England. Stream sediment collected from water below a molybdenum mine and mill in Colorado had molybdenum concentrations ranging from 50 to 1,800 mg/kg (mean of 530 mg/kg). Molybdenum content in stream sediments have been shown to reflect mineralization, as the concentration increases with decreasing sediment grain size (EPA 1979). FOREGS collected 848 freshwater sediment

samples from 26 European countries and reported molybdenum concentrations ranging from 0.12 to 117 mg/kg (mean 1.34 mg/kg) (FOREGS 2005). An analysis of 65,477 stream sediments in the British Geological Survey G-Base reported a range of molybdenum concentrations of <0.1–309 mg/kg, with a median of 0.4 mg/kg (Smedley and Kinniburgh 2017). Sediment samples collected from river/streams in Tibet close in proximity to mining operations had molybdenum levels of 9.1–20.8 mg/kg (Huang et al. 2010).

5.5.4 Other Media

In a study detecting and comparing trace elements in the milk of guinea pigs (n=87), dairy cattle (n=48), horses (n=35), and humans (n=84), the average molybdenum concentrations measured were 26, 22, 16, and 17 µg/L, respectively (Anderson 1992). Average concentrations of molybdenum detected in six kinds of milk, including cow's milk-based formula, breast milk, soya milk, bottled milk, dried milk, and evaporated milk, were 18, 4, 160, 34, 35, and 29 µg/L, respectively (Biego et al. 1998). Most of the molybdenum is in the cream fraction (Archibald 1951).

Food derived from aboveground plants, such as legumes, leafy vegetables, and cauliflower generally has a relatively higher concentration of molybdenum in comparison to food from tubers or animals. Beans, cereal grains, leafy vegetables, legumes, liver, and milk are reported as the richest sources of molybdenum in the average diet (Barceloux 1999).

Typical concentrations of molybdenum in plants are 1–2 mg/kg; however, a range of tenths to hundreds of mg/kg have been reported (EPA 1979). Tobacco contains molybdenum concentrations of 0.3–1.76 mg/kg (Barceloux 1999).

5.6 GENERAL POPULATION EXPOSURE

Molybdenum exposure to the general population via ambient air and drinking water is expected to be negligible compared with exposure through food (Barceloux 1999). Molybdenum does not occur naturally in the pure metallic form. It is principally found as oxide or sulfide compounds (Barceloux 1999; EPA 1979). Therefore, almost all exposure is to a molybdenum compound rather than the metal alone. The average dietary intake of molybdenum in the United States by adult men and women are 109 and 76 µg/day, respectively (NAS 2001). A study of the dietary intake of adult residents in Denver, Colorado reported a mean molybdenum ingestion rate of 180 µg/day (range 120–240 µg/day) (Barceloux

1999). Daily intakes ranged from 74 to 126 µg molybdenum in a study of older children and adults in the northeastern United States (Barceloux 1999).

The European Food Safety Authority (EFSA) used dietary intake studies to derive estimates of which foods were most responsible for molybdenum intake in European populations (EFSA 2013). Cereals and cereal-based products (including bread) are the largest contributors to molybdenum intake in a Western diet; these products contribute one-third to one-half of the total molybdenum intake. Other contributors to total molybdenum intake include dairy products and vegetables.

A summary of molybdenum concentrations positively identified in foods analyzed during the FDA Total Diet Study (TDS) of 2006–2011 and 2013–2014 is summarized in Table 5-8 (FDA 2017). The data for molybdenum arose from Market Basket Surveys conducted in 2010 and 2011 and 2013–2014, in which 382 store-bought foods purchased in four geographic regions of the United States (northeast, southeast, central, and west) were analyzed. Only those food items in which the molybdenum content of at least one sample was above the detection limit of the analytical method are reported. Another survey of levels of molybdenum in food found the highest molybdenum concentrations in legumes; grains and grain products; nuts; meat, fish, and poultry (including liver); eggs; and milk, yogurt, and cheese (76.7, 30.0, 29.5, 8.9, 6.3, and 4.6 µg/100 g, respectively) (Pennington and Jones 1987).

Table 5-8. Molybdenum Levels Detected in Foods in the 2006–2011 and 2013–2014 Market Basket Surveys[a]

Food	Number of samples	Positive detections	Mean (mg/kg)	Median (mg/kg)	Maximum (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
Liver (beef/calf), pan-cooked with oil	8	8	1.500	1.400	1.700	0.700	3.000
Pinto beans, dry, boiled	8	8	1.300	1.270	1.600	0.700	3.000
Pork and beans, canned	8	1	0.088	0	0.700	0.700	3.000
Peanut butter, smooth/creamy	8	3	0.508	0	1.900	0.900	3.000
Shredded wheat cereal	8	5	0.554	0.883	0.984	0.700	3.000
Raisin bran cereal	8	1	0.088	0	0.701	0.700	3.000
Crisped rice cereal	8	8	0.898	0.837	11.300	0.700	3.000
Granola with raisins	8	6	0.589	0.772	0.815	0.700	3.000
Oat ring cereal	8	8	1.300	1.300	1.400	0.700	3.000
Collards, fresh/frozen, boiled	8	2	0.262	0	1.600	0.500	2.000
Chili con carne with beans, canned	8	2	0.179	0	0.730	0.700	3.000
Refried beans, canned	8	2	0.254	0	1.100	0.800	3.000

Table 5-8. Molybdenum Levels Detected in Foods in the 2006–2011 and 2013–2014 Market Basket Surveys[a]

Food	Number of samples	Positive detections	Mean (mg/kg)	Median (mg/kg)	Maximum (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
White beans, dry, boiled	8	8	1.100	1.116	1.800	0.700	3.000
Granola bar, with raisins	8	1	0.164	0	1.300	0.800	3.000
Candy bar, chocolate, nougat, and nuts	8	1	0.115	0	0.922	0.800	3.000

[a]Trace values were defined as results ≥LOD and <LOQ. Results ≥LOD and <LOQ (trace values) were used as reported when calculating the means.

LOD = limit of detection; LOQ = limit of quantification

Source: FDA 2017 (Data were initially released in 2014 and revised April 2017.)

Molybdenum is an essential dietary element and is often included in nutritional supplements. Based on data from NHANES, the median molybdenum intake from dietary supplements was about 23 and 24 µg/day for men and women who reported supplement use, respectively. Dietary supplements generally contain molybdenum in the form of sodium molybdate or ammonium molybdate (Momcilovic 1999; NAS 2001), although the molybdenum can also be in the form of molybdenum chloride, molybdenum glycinate, and molybdenum amino acid chelate (NIH 2019).

It was reported in 1979 that in the United States, the average human intake of molybdenum via drinking water was <5 µg/day (EPA 1979). Drinking water coming from sources close to areas with high molybdenum contamination from industrial effluents may contain a higher concentration of molybdenum (>50 µg/L) (EPA 1979).

Urinary levels of molybdenum were measured for the U.S. population from NHANES studies from 1999 to 2016 (CDC 2019) and are summarized in Table 5-9.

Table 5-9. Urinary Molybdenum Levels in U.S. Adults

Survey years	Geometric mean	50th percentile	95th percentile	Sample size
		Urinary molybdenum (µg/L)[a]		
1999–2000	41.7 (36.7–47.4)	46.6 (40.5–52.5)	168 (143–206)	1,299
2001–2002	41.1 (38.3–44.1)	47.6 (43.7–51.2)	150 (130–166)	1,560
2003–2004	35.9 (34.0–38.0)	40.3 (37.6–42.1)	133 (119–144)	1,543
2005–2006	41.3 (38.7–44.0)	46.0 (41.7–49.6)	153 (135–171)	1,520
2007–2008	40.8 (38.7–43.0)	44.5 (42.2–47.8)	152 (145–164)	1,857

Table 5-9. Urinary Molybdenum Levels in U.S. Adults

Survey years	Geometric mean	50th percentile	95th percentile	Sample size
2009–2010	39.6 (37.5–41.8)	42.0 (39.8–43.9)	144 (130–163)	2,019
2011–2012	34.1 (31.8–36.5)	37.3 (33.6–39.8)	136 (120–146)	1,715
2013–2014	30.8 (28.58–33.3)	32.7 (28.3–36.0)	129 (116–137)	1,811
2015–2016	32.0 (29.9–34.1)	35.9 (33.1–37.8)	124 (112–136)	1,793
Creatinine corrected urinary molybdenum (µg/g creatinine)				
1999–2000	39.6 (36.9–42.6)	38.5 (36.1–41.0)	122 (116–147)	1,299
2001–2002	39.3 (36.8–42.0)	39.6 (36.4–42.1)	123 (109–139)	1,559
2003–2004	36.9 (35.0–38.9)	37.0 (35.7–38.4)	118 (101–134)	1,543
2005–2006	41.2 (39.3–43.1)	40.5 (38.8–42.7)	119 (103–132)	1,520
2007–2008	43.5 (42.1–44.9)	42.9 (41.3–44.7)	122 (110–132)	1,857
2009–2010	41.9 (40.0–43.9)	41.2 (39.4–43.0)	127 (115–141)	2,019
2011–2012	38.6 (37.5–42.2)	40.0 (36.0–43.6)	118 (108–131)	1,261
2013–2014	35.9 (33.7–38.2)	36.9 (35.1–38.4)	97.8 (88.5–111)	1,810
2015–2016	34.9 (33.3–36.6)	36.3 (34.4–38.1)	97.4 (85.5–102)	1,791

[a]Limit of detection for survey years 1999–2001, 2001–2002, 2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016 were 0.8, 0.8, 1.5, 0.92, 0.92, 0.99, 0.8, and 0.8 µg/L, respectively.

Source: CDC 2019

Paschal et al. (1998) analyzed the levels of molybdenum and 12 other metals in the urine of 496 residents of the United States obtained from the NHANES III survey conducted from 1988 to 1994. The specimens randomly selected were from a broad spectrum of the population (e.g., both urban and rural communities, both males and females, and persons aged 6–88 years from all major ethnicities). The geometric mean molybdenum concentration of the samples was 46.8 µg/L and the 25th, 50th, 75th, and 95th percentiles were 27.9, 56.5, 93.9, and 168.0, µg/L, respectively. The creatinine-adjusted 25th, 50th, 75th, and 95th percentiles were 30.9, 45.7, 64.3, and 133.8 µg/g, respectively, with a geometric mean of 39.6 µg/g.

Molybdenum levels in whole blood are typically <5 ng/mL in the general population; however, blood samples from persons from areas with natural molybdenum deposits or from molybdenum mining areas may have concentrations of up to 150 µg/mL (Barceloux 1999).

Blood samples collected from 18 miners at a molybdenum mine in New Mexico had plasma molybdenum levels <5 µg/L in 12 of the 18 samples and 6–18 µg/L in the remaining 6 samples. The concentration of molybdenum in urine collected from 11 of the miners ranged from 20 to 74 µg/L. It was noted that

molybdenum levels in urine and blood of miners mainly exposed to molybdenite may not be above average, since molybdenite is a relatively insoluble compound (EPA 1979).

In a survey of a molybdenite mining, crushing, and milling operation in Colorado, mean molybdenum levels in respirable dust samples were 0.471, 1.318, 0.142, and 0.318 mg/m^3 during mining, crushing, milling, and open pit operations, respectively (EPA 1979). In settled dust and air samples collected from a molybdenum smelting operation, concentrations of molybdenum, in the form of molybdenum trioxide, were 57–61% and 3–33 mg/m^3, respectively (EPA 1979). Forty air samples collected above a crucible in a molybdenum trioxide smelting plant contained a mean molybdenum concentration of 0.22 mg/m^3, while air samples collected in the breathing zone of workers had molybdenum concentrations ranging from 1.4 to 5.4 mg/m^3 (EPA 1979). The air concentrations of molybdenum in two plants that produce molybdenum salts were 0.5–200 and 0.2–30 mg/m^3 (EPA 1979). More recent monitoring data for mining and milling operations were not located; current levels may be lower due to possible changes in occupational standards, engineering and administrative controls, and personal protective equipment requirements.

Workers involved in metal refining and metal working may be exposed to airborne particulates containing molybdenum. In a study assessing the exposure of a group of 20 workers performing welding, polishing, and assembly of stainless steel constructions, molybdenum was detected in personal air samplers at concentrations of 0.27–9.7, 0.03–4.2, and 0.14–0.60 µg/m^3, respectively. Stationary air samplers in the facility detected course (equivalent aerodynamic diameter [EAD] 2–10 µm) and fine (EAD <2 µm) molybdenum particles at concentrations of 0.015–0.087 and 0.093–0.54 µg/m^3, respectively (Kucera et al. 2000).

The National Occupational Exposure Survey (NOES) conducted by NIOSH in 1983 estimated that 245,024 workers employed at 15,996 facilities were potentially exposed to molybdenum (pure, powder, and unknown forms) in the United States (RTECS 2009). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace.

The extensive nationwide use of radioactive ^{99}Mo in generators that produce ^{99m}Tc for nuclear medicine imaging scans can expose medical staff and the public in medical facilities to low levels of ionizing radiation. The extent of those exposures is limited by the USNRC and agreement state regulations (USNRC 2016a, 2016b).

Breast milk and infant formula are the primary sources of molybdenum in infants aged 0–6 months (NAS 2001). The primary source of dietary molybdenum intake among children in the United States is milk (EPA 1979). Several studies have measured molybdenum levels in human breast milk; average molybdenum levels ranged from 1.5 to 17 µg/L (Anderson 1992; Aquilio et al. 1996; Biego et al. 1998; Bougle et al. 1988). As shown in Table 5-10, the highest molybdenum concentrations occur within the first week after birth and tend to be higher in the mothers of term infants, as compared to preterm infants (Aquilio et al. 1996; Bougle et al. 1988).

Table 5-10. Molybdenum Levels in Breast Milk in Mothers of Term and Preterm Infants

	Molybdenum levels in breast milk (µg/L)		
Lactation day	Term infants	Preterm infants	Reference
2–6	6.8	3.9[a]	Aquilio et al. 1996
12–16[b]	5.7	2.4[a]	
21[c]	3.6	1.9[a]	
3–5	10.2	4.0[a]	Bougle et al. 1988
7–10[d]	4.8	3.7	
14[d]	1.5	1.4	
30[d]	2.6	1.9	
60[e]	No data	1.2	

[a]Significantly different from term infant levels ($p<0.05$).
[b]Significantly different from molybdenum concentration at 2-6 days ($p<0.01$).
[c]Significantly different from molybdenum concentration at 2-6 days ($p<0.05$).
[d]Significantly different from molybdenum concentration for whole group at 3-5 days ($p<0.01$).
[e]Significantly different from molybdenum concentration at for whole group at 3-5 days ($p<0.05$).

Krachler and colleagues studied the trace element concentrations in human milk during the course of lactation (Krachler et al. 1998; Rossipal and Krachler 1998). In total, 79 samples of human milk from 46 healthy mothers were sampled in Austria in 1995 and 1996 at 1–293 days after the mothers gave birth (Rossipal and Krachler 1998). In colostrum milk (1–3 days postpartum), the molybdenum concentration was 8.88±3.74 µg/L. In samples collected 42–60 days postpartum, the concentration was 1.43±1.77 µg/L, and at 97–293 days, the milk contained 1.78±1.62 µg/L. In a later study, the same group analyzed a further set of samples of colostrum milk only (Krachler et al. 1999). Previous results were confirmed, with the mean concentration being reported as 7.0±3.8 µg/L (median 5.7 µg/L, range 3.4–18.8 µg/L). Another study from Europe reported molybdenum concentrations in human milk (Wappelhorst et al. 2002). In samples taken daily in 2002 from 19 mothers from Germany, Poland, and

the Czech Republic, for sample periods between 2 and 8 weeks for each mother and covering weeks 3–68 of lactation, the median molybdenum concentration was 0.53 µg/L (mean 0.72 µg/L, range 0.27–1.61 µg/L). Data on molybdenum concentrations in human milk are also available from Japan (Hattori et al. 2004). In 17 samples provided by three mothers during days 96 and 327 after delivery, the molybdenum concentrations ranged from 1.97 to 8.93 µg/L, with an estimated average of ~4.3 µg/L (estimated from three median values given for the individual mothers). In comparison, the concentration of molybdenum in formula milk after preparation is reported as 2.38±0.75 µg/L (n=6). In Canada, 20 samples of mother's milk were analyzed for molybdenum in the context of a balance study on low-birth-weight infants on parenteral and enteral nutrition (Friel et al. 1999b). The median molybdenum concentration is reported as 5 µg/L, with a range of 2.1–23 µg/L.

Urinary levels of molybdenum in children 6–11 and 12–19 years old were measured during the NHANES study assessing exposure from 1999 to 2016 (CDC 2019) and in children 3–5 years of age during NHANES 2015–2016; these data are summarized in Table 5-11.

Table 5-11. Urinary Molybdenum Levels in U.S. Children and Adolescents

Survey years	Geometric mean	50th percentile	95th percentile	Sample size
	Urinary molybdenum (µg/L)[a]			
1999–2000				
Age 6–11 years	78.2 (61.0–100)	84.8 (67.7–105)	267 (159–840)	310
Age 12–19 years	54.3 (47.6–62.0)	60.6 (52.2–70.3)	188 (146–216)	648
2001–2002				
Age 6–11 years	63.3 (53.4–75.0)	69.2 (63.0–77.6)	197 (161–291)	368
Age 12–19 years	60.6 (55.5–66.2)	65.7 (58.7–73.1)	179 (155–227)	762
2003–2004				
Age 6–11 years	62.2 (56.7–68.3)	71.3 (55.7–84.1)	181 (138–235)	290
Age 12–19 years	52.5 (49.0–56.3)	59.6 (55.5–65.1)	143 (130–156)	725
2005–2006				
Age 6–11 years	65.6 (56.6–76.0)	73.5 (62.8–85.5)	181(154–205)	355
Age 12–19 years	59.1 (53.7–65.1)	63.8 (57.9–69.4)	173 (148–202)	701
2007–2008				
Age 6–11 years	69.3 (60.8–79.0)	72.8 (62.1–83.9)	235 (169–282)	394
Age 12–19 years	64.1 (58.6–70.2)	68.6 (63.7–80.2)	174 (151–196)	376
2009–2010				
Age 6–11 years	65.0 (57.8–73.2)	69.7 (61.1–84.2)	218 (180–263)	378
Age 12–19 years	52.4 (47.5–57.7)	58.5 (51.4–65.6)	178 (151–201)	451

Table 5-11. Urinary Molybdenum Levels in U.S. Children and Adolescents

Survey years	Geometric mean	50th percentile	95th percentile	Sample size
2011–2012				
Age 6–11 years	58.4 (51.5–66.2)	65.1 (52.8–74.5)	211 (187–283)	399
Age 12–19 years	46.4 (40.2–53.7)	51.0 (44.2–64.8)	163 (145–173)	390
2013–2014				
Age 6–11 years	51.7 (47.1–56.6)	54.7 (49.3–61.9)	182 (159–210)	402
Age 12–19 years	48.2 (41.5–55.8)	55.9 (49.0–64.8)	156 (136–180)	451
2015–2016				
Age 3–5 years	47.3 (43.9–50.8)	51.6 (45.8–61.1)	191 (146–218)	486
Age 6–11 years	56.2 (51.3–61.5)	57.5 (50.2–69.1)	173(165–224)	379
Age 12–19 years	47.7 (43.3–52.6)	53.0 (45.9–57.0)	149 (135–166)	402
Creatinine corrected urinary molybdenum (µg/g creatinine)				
1999–2000				
Age 6–11 years	85.9 (73.7–100)	79.3 (71.6–88.4)	214 (154–1,040)	310
Age 12–19 years	41.9 (39.3–44.6)	40.5 (37.7–44.4)	112 (78.4–185)	648
2001–2002				
Age 6–11 years	77.2 (73.1–81.5)	77.6 (71.8–84.5)	185 (165–219)	368
Age 12–19 years	43.4 (40.8–46.1)	44.1 (40.8–47.2)	106 (94.8–118)	762
2003–2004				
Age 6–11 years	72.5 (65.2–80.7)	73.5 (65.1–79.9)	160 (129–257)	290
Age 12–19 years	37.5 (35.4–39.8)	38.9 (36.9–41.8)	81.0 (74.3–102)	725
2005–2006				
Age 6–11 years	81.0 (71.9–91.3)	78.6 (72.1–89.0)	201(160–261)	355
Age 12–19 years	45.5 (42.5–48.7)	45.7 (41.3–49.2)	109 (95.0–131)	701
2007–2008				
Age 6–11 years	90.4 (81.8–99.8)	88.2 (79.2–101)	274 (224–354)	394
Age 12–19 years	50.1 (47.2–53.2)	50.1 (44.2–53.4)	129 (99.5–138)	376
2009–2010				
Age 6–11 years	88.6 (81.9–95.4)	89.0 (79.2–95.4)	195 (178–216)	378
Age 12–19 years	49.0 (45.3–53.0)	50.7 (44.6–56.2)	126 (96.4–134)	451
2011–2012				
Age 6–11 years	83.5 (76.1–91.6)	81.7 (74.3–91.2)	259 (185–300)	398
Age 12–19 years	44.4 (40.8–48.4)	43.7 (39.1–48.0)	109 (92.4–131)	390
2013–2014				
Age 6–11 years	77.0 (73.5–80.8)	73.5 (70.0–81.4)	184 (164–225)	402
Age 12–19 years	43.6 (40.3–47.2)	44.0 (39.1–47.3)	113 (95.9–140)	451

Table 5-11. Urinary Molybdenum Levels in U.S. Children and Adolescents

Survey years	Geometric mean	50th percentile	95th percentile	Sample size
2015–2016				
Age 3–5 years	109 (102–116)	107 (98.4–117)	275 (239–329)	485
Age 6–11 years	79.7 (75.5–84.1)	79.6 (69.9–86.7)	200(171–229)	379
Age 12–19 years	44.6 (41.9–47.5)	45.0 (41.8–48.7)	107 (89.3–133)	402

[a]Limit of detection for survey years 1999–2001, 2001–2002, 2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016 were 0.8, 0.8, 1.5, 0.92, 0.92, 0.99, 0.8, and 0.8 µg/L, respectively.

NHANES = National Health and Nutrition Examination Survey

Source: CDC 2019; NHANES data are periodically updated, and the most recent information can be found at https://cdc.gov/exposurereport/index.html.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers in an industrial setting such as mining, metal refining, and metal working can be exposed to significant levels of molybdenum (Kucera et al. 2000). Populations living close to areas with high molybdenum contamination from industrial effluents and high mineral deposits are at risk for higher exposures (EPA 1979).

^{99}Mo generators are the major source of ionizing radiation exposure to nuclear medicine staff in medical facilities that perform ^{99m}Tc diagnostic imaging scans (Ahasan 2004).

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of molybdenum is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of molybdenum.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to molybdenum that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of molybdenum. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

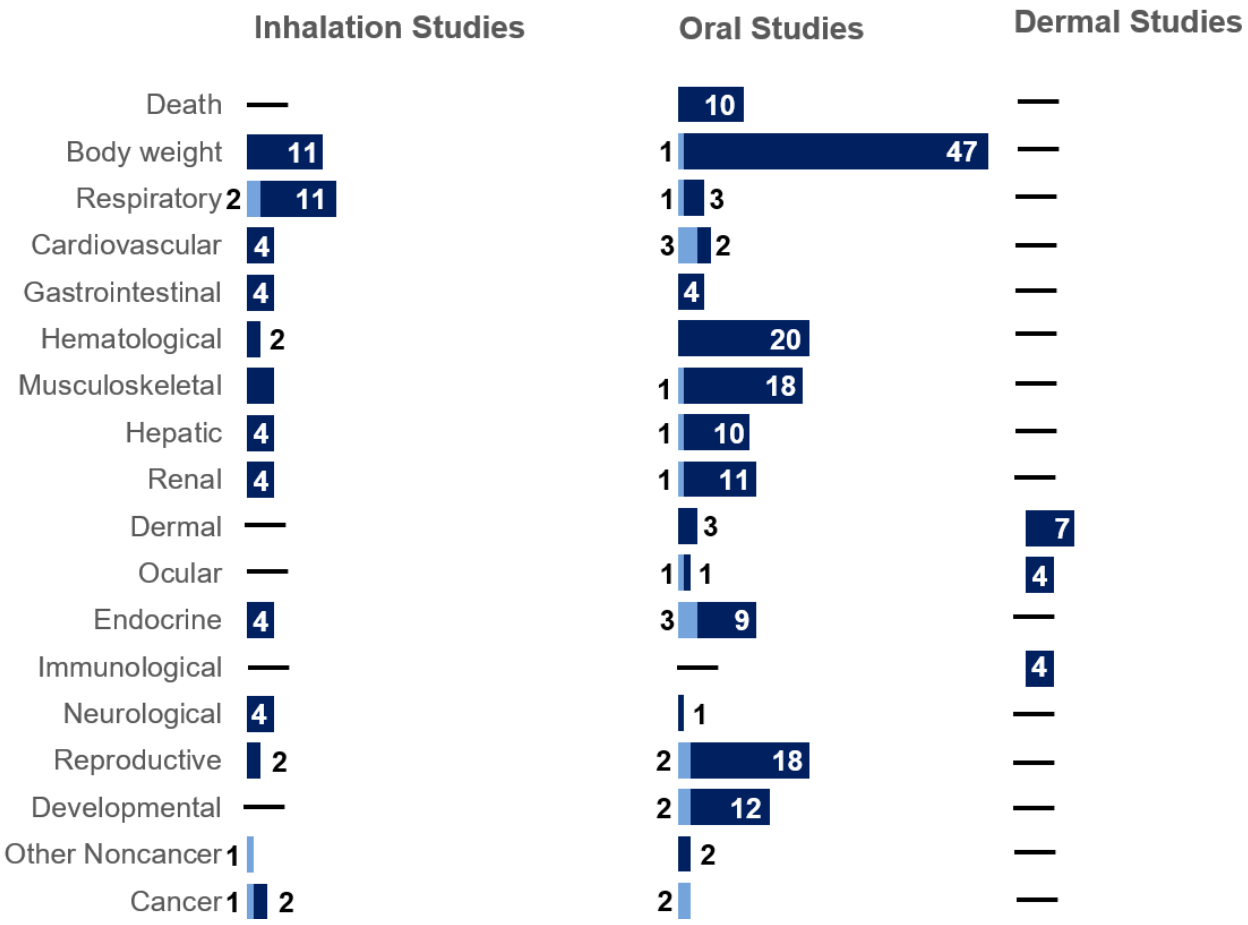
6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 6-1. Summary of Existing Health Effects Studies on Molybdenum By Route and Endpoint*

Potential body weight, hematological, musculoskeletal, and reproductive effects were the most studied endpoints

The majority of the studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2; the number of studies include those finding no effect and studies may have examined more than one endpoint.

Acute-Duration MRLs. No data were located regarding health effects after acute inhalation exposure to molybdenum in humans. In laboratory animals, the inhalation exposure data are limited to studies of molybdenum trioxide conducted in rats and mice (NTP 1997); however, the studies only examined the nasal cavity and body weight. Although increased mortality and decreases in body weight gain were observed, the studies are not adequate for identifying the primary target of toxicity. Thus, they were not considered adequate for derivation of an acute-duration inhalation MRL. Additional studies examining a wide-range of endpoints and several different molybdenum compounds would be useful for characterizing the hazard of molybdenum following acute inhalation exposure.

In an acute oral exposure experiment, no alterations in uric acid levels were observed in volunteers (Deosthale and Gopalan 1974); the study did not examine other potential endpoints. A small number of studies have examined the acute oral toxicity in laboratory animals, and none of them examined a wide-range of endpoints. One study found an increase in serum triglyceride levels in rabbits but did not find any histological alterations in the liver or kidneys (Bersenyi et al. 2008). Three acute laboratory animal studies have reported reproductive effects (Bersenyi et al. 2008; Zhai et al. 2013; Zhang et al. 2013). However, interpretation of the results is limited by the lack of statistical analyses (Bersenyi et al. 2008) or limited information on molybdenum and copper intake (Zhai et al. 2013; Zhang et al. 2013). Additionally, reproductive effects have not been reported in high-quality intermediate-duration studies (Murray et al. 2014a, 2019). Given these limitations, the database was not considered suitable for derivation of an acute-duration oral MRL. Additional studies that report molybdenum doses and copper content of the diet, and evaluate a wide range of endpoints, including the reproductive system, are needed.

Intermediate-Duration MRLs. The available data on the toxicity of molybdenum following intermediate-duration inhalation exposure are limited to 90-day studies of molybdenum trioxide examining a wide range of potential targets of toxicity in rats and mice (NTP 1997). No adverse effects were observed in these studies, and the studies were not considered suitable for derivation of an intermediate-duration inhalation MRL for molybdenum. Additional studies testing higher concentrations and several molybdenum compounds may identify sensitive targets.

A number of studies have examined the intermediate-duration toxicity of ingested molybdenum. Among studies in which the laboratory animals were provided a diet with adequate levels of copper, a number of targets of toxicity were identified including the kidney, hematological system, reproductive system, and the developing organism (Bompart et al. 1990; Fungwe et al. 1990; Jeter and Davis 1954; Lyubimov et al. 2004; Murray et al. 2014a, 2019; Pandey and Singh 2002). The lowest LOAEL values were identified for

reproductive and developmental effects. However, these values were identified in lower quality studies and were not confirmed in higher quality studies; thus, they were not considered suitable as a point of departure (POD) for an MRL. An intermediate-duration oral MRL was derived based on kidney effects in a high-quality study (Murray et al. 2014a). Additional studies are needed to confirm that the kidney is the most sensitive target of oral molybdenum toxicity.

Chronic-Duration MRLs. Two occupational exposure studies have reported mixed results on the effect of molybdenum on the respiratory tract (Ott et al. 2004; Walravens et al. 1979). There is insufficient information on the specific molybdenum compounds involved and limited data on exposure levels. Chronic exposure studies in rats and mice have identified the respiratory tract as a sensitive target of molybdenum trioxide toxicity (NTP 1997), and an inhalation MRL was derived based on the findings in the animal studies. Additional studies are needed to evaluate the inhalation toxicity of other molybdenum compounds.

A number of studies have evaluated the chronic toxicity of ingested molybdenum in humans. Studies of populations potentially exposed to high concentrations of molybdenum have evaluated potential alterations in uric acid levels (EPA 1979; Koval'skiy et al. 1961); there are a number of limitations with both of these studies restricting their usefulness in evaluating the chronic toxicity of molybdenum in humans. Epidemiological studies that examined the potential of molybdenum to induce adverse health effects presumably involved background environmental exposure (Meeker et al. 2008, 2010; Mendy et al. 2012; Schroeder and Kraemer 1974; Shiue and Hristova 2014; Vazquez-Salas et al. 2014; Yorita Christensen 2013). Although some of these studies reported statistically significant associations between biomarkers of molybdenum exposure (plasma or urine levels) and adverse effects, the studies do not establish causality and there may have been factors other than molybdenum exposure. No laboratory animal studies evaluated the chronic oral toxicity of molybdenum. Additional studies examining a wide range of potential endpoints are needed to identify the hazards associated with chronic ingestion of high levels of molybdenum and establish dose-response relationships; these data could be used to derive a chronic-duration oral MRL.

Health Effects.

Reproductive. A study of men at an infertility clinic found associations between blood molybdenum levels and altered sperm parameters and reproductive hormone levels (Meeker et al. 2008, 2010). These studies do not establish causality. Oral exposure studies in laboratory animals have provided mixed results on whether the reproductive system is a target of

molybdenum toxicity (Bersenyi et al. 2008; Fungwe et al. 1990; Lyubimov et al. 2004; Murray et al. 2014a, 2019; Pandey and Singh 2002; Zhai et al. 2013; Zhang et al. 2013). High-quality studies did not find any significant alterations in sperm parameters, estrous cycling, or male or female reproductive tissue (Murray et al. 2014a, 2019), and no effects on fertility were found in a 2-generation study (Murray et al. 2019). In contrast, other studies have found alterations in estrous cycling (Fungwe et al. 1990), sperm parameters (Pandey and Singh 2002; Zhai et al. 2013), oocytes (Zhang et al. 2013), and male fertility (Pandey and Singh 2002). Interpretation of the results of these studies was limited by inadequate information on molybdenum doses (the investigators did not provide adequate information on body weight or water consumption, which could be used to estimate doses) or did not report the copper content of the commercial diet used. Additional studies are needed to provide insight into the apparent conflicting results for reproductive toxicity.

Immunotoxicity. The immunotoxicity of molybdenum has not been adequately addressed. No inhalation or oral exposure studies addressed immune function; intermediate- and chronic-duration inhalation studies did not find histological alterations in the thymus or spleen (NTP 1997). Very low levels of positive results of patch tests were observed in patients undergoing hip or knee replacements (Koster et al. 2000; Menezes et al. 2004; Zeng et al. 2014). In animals, contact sensitization was observed in a guinea pigs in a sensitization assay with molybdenum pentachloride (Boman et al. 1979); other studies with other molybdenum compounds—ammonium dimolybdate, molybdenum trioxide, and sodium molybdate—have not found evidence of skin sensitization (Allan et al. 1996, 1996b, 1996c, 1996d). Studies examining immune function and systemic immunological endpoints (e.g., changes in white cell populations, cytokine levels, macrophage infiltration) would be useful in evaluating whether this is a target of molybdenum toxicity; it would be useful if the studies evaluated different molybdenum compounds.

Mechanisms of Action. The mechanisms of molybdenum toxicity are poorly understood. Although there are data suggesting that molybdenum toxicity may be related to alterations in copper utilization, it is also likely that other mechanisms, such as oxidative damage, are also involved. Studies examining the mode of action are needed to support the identification of critical endpoints and derivation of MRLs.

Epidemiology and Human Dosimetry Studies. A small number of epidemiology studies were identified for molybdenum; however, most of these studies presumably involved background environmental exposure to molybdenum. Two occupational exposure studies found conflicting results regarding the respiratory toxicity of molybdenum (Walravens et al. 1979; Ott et al. 2004). Additional studies of worker populations examining a wide range of potential endpoints, including the respiratory tract, would provide valuable information on the toxicity of inhaled molybdenum. General population studies have identified a number of potential targets of toxicity of ingested molybdenum including blood pressure (Shiue and Hrisova 2014), liver (Mendy et al. 2012), the reproductive system (Meeker et al. 2008, 2010), and the developing organism (Shirai et al. 2010); however, none of the studies established causality. Studies of populations exposed to high levels of molybdenum in drinking water or from foods grown in molybdenum-rich soil would provide support for establishing sensitive targets of molybdenum toxicity.

Biomarkers of Exposure and Effect.

Exposure. Molybdenum levels can be measured in blood, tissues, and excreta, and background urinary levels of molybdenum have been established in healthy individuals (CDC 2019). Blood and urinary levels have been shown to increase in response to increased molybdenum ingestion (Turnland and Keyes 2004), although plasma molybdenum levels are likely to be reflective of recent dietary intake. Studies that quantified the relationship between blood and/or urinary levels and intake would provide valuable information on screening and comparison with adverse effect levels. Studies evaluating biochemical and/or genomic biomarkers of exposure would also be useful for evaluating potential inhalation and/or oral exposure.

Effect. No biomarkers of effect were identified. The available data have identified the following sensitive targets: respiratory tract (inhalation only), kidney, and possibly the reproductive system. Studies examining the possible relationship between blood or urinary levels of molybdenum with these adverse health effects could facilitate medical surveillance leading to early detection and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. For humans, detailed quantitative information is available regarding the absorption, distribution, and excretion of ingested molybdate ($Mo^{[VI]}O_4^{2-}$) and molybdenum incorporated into food. Although molybdate is most likely the dominant chemical species of molybdenum in the body, there are no data for humans on toxicokinetics following

exposures to other forms of molybdenum that could occur in the environment, such as Mo^{IV} compounds. No quantitative information is available on the toxicokinetics of molybdenum in humans following chronic oral exposure. There is no information on inhalation, and dermal toxicokinetic data are limited to an *in vitro* percutaneous absorption study (Roper 2009). A study conducted in mice showed that molybdenum is absorbed following inhalation exposure to molybdenum trioxide (NTP 1997).

Limited information was identified on the relative bioavailability of different molybdenum compounds following inhalation or oral exposure. It is likely that the solubility of the molybdenum compound would greatly influence the amount that is absorbed through the lungs or gastrointestinal tract. Studies examining relative bioavailability would provide valuable information on extrapolating data across molybdenum compounds and species.

Studies conducted in humans have provided data for the development of PBPK models of molybdenum kinetics in humans (Giussani 2008; Novotny and Turnlund 2007). Models have not been developed for rodents or other animal species that could be used in dosimetry extrapolation of animal bioassay results.

Comparative Toxicokinetics. The available data on the toxicity of molybdenum in humans and laboratory animals suggest that they have similar targets of toxicity; however, there are limited epidemiology data. The available data suggest similarities in the absorption, distribution, and elimination of ingested molybdenum in humans and rats. Additional studies are needed to compare the toxicokinetics of inhaled molybdenum and to assess whether there are species differences.

Children's Susceptibility. Two epidemiological studies have examined possible developmental effects associated with maternal urinary molybdenum levels (Shirai et al. 2010; Vazquez-Salas et al. 2014); interpretation of the results of these studies is limited. Studies in laboratory animals have not reported alterations in pup survival, body weight, occurrence of malformations, or developmental landmarks in rats orally exposed to molybdenum (Jeter and Davis 1954; Murray et al. 2014, 2019). There are limited data on the toxicity of molybdenum in children; studies are needed to evaluate whether the susceptibility of children differs from adults.

Physical and Chemical Properties. The physical-chemical properties of molybdenum are provided in Chapter 4. No data needs are identified.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI is updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. Molybdenum is a naturally occurring trace element that can be found extensively in nature (EPA 1979). Its transport and partitioning are well understood. No data needs are identified.

Bioavailability from Environmental Media. Biologically, molybdenum plays an important role as a micronutrient in plants and animals, including humans (EPA 1979). Its bioavailability is well documented. No data needs are identified.

Food Chain Bioaccumulation. Measured BCFs of molybdenum in fish suggest that bioaccumulation in aquatic organisms is not high. No data needs are identified.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of molybdenum in contaminated media at hazardous waste sites are needed so that the information obtained on levels of molybdenum in the environment can be used in combination with the known body burden of molybdenum to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Exposure to molybdenum to the general population is almost entirely through food. Food derived from aboveground plants, such as legumes, leafy vegetables, and cauliflower generally has a relatively higher concentration of molybdenum in comparison to food from tubers or animals. Beans, cereal grains, leafy vegetables, legumes, liver, and milk are reported as the richest sources of molybdenum in the average diet. Nutritional supplements are also a source of dietary exposure. Drinking water coming from sources close to areas with high molybdenum contamination from industrial effluents may contain a higher concentration of molybdenum. Exposure to molybdenum in an industrial setting such as mining can be significant (Barceloux 1999; EPA 1979; Momcilovic 1999; NAS 2001).

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. There are limited data on estimates of molybdenum exposure in children. Milk is reported to be the primary source of dietary molybdenum intake among children in the United States (Biego et al. 1998; EPA 1979); however, this is based on older data. More recent monitoring data would be valuable in assessing whether molybdenum exposure sources vary between children and adults.

6.3 ONGOING STUDIES

No ongoing studies on the toxicity of molybdenum or its toxicokinetic properties were identified in the National Institute of Health (NIH) RePORTER (2019) database.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding molybdenum in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for molybdenum.

Table 7-1. Regulations and Guidelines Applicable to Molybdenum

Agency	Description	Information	Reference
	Air		
EPA	RfC	No data	IRIS 2003
WHO	Air quality guidelines	Not listed	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories for molybdenum		EPA 2018a
	1-Day health advisory (10-kg child)	0.08 mg/L	
	10-Day health advisory (10-kg child)	0.08 mg/L	
	DWEL	0.2 mg/L	
	Lifetime health advisory	0.04 mg/L	
	National primary drinking water regulations	Not listed	EPA 2009b
	RfD (molybdenum)	$5x10^{-3}$ mg/kg/day[a]	IRIS 2003
WHO	Drinking water quality guidelines	Not established[b]	WHO 2017
FDA	Substances added to food	Not listed[c]	FDA 2018
USNRC	Annual limit on intake, oral ingestion		NRC 2018
	^{99}Molybdenum compounds except oxides, hydroxides, and molybdenum disulfide	$2x10^3$ µCi	
	Cancer		
HHS	Carcinogenicity classification	No data	NTP 2016
EPA	Carcinogenicity classification	No data	IRIS 2003
IARC	Carcinogenicity classification		IARC 2018
	Molybdenum trioxide	Group 2B[d]	
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards and construction (molybdenum, as molybdenum)		OSHA 2018a, 2018b, 2018c
	Soluble compounds	5 mg/m^3	
	Insoluble compounds, total dust	15 mg/m^3	

Table 7-1. Regulations and Guidelines Applicable to Molybdenum

Agency	Description	Information	Reference
NIOSH	REL (up to 10-hour TWA)	Not established[e]	NIOSH 2016a, 2016b
	IDLH (molybdenum, as molybdenum)		
	Soluble compounds	1,000 mg Mo/m^3	NIOSH 1994a
	Insoluble compounds	5,000 mg Mo/m^3	NIOSH 1994b
USNRC	Annual limit on intake, inhalation		NRC 2018
	^{99}Molybdenum compounds except oxides, hydroxides, and molybdenum disulfide	3x10^3 µCi	
	Derived air concentration		
	^{99}Molybdenum compounds except oxides, hydroxides, and molybdenum disulfide	1x10^{-6} µCi/mL	
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2016
DOE	PACs-air		DOE 2018b
	PAC-1[f]		
	Molybdenum	30 mg/m^3	
	Ammonium heptamolybdate	2.6 mg/m^3	
	Ammonium molibdate	3.5 mg/m^3	
	Ammonium molybdate(VI) tetrahydrate	2.8 mg/m^3	
	Diammonium dimolybdate	2.6 mg/m^3	
	Diammonium molybdate	3.1 mg/m^3	
	Disodium molybdate	3.2 mg/m^3	
	Molybdenum carbide	34 mg/m^3	
	Molybdenum dioxide	40 mg/m^3	
	Molybdenum hexacarbonyl	83 mg/m^3	
	Molybdenum pentachloride	4.3 mg/m^3	
	Molybdenum trioxide	2.3 mg/m^3	
	Molybdenum(IV) sulfide	50 mg/m^3	
	Sodium molybdate dihydrate	3.8 mg/m^3	
	PAC-2[f]		
	Molybdenum	330 mg/m^3	
	Ammonium heptamolybdate	230 mg/m^3	
	Ammonium molibdate	290 mg/m^3	
	Ammonium molybdate(VI) tetrahydrate	30 mg/m^3	
	Diammonium dimolybdate	29 mg/m^3	
	Diammonium molybdate	22 mg/m^3	
	Disodium molybdate	17 mg/m^3	
	Molybdenum carbide	360 mg/m^3	
	Molybdenum dioxide	430 mg/m^3	
	Molybdenum hexacarbonyl	920 mg/m^3	
	Molybdenum pentachloride	410 mg/m^3	
	Molybdenum trioxide	43 mg/m^3	

Table 7-1. Regulations and Guidelines Applicable to Molybdenum

Agency	Description	Information	Reference
	Molybdenum(IV) sulfide	260 mg/m^3	
	Sodium molybdate dihydrate	34 mg/m^3	
	PAC-3[f]		
	Molybdenum	2,000 mg/m^3	
	Ammonium heptamolybdate	1,400 mg/m^3	
	Ammonium molibdate	1,700 mg/m^3	
	Ammonium molybdate(VI) tetrahydrate	180 mg/m^3	
	Diammonium dimolybdate	170 mg/m^3	
	Diammonium molybdate	130 mg/m^3	
	Disodium molybdate	100 mg/m^3	
	Molybdenum carbide	2,200 mg/m^3	
	Molybdenum dioxide	2,600 mg/m^3	
	Molybdenum hexacarbonyl	5,500 mg/m^3	
	Molybdenum pentachloride	2,400 mg/m^3	
	Molybdenum trioxide	260 mg/m^3	
	Molybdenum(IV) sulfide	1,600 mg/m^3	
	Sodium molybdate dihydrate	210 mg/m^3	

[a]The RfD is based on a LOAEL of 0.14 mg/kg/day for increased uric acid levels in humans (Koval'skiy et al. 1961).
[b]Reason for not establishing guideline value: occurs in drinking water at concentrations well below those of health concern.
[c]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited in food, delisted color additives, and some substances "no longer FEMA GRAS."
[d]Group 2B: possibly carcinogenic to humans.
[e]In 1988, NIOSH provided comments to OSHA in which NIOSH questioned whether proposed PELs for particular substances, including the TWA 5 mg/m^3 PEL for molybdenum (soluble compounds as molybdenum), were adequate to protect workers from recognized health hazards. At that time, NIOSH also concluded that the documentation cited by OSHA was inadequate to support a proposed PEL of 10 mg/m^3 for particular substances including molybdenum (insoluble compounds as molybdenum) (NIOSH 2018).
[f]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018a).

AEGL = acute exposure guideline level; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOAEL = lowest-observed-adverse-effect level; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfD = oral reference dose; TWA = time-weighted average; USNRC = U.S. Nuclear Regulatory Commission; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Molybdenum
CAS Numbers: 7439-98-7
Date: May 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for molybdenum due to the limited number of endpoints examined in the only available animal studies.

Rationale for Not Deriving an MRL: The database on the acute inhalation toxicity of molybdenum is limited to several 4-hour studies in rats exposed to ammonium dimolybdate (Jackson et al. 1991a), molybdenum trioxide (Jackson et al. 1991b, 1991d; Leuschner 2010), or sodium molybdate (Jackson et al. 1991c) and a 14-day study in rats and mice exposed to molybdenum trioxide (NTP 1997). No effects on lethality or the respiratory tract (most only examined the lungs) were observed at concentrations of 1,200 mg molybdenum/m^3 and higher (Jackson et al. 1991a, 1991b, 1991c, 1991d; Leuschner 2010); several of the studies reported decreases in body weight on days 2–3 post-exposure (Jackson et al. 1991b, 1991c, 1991d). The NTP (1997) study evaluated the effect of molybdenum trioxide on the nasal cavity and on body weight in rats and mice exposed 6 hours/day, 5 days/week for 14 days. No adverse effects were observed in the nasal cavity. However, weight loss was observed at the highest concentration tested (200 mg molybdenum/m^3); decreases in body weight gain were observed in male rats exposed to 67 mg molybdenum/m^3 and in female rats and mice exposed to 200 mg/m^3. Given the limited number of endpoints examined, the decrease in body weight gain was not considered a suitable basis for an acute-duration inhalation MRL because the database is inadequate for identifying the critical target of molybdenum toxicity following acute-duration inhalation exposure.

Agency Contacts (Chemical Managers): G. Daniel Todd

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Molybdenum
CAS Numbers: 7439-98-7
Date: May 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for molybdenum due to the lack of studies identifying a critical target of toxicity.

Rationale for Not Deriving an MRL: Information on the intermediate-duration toxicity of molybdenum is limited to 90-day studies of molybdenum trioxide in rats and mice conducted by NTP (1997) that examined a wide range of potential targets, including reproductive endpoints. No toxicologically significant alterations were observed at concentrations of molybdenum trioxide as high as 67 mg/m^3. Consistent with ATSDR's practice of not using free-standing NOAELs as a POD, an intermediate-duration inhalation MRL was not derived.

Agency Contacts (Chemical Managers): G. Daniel Todd

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Molybdenum trioxide
CAS Numbers: 1313-27-5
Date: May 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic
MRL 0.002 mg molybdenum/m^3
Critical Effect: Respiratory effect, squamous metaplasia of the epiglottis in female rats
Reference: NTP 1997
Point of Departure: $BMCL_{10}$ of 1.60 mg molybdenum/m^3 ($BMCL_{HEC}$ of 0.071 mg Mo/m^3)
Uncertainty Factor: 30
LSE Graph Key: 11
Species: Rat

MRL Summary: A chronic-duration inhalation MRL of 0.002 mg molybdenum/m^3 was derived for molybdenum trioxide based on an increased incidence of squamous metaplasia of the epiglottis in female rats exposed to 6.7 mg molybdenum/m^3 as molybdenum trioxide 6 hours/day, 5 days/week for 2 years (NTP 1997). The MRL is based on a $BMCL_{10}$ of 1.60 mg molybdenum/m^3 (human equivalent concentration [HEC] of 0.071 mg molybdenum/m^3) and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

Selection of the Critical Effect: There are limited data on the toxicity of inhaled molybdenum in humans. A study of workers at a molybdenite roasting facility exposed to molybdenum trioxide and other oxides found no alterations in lung function but did find increases in serum uric acid levels (Walravens et al. 1979); the TWA molybdenum concentration was 9.46 mg molybdenum/m^3. Another study of workers exposed to ultrafine molybdenum trioxide dust reported respiratory symptoms (dyspnea and cough), radiographic abnormalities, and impaired lung function (Ott et al. 2004); the study did not provide monitoring data. Confidence in these cohort studies was considered very low (see Appendix C for additional information).

Data on the chronic toxicity of molybdenum in laboratory animals is limited to 2-year studies in rats and mice exposed to molybdenum trioxide (NTP 1997). In these studies, NTP (1997) examined a wide range of potential targets of toxicity. Adverse effects were limited to the respiratory tract, specifically the nasal respiratory and olfactory epithelium, epiglottis, and lungs. The respiratory tract was considered the critical target of molybdenum trioxide toxicity.

Selection of the Principal Study: The NTP (1997) study was selected as the principal study.

Summary of the Principal Study:

NTP. 1997. Toxicology and carcinogenicity studies of molybdenum trioxide (CAS No. 1313-27-5) in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program, Research Triangle Park, NC. NT PTR 462.

Groups of male and female F344/N rats and B6C3F1 mice (50/sex/species/group) were exposed to target concentrations of 0, 10, 30, or 100 mg/m^3 molybdenum trioxide (0, 6.7, 20, and 67 mg molybdenum/m^3) 6 hours/day, 5 days/week for 106 (rats) or 105 (mice) weeks; actual concentrations were within 15% of the target level. The average mass median aerodynamic diameter particle sizes (and geometric standard deviation, σ_g) were 1.5 (1.8), 1.6 (1.8), and 1.7 (1.8) µm for the 6.7, 20, and 67 mg/m^3 concentrations,

respectively. The following parameters were used to assess toxicity: twice daily cage-side observations, body weights (weekly for 12 weeks, at 15 weeks, monthly thereafter, and at termination), and histopathological examination of major tissues and organs. In addition, bone density and femoral curvature studies were conducted in 10 animals/sex/species/group.

No significant alterations in survival rates or body weight gain and no toxicologically significant alterations in bone density or curvature were found. Non-neoplastic lesions were only observed in the nose, larynx, and lungs; a summary of the type of lesions and incidences is presented in Table A-1. The severity of the respiratory lesions was concentration related. Significant increases in the incidence of alveolar/bronchiolar carcinoma and/or adenoma were observed in mice: carcinoma in male mice at ≥6.7 mg/m^3, adenoma or carcinoma (combined) in male mice at 6.7 and 20 mg/m^3, adenoma in female mice at ≥20 and 67 mg/m^3, and adenoma or carcinoma (combined) in female mice at 67 mg/m^3. In rats, the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was increased in males; however, the incidences (0/50, 1/49, 1/49, 4/60) were within the range of historical controls and NTP considered this to be equivocal evidence of carcinogenic activity.

Table A-1. Incidence of Non-Neoplastic Respiratory Tract Lesions in Rats and Mice Exposed to Molybdenum Trioxide for 2 Years

	Concentration (mg molybdenum/m^3)			
	0	6.7	20	67
Male rats				
Hyaline degeneration of nasal respiratory epithelium	2/50	7/49	48/49[a]	49/50[a]
Squamous metaplasia of epiglottis	0/49	11/48[a]	16/49[a]	39/49[a]
Chronic lung inflammation in alveolus	2/50	3/50	25/50[a]	47/50[a]
Female rats				
Hyaline degeneration of nasal respiratory epithelium	1/48	13/49[a]	50/50[a]	50/50[a]
Hyaline degeneration of nasal olfactory epithelium	39/48	47/49[b]	50/50[a]	50/50[a]
Squamous metaplasia of epiglottis	0/49	18/49[a]	29/49[a]	49/50[a]
Chronic lung inflammation	14/50	13/50	43/50[a]	49/50[a]
Male mice				
Nasal suppurative inflammation	2/50	6/50	10/49[b]	8/50[b]
Nasal olfactory epithelium atrophy	3/50	5/50	3/49	10/50[b]
Hyaline degeneration of nasal respiratory epithelium	11/50	13/50	11/49	41/50[a]
Squamous metaplasia of epiglottis	0/50	26/49[a]	37/48[a]	49/50[a]
Laryngeal hyperplasia	1/50	3/49	6/48	41/50
Histiocyte infiltration in the lungs	2/50	16/50[a]	9/49[b]	9/50[b]
Alveolar epithelial metaplasia	0/50	32/50[a]	36/49[a]	49/50[a]
Female mice				
Hyaline degeneration of nasal respiratory epithelium	26/49	23/50	28/49	48/49[a]
Hyaline degeneration of nasal olfactory epithelium	22/49	14/50	14/49	36/49[a]

Table A-1. Incidence of Non-Neoplastic Respiratory Tract Lesions in Rats and Mice Exposed to Molybdenum Trioxide for 2 Years

	Concentration (mg molybdenum/m^3)			
	0	6.7	20	67
Squamous metaplasia of epiglottis	1/49	36/50[a]	43/49[a]	49/50[a]
Laryngeal hyperplasia	1/49	1/50	7/49	35/50
Alveolar epithelial metaplasia	2/50	26/50[a]	39/49[a]	46/49[b]

[a]Significantly different from controls; p≤0.01.
[b]Significantly different from controls; p≤0.05.

Source: NTP 1997

Selection of the Point of Departure for the MRL: The MRL was based on a $BMCL_{10}$ of 1.60 mg molybdenum/m^3 for squamous metaplasia of the epiglottis in female rats.

Benchmark dose (BMD) modeling was conducted for the respiratory tract lesions with statistically significant increases in incidence at ≥6.7 mg/m^3 (squamous metaplasia of the epiglottis in male and female rats and mice, hyaline degeneration of the nasal respiratory and olfactory epithelium in female rats, histiocyte infiltration in the lungs in male mice, and alveolar epithelial metaplasia in male and female mice). The incidence data (Table A-1) provided adequate fit for four endpoints (squamous metaplasia in male rats, female rats, and female mice and hyaline degeneration of the nasal respiratory epithelium in female rats). The results of the BMD modeling are presented in the Benchmark Dose Modeling subsection and are summarized in Table A-2.

Table A-2. Summary of Benchmark Dose Modeling

Endpoint	Selected model	BMC_{10} (mg Mo/m^3)	$BMCL_{10}$ (mg Mo/m^3)
Squamous metaplasia of the epiglottis in male rats	Multistage, 2-degree (Table A-4 and Figure A-1)	4.36	3.53
Hyaline degeneration of the respiratory epithelium in female rats	Log-logistic (Table A-5 and Figure A-2)	5.87	4.82
Squamous metaplasia of the epiglottis in female rats	Weibull (Table A-6 and Figure A-3)	1.97	1.60
Squamous metaplasia of the epiglottis in male mice	Gamma (Table A-7 and Figure A-4)	1.30	1.06

BMC = benchmark concentration; BMCL = 95% lower confidence limit on the benchmark concentration

A summary of the potential POD values is presented in Table A-3. Because there are dosimetric differences in regional respiratory tract deposition of aerosols between animal species, a comparison was made between the human equivalent concentration PODs (POD_{HEC}). The lowest POD_{HEC}, $BMCL_{HEC}$ of 0.071 mg molybdenum/m^3 for squamous metaplasia of the epiglottis in female rats, was selected as the POD for the MRL.

Table A-3. Summary of PODs and HECs

Endpoint	PODs (mg Mo/m^3)	RDDR values[a]	HECs[b] (mg Mo/m^3)
Squamous metaplasia of the epiglottis in male rats	3.53 (BMCL)	0.459	0.28
Hyaline degeneration of the respiratory epithelium in female rats	4.82 (BMCL)	0.248	0.21
Hyaline degeneration of the olfactory epithelium in female rats	6.7 (LOAEL)	0.248	0.30
Squamous metaplasia of the epiglottis in female rats	1.60 (BMCL)	0.248	0.071
Squamous metaplasia of the epiglottis in male mice	1.06 (BMCL)	0.441	0.08
Histiocyte infiltration in the lungs of male mice	6.7 (LOAEL)	1.046	1.3
Alveolar epithelial metaplasia in male mice	6.7 (LOAEL)	1.046	1.3
Squamous metaplasia of the epiglottis in female mice	6.7 (LOAEL)	0.367	0.44
Alveolar epithelial metaplasia in female mice	6.7 (LOAEL)	3.067	3.7

[a]RDDR values specific for each region of the respiratory tract (extrathoracic, tracheobronchial, and pulmonary) were calculated using EPA's RDDR calculator with reference body weights of 0.40, 0.25, 0.040, and 0.035 kg for male rats, female rats, male mice, and female mice, respectively, and reported particle sizes and particle size distributions.
[b]HEC calculated by multiplying the duration-adjusted POD (POD x 6 hours/24 hours x 5 days/7days) by the RDDR value.

BMCL = 95% lower confidence limit on the benchmark concentration; HEC = human equivalent concentration; LOAEL = lowest observed adverse effect level; POD = point of departure; RDDR = regional deposited dose ratio for the specific region of the respiratory tract

Adjustment for Intermittent Exposure: The PODs were adjusted for intermittent exposure (6 hours/day, 5 days/week).

Calculation of Human Equivalent Concentration: HECs were calculated for each potential POD by multiplying the duration-adjusted POD by the regional deposited dose ratio (RDDR) for the specific region of the respiratory tract. The RDDR is a factor used to adjust particulate exposure concentration in animals to a predicted concentration in humans that would be associated with the same dose delivered to a specific region of the respiratory tract or to the blood (EPA 1994). The RDDRs were calculated using EPA's RDDR calculator with reference body weights of 0.40, 0.25, 0.040, and 0.035 kg for the male rats, female rats, male mice, and female mice, respectively, the reported particle sizes, and particle size distributions. The particles were assumed to be monodispersed given that the σ_g was 1.8.

Uncertainty Factor: The $BMCL_{HEC}$ is divided by a total uncertainty factor (UF) of 30.

- 3 for extrapolation from animals to humans with dosimetric adjustments
- 10 for human variability

$$BMCL_{HEC} \div UFs = MRL$$
$$0.071 \text{ mg molybdenum/m}^3 \div 30 = 0.002 \text{ mg molybdenum/m}^3$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: This MRL is specific to molybdenum trioxide; there are insufficient data to evaluate the health effects associated with inhalation exposure to other molybdenum compounds.

Benchmark Dose Modeling: The incidence data (Table A-1) for respiratory tract lesions, which had significant increases in incidence at ≥6.7 mg/m^3 (squamous metaplasia of the epiglottis in male and

female rats and mice, hyaline degeneration of the nasal respiratory and olfactory epithelium in female rats, histiocyte infiltration in the lungs in male mice, and alveolar epithelial metaplasia in male and female mice), were fit to all available dichotomous models in EPA's Benchmark Dose Software (BMDS, version 3.1) using the extra risk option. Adequate model fit was judged by three criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all of the models providing adequate fit to the data, the lowest BMCL was selected as the POD when the difference between the BMCLs estimated from these models was >3-fold; otherwise, the BMCL from the model with the lowest Akaike's Information Criterion (AIC) was chosen. For all lesion types, a BMR of 10% was used. Since the incidence of hyaline degeneration in the olfactory epithelium of female rats was essentially the same response level across groups, the data were not modeled since they provide limited information on the dose-response relationship. The incidence data for histiocyte infiltration in the lungs in male mice, alveolar epithelial metaplasia in male mice, squamous metaplasia in female mice, and alveolar epithelial metaplasia in female mice did not fit any of the available dichotomous models. The model predictions for the other endpoints are presented in Tables A-4, A-5, A-6, and A-7 and the fits of the selected models are presented in Figures A-1, A-2, A-3, and A-4.

Table A-4. Model Predictions for Squamous Metaplasia of the Epiglottis in Male Rats Exposed to Molybdenum Trioxide (NTP 1997)

Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMC	Scaled residuals[b] Dose above BMC	Scaled residuals[b] Overall largest	AIC	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Gamma[c]	2	3.07	0.22	0.00	1.55	1.55	169.98	4.36	3.53
Logistic	2	9.45	0.01	1.50	0.93	-2.47	181.70	ND	ND
LogLogistic[d]	2	3.56	0.17	0.00	0.98	-1.42	170.75	3.80	2.23
LogProbit[d]	2	3.74	0.15	-0.00	0.93	-1.51	170.95	ND	ND
Multistage (1-degree)[e]	3	3.07	0.38	0.00	1.55	1.55	167.98	4.36	3.53
Multistage (2-degree)[e,f]	***3***	***3.07***	***0.38***	***0.00***	***1.55***	***1.55***	***167.98***	***4.36***	***3.53***
Multistage (3-degree)[e]	3	3.07	0.38	0.00	1.55	1.55	167.98	4.36	3.53
Probit	2	9.17	0.01	1.60	0.90	-2.37	181.01	ND	ND
Weibull[c]	2	3.07	0.22	0.00	1.55	1.55	169.98	4.36	3.53

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMC; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model. BMCLs for models providing adequate fit were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND = not determined, goodness-of-fit criteria, p<0.10

Figure A-1. Fit of 2-Degree Multistage Model to Data on Incidence of Squamous Metaplasia of the Epiglottis in Male Rats Exposed to Molybdenum Trioxide

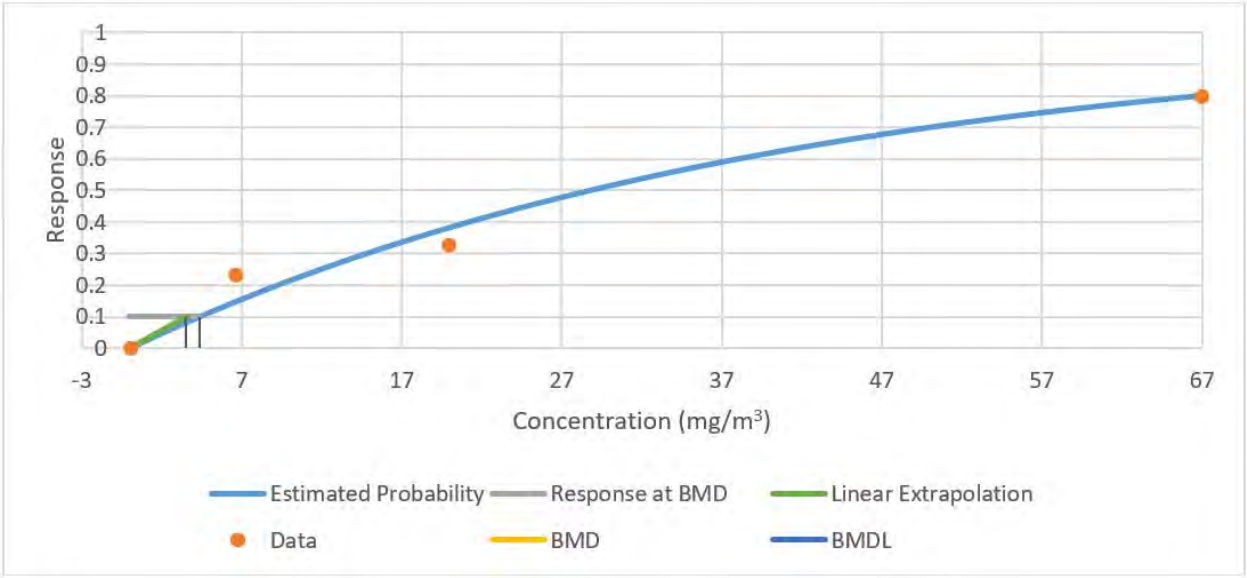


Table A-5. Model Predictions for Hyaline Degeneration of the Nasal Respiratory Epithelium in Female Rats Exposed to Molybdenum Trioxide (NTP 1997)

Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMC	Dose above BMC	Overall largest	AIC	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Gamma[c]	2	4.41	0.11	0.14	-1.03	1.82	77.98	3.69	2.85
Logistic	3	5.04	0.17	-1.20	-0.37	1.86	77.15	3.78	2.95
LogLogistic[d,e]	***2***	***0.02***	***0.99***	***0.00***	***-0.00***	***0.13***	***70.45***	***5.87***	***4.82***
LogProbit[d]	1	0.00	0.99	-0.00	-0.00	-0.00	72.42	5.92	4.73
Multistage (1-degree)[f]	2	18.41	0.00	0.28	-3.28	-3.28	95.80	ND	ND
Multistage (2-degree)[f]	2	2.81	0.24	0.20	-1.21	-1.21	74.57	3.40	2.54
Multistage (3-degree)[f]	2	0.02	0.99	0.01	-0.05	0.15	70.46	4.77	2.39
Probit	2	0.48	0.79	0.49	-0.28	0.49	71.03	4.09	3.12

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMC; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Selected model. BMCLs for models providing adequate fit were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected.
[f]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND = not determined, goodness-of-fit criteria, p<0.10

Figure A-2. Fit of Log-logistic Model to Data on Incidence of Hyaline Degeneration of the Nasal Respiratory Epithelium in Female Rats Exposed to Molybdenum Trioxide

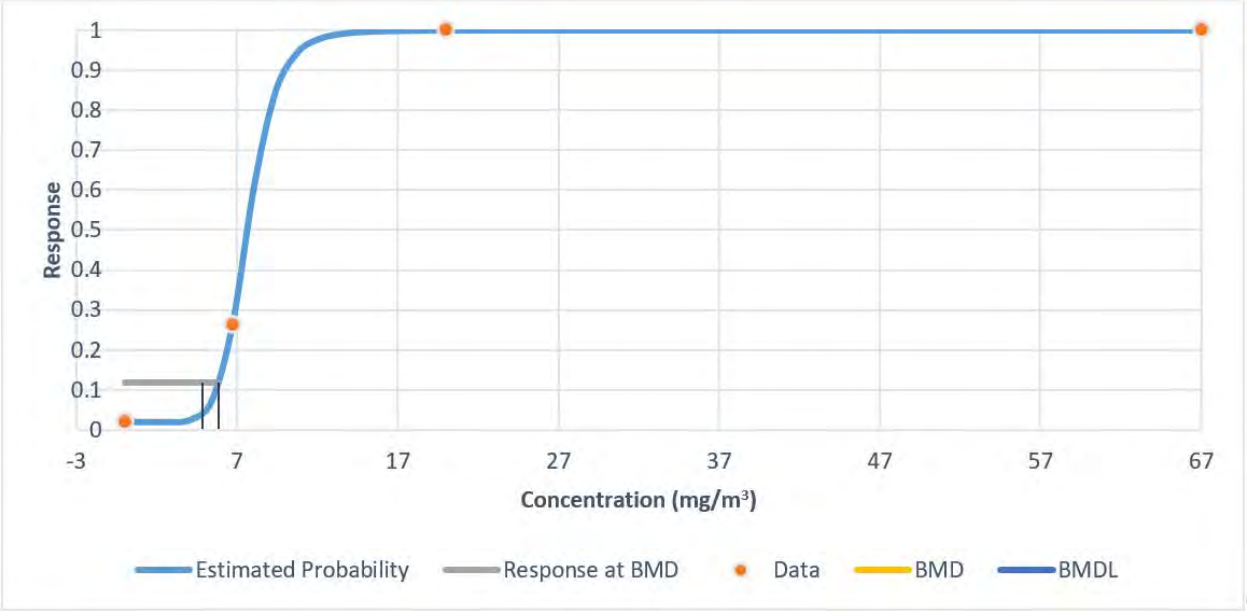


Table A-6. Model Predictions for Squamous Metaplasia of the Epiglottis in Female Rats Exposed to Molybdenum Trioxide (NTP 1997)

Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMC	Dose above BMC	Overall largest	AIC	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Gamma[c]	2	2.05	0.36	0.00	1.00	1.00	146.51	1.97	1.60
Logistic	2	15.55	0.00	-2.67	2.17	-2.67	163.85	ND	ND
LogLogistic[d]	1	5.02	0.03	-0.00	0.82	-1.58	152.04	ND	ND
LogProbit[e]	2	4.16	0.12	-0.00	0.79	-1.51	148.92	2.76	1.41
Multistage (1-degree)[e]	3	2.05	0.56	-0.00	1.00	1.00	144.51	1.97	1.60
Multistage (2-degree)[e]	1	2.05	0.15	-0.00	1.04	1.04	148.50	1.99	1.60
Multistage (3-degree)[e]	1	1.98	0.16	-0.00	1.11	1.11	148.42	2.02	1.61
Probit	2	17.51	0.00	-2.85	2.00	-2.13	166.05	ND	ND
Weibull[f]	***3***	***2.05***	***0.56***	***-0.00***	***1.00***	***1.00***	***144.51***	***1.97***	***1.60***

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMC; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model. BMCLs for models providing adequate fit were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND = not determined, goodness-of-fit criteria, p<0.10

Figure A-3. Fit of Weibull Model to Data on Incidence of Squamous Metaplasia of the Epiglottis in Female Rats Exposed to Molybdenum Trioxide

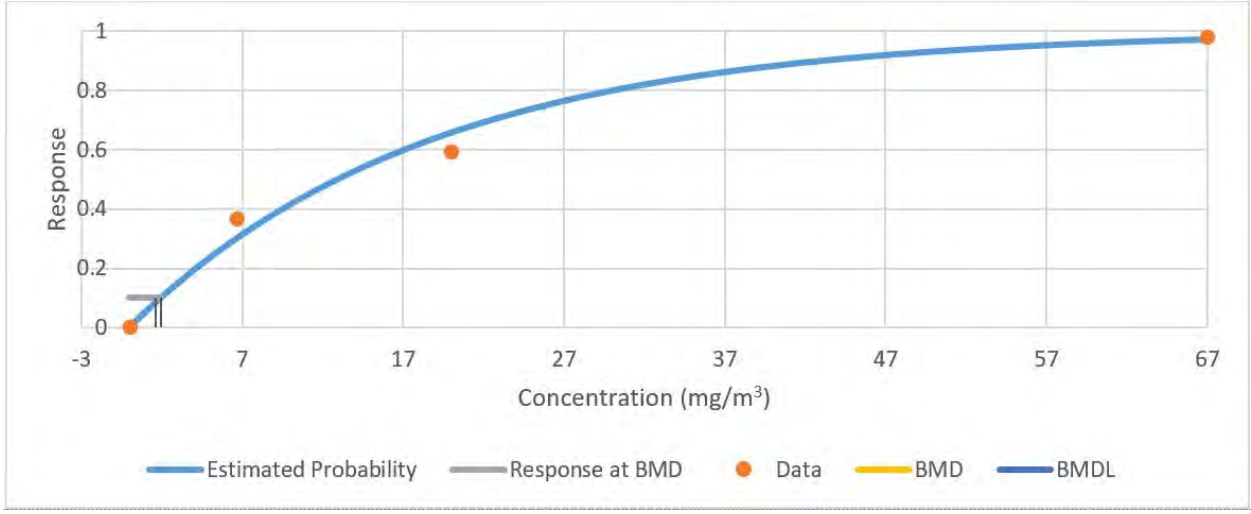


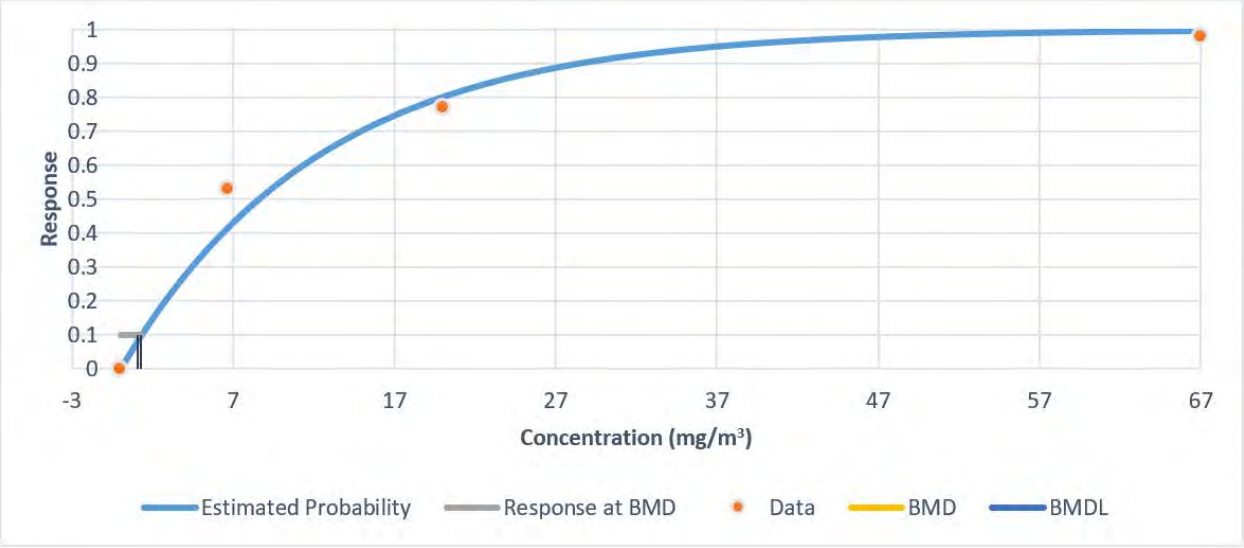
Table A-7. Model Predictions for Squamous Metaplasia of the Epiglottis in Male Mice Exposed to Molybdenum Trioxide (NTP 1997)

Model	DF	χ^2	χ^2 Goodness of fit p-value[a]	Scaled residuals[b] Dose below BMC	Dose above BMC	Overall largest	AIC	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Gamma[c,d]	***3***	***5.55***	***0.14***	***-0.00***	***1.60***	***-1.65***	***135.46***	***1.30***	***1.06***
Logistic	2	61.77	0.00	-3.19	2.80	-6.62	164.85	ND-1	ND-1
LogLogistic[e]	1	1.42	0.23	-0.00	0.34	-0.85	134.73	ND-2	ND-2
LogProbit[d]	1	0.88	0.35	-0.00	0.31	-0.70	136.12	ND-2	ND-2
Multistage (1-degree)[f]	2	5.55	0.06	-0.00	1.60	-1.65	137.46	ND-1	ND-1
Multistage (2-degree)[f]	3	5.55	0.14	-0.00	1.60	-1.65	135.46	1.30	1.06
Multistage (3-degree)[f]	3	5.55	0.14	-0.00	1.60	-1.65	135.46	1.30	1.06
Probit	2	90.03	0.00	-3.63	2.65	-8.24	171.89	ND-1	ND-1
Weibull[c]	3	5.55	0.14	-0.00	1.60	-1.65	135.46	1.30	1.06

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMC; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Selected model. BMCLs for models providing adequate fit were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND-1 = not determined, goodness-of-fit criteria, p<0.10; ND-2 = not determined, BMCL was 10 times lower than lowest non-zero dose

Figure A-4. Fit of Gamma Model to Data on Incidence of Squamous Metaplasia of the Epiglottis in Male Mice Exposed to Molybdenum Trioxide



Agency Contacts (Chemical Managers): G. Daniel Todd

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Molybdenum
CAS Numbers: 7439-98-7
Date: May 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for molybdenum due inadequate information on the molybdenum and copper intake in the acute-duration studies reporting adverse reproductive effects and the conflicting results between the acute-duration studies and high-quality, intermediate-duration studies.

Rationale for Not Deriving an MRL: A small number of studies have evaluated the acute toxicity of molybdenum. One human study (Deosthale and Gopalan 1974) examining a limited number of potential endpoints did not find alterations in urinary uric acid levels in subjects exposed to doses as high as 0.022 mg molybdenum/kg/day for 10 days. In rabbits, exposure to 1.2 mg molybdenum/kg/day as ammonium heptamolybdate in the diet for 14 days resulted in a 60% increase in serum triglyceride levels (Bersenyi et al. 2008); no histological alterations were observed in the liver or kidneys. The toxicological significance of this finding is not known and has not been reported in a study of male rabbits exposed to 0.58 mg molybdenum/kg/day as ammonium heptamolybdate (Bersenyi et al. 2008) or rats exposed to 60 mg molybdenum/kg/day as sodium molybdate for 90 days (Murray et al. 2014a).

Reproductive effects have been observed in male and female mice and rabbits. In female mice, an increased rate of abnormal MII oocytes was observed at 11 mg molybdenum/kg/day (Zhang et al. 2013). A second acute-exposure study in rabbits exposed to 1.2 mg molybdenum/kg/day as ammonium heptamolybdate for 14 days (Bersenyi et al. 2008) and an intermediate-duration oral study in rats exposed to 60 mg molybdenum/kg/day as sodium molybdate for 90 days (Murray et al. 2014a) did not find histological alterations in the ovaries. In males, a significant decrease in sperm concentration and motility and an increase in sperm abnormalities were observed at 25 mg molybdenum/kg/day in mice (Zhai et al. 2013). A rabbit study reported a reduction in mature spermatocytes in rabbits exposed to 0.58 mg molybdenum/kg/day, but did not report the incidence or statistical significance (Bersenyi et al. 2008). Intermediate-duration studies in rats did not find significant alterations in sperm parameters in rats exposed to 60 mg molybdenum/kg/day as sodium molybdate for 90 days (Murray et al. 2014a) or in rats exposed to 40 mg molybdenum/kg/day as sodium molybdate in a 2-generation study (Murray et al. 2019). Interpretation of the Zhang et al. (2013) and Zhai et al. (2013) studies is limited by the lack of information on the copper content of the "commercial standard pellet" diet used in these studies and the lack of information on molybdenum doses. ATSDR estimated doses using the reported molybdenum concentration in the drinking water and reference values for water consumption and body weight (Zhang et al. 2013) or the midpoint of the reported body weights and an estimated water consumption based on this body weight (Zhai et al. 2013).

The acute-duration oral database was not considered suitable for derivation of an MRL due to the limited information on the molybdenum and copper intake and the conflicting results between the findings of the Zhang et al. (2013) and Zhai et al. (2013) studies with the intermediate-duration Murray et al. (2014a, 2019) studies.

Agency Contacts (Chemical Managers): G. Daniel Todd

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:**	Molybdenum
CAS Numbers:	7439-98-7
Date:	May 2020
Profile Status:	Final
Route:	Oral
Duration:	Intermediate
MRL	0.06 mg molybdenum/kg/day
Critical Effect:	Renal effect, proximal tubule hyperplasia
Reference:	Murray et al. 2014a
Point of Departure:	NOAEL of 17 mg molybdenum/kg/day
Uncertainty Factor:	100
Modifying Factor:	3
LSE Graph Key:	14
Species:	Rat

MRL Summary: An intermediate-duration oral MRL of 0.06 mg molybdenum/kg/day was derived for molybdenum based on an increased incidence of renal proximal tubule hyperplasia in rats exposed to sodium molybdate in the diet for 90 days (Murray et al. 2014a). The MRL is based on a NOAEL of 17 mg molybdenum/kg/day, a total uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability), and a modifying factor of 3 (to address concern that reproductive/developmental alterations may be sensitive outcomes in populations with marginal copper intakes). The MRL is calculated based on the assumption of healthy dietary levels of molybdenum and copper and represents the level of exposure above and beyond the normal diet.

Selection of the Critical Effect: Several adverse effects have been reported in intermediate-duration oral studies in laboratory animals. Observed effects include kidney damage (Bompart et al. 1990; Murray et al. 2014a, 2019), decreased body weight gain (Bompart et al. 1990; Lyubimov et al. 2004; Mills et al. 1958; Murray et al. 2014a; Van Reen and Williams 1956; Williams and Van Reen 1956), hematological effects (Arrington and Davis 1953; Lyubimov et al. 2004), reproductive effects (Fungwe et al. 1990; Jeter and Davis 1954; Lyubimov et al. 2004; Murray et al. 2014a; Pandey and Singh 2002; Wang et al. 2016), and developmental effects (Pandey and Singh 2002).

The toxicity of molybdenum can be influenced by several factors including animal species; previous dietary history; relative amounts of dietary molybdenum, copper, and sulfur; and the form of molybdenum. Copper nutritional status is particularly important in evaluating the relevance of animal toxicity studies for establishing an MRL. Marked differences in the distribution of molybdenum and copper and the toxicity of molybdenum have been observed in rats exposed to high doses of molybdenum and maintained on a copper-deficient diet compared to those maintained on a copper-adequate diet (Brinkman and Miller 1961; Johnson et al. 1969; Nederbragt 1980, 1982; Sasmal et al. 1968). Since the average copper intake of the U.S. population exceeds the dietary requirements (NAS 2001), studies in which animals were fed inadequate levels of copper were not considered relevant for MRL derivation and were excluded from further consideration. Similarly, studies in which the molybdenum was administered as ammonium tetrathiomolybdate were also excluded since administration of tetrathiomolybdate compounds can result in shifts in the copper levels in rats fed copper-adequate diets (increases in serum and kidney copper levels and decreases in liver copper levels) (Mills et al. 1981a), and copper supplementation of rats exposed to ammonium tetrathiomolybdate resulted in a reversal of adverse effects (Lyubimov et al. 2004). A summary of the NOAEL and LOAEL values for studies with adequate copper in the diet is presented in Table A-8.

Table A-8. Summary of Health Effects Following Intermediate-Duration Oral Exposure to Molybdenum

Species, duration (route)	NOAEL	LOAEL	Effect	Reference (compound)
Body weight				
Rat 147–158 days (diet)		40	22% decrease in maternal body weight gain on GDs 0–7; <10% decrease over entire study	Murray et al. 2019 (sodium molybdate)
Rat 90 days (diet)	17	60	Decrease in body weight gain in males; terminal weights 15.2% less than controls	Murray et al. 2014a (sodium molybdate)
Rat 5 weeks (diet)		74	36% decrease in body weight gain	Mills et al. 1958 (sodium molybdate)
Rat 8 weeks (gavage)	40	80	Decrease in body weight gain; terminal body weight was 26% lower than in controls	Bompart et al. 1990 (ammonium heptamolybdate)
Rat 6 weeks (diet)	85			Williams and Van Reen 1956 (sodium molybdate)
Rat 6 weeks (diet)		90	22% decrease in body weight gain	Williams and Van Reen 1956 (sodium molybdate)
Rat 4–5 weeks (diet)		110	46–48% decrease in body weight gain	Van Reen and Williams 1956 (sodium molybdate)
Rat 147–158 days (drinking water)	40			Murray et al. 2019 (sodium molybdate)
Hematological effects				
Rabbit 30–84 days (diet)	25	54	Anemia	Arrington and Davis 1953 (sodium molybdate)
Rabbit ≥8 weeks (diet)	7			Jeter and Davis 1954 (sodium molybdate)
Rat 90 days (diet)	60			Murray et al. 2014a (sodium molybdate)
Rat 6 weeks (diet)	70			Gray and Daniel 1954 (sodium molybdate)
Kidney effects				
Rat 90 days (diet)	17	60	Slight diffuse hyperplasia in proximal tubules	Murray et al. 2014a (sodium molybdate)
Rat 8 weeks (gavage)	40	80	Diuresis and creatinuria and decreases in creatinine clearance	Bompart et al. 1990 (ammonium heptamolybdate)
Rat 147–158 days (diet)	40			Murray et al. 2019 (sodium molybdate)
Rats 147–158 days (drinking water)	40			Murray et al. 2019 (sodium molybdate)

Table A-8. Summary of Health Effects Following Intermediate-Duration Oral Exposure to Molybdenum

Species, duration (route)	NOAEL	LOAEL	Effect	Reference (compound)
Reproductive effects				
Rat 8 weeks (drinking water)	0.76	1.5	Prolonged estrus phase; no effect on female fertility	Fungwe et al. 1990 (sodium molybdate)
Rat 60 days (gavage)	3.4[a]	10[a]	Decreases in sperm count and motility; increases in sperm abnormalities	Pandey and Singh 2002 (sodium molybdate)
Rat 60 days (gavage)		10[a]	Decreases in male fertility	Pandey and Singh 2002 (sodium molybdate)
Mouse 100 days (drinking water)		100	Decreased sperm density and motility	Wang et al. 2016 (unspecified molybdenum compound)
Rat 90 days (diet)	60		No treatment-related alterations in sperm parameters; no alterations in vaginal cytology, estrus cycle, or histology of male or female reproductive tissues	Murray et al. 2014a (sodium molybdate)
Rat ≥8 weeks (diet)	7		No effect on fertility	Jeter and Davis 1954 (sodium molybdate)
Rat 2 generations (diet)	40		No effects on sperm parameters, estrous cycling, or fertility	Murray et al. 2019 (sodium molybdate)
Rat 2 generations (drinking water)	40		No effects on sperm parameters, estrous cycling, or fertility	Murray et al. 2019 (sodium molybdate)
Developmental effects[b]				
Rat (males only) 60 days (gavage)		10[a]	Increased post-implantation losses, increased resorptions, decreased number of live fetuses, and decreases in fetal weight and crown-rump length	Pandey and Singh 2002 (sodium molybdate)
Rat ≥8 weeks (diet)	7			Jeter and Davis 1954 (sodium molybdate)
Rat GDs 6–20 (diet)	37.5			Murray et al. 2014b (sodium molybdate)
Rat 2 generations (diet)	40			Murray et al. 2019 (sodium molybdate)
Rat 2 generations (drinking water)	40			Murray et al. 2019 (sodium molybdate)

[a]Adjusted for intermittent exposure (5 days/week).
[b]The copper content of the basal diet (6 g/kg diet) in the Fungwe et al. (1990) study is below the recommended level of 8 g/kg required for pregnancy and lactation. Thus, the observed developmental effects are not included in this table.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

The Fungwe et al. (1990) study identified the lowest LOAEL value: prolonged estrus phase without an effect on fertility in rats exposed to 1.5 mg molybdenum/kg/day as sodium molybdate in drinking water for 8 weeks (Fungwe et al. 1990). However, this finding was not selected as the critical effect because other high-quality studies have not reported estrus cycle alterations in a 90-day (Murray et al. 2014a) study or 2-generation study (Murray et al. 2019). Additionally, confidence in this study is decreased by the limited information on doses. The study reported molybdenum drinking water concentrations but did not calculate doses. ATSDR estimated doses using reference values for body weight and drinking water consumption. As presented in Table A-9, a comparison with the molybdenum liver concentrations in this study with levels reported in the Murray et al. (2014a, 2019) studies suggested that these estimated doses may have underestimated the actual doses. In the Fungwe et al. (1989) study, the average liver molybdenum level was 10.76 µg/g in the 15 mg/kg/day group; in the Murray et al. (2014a, 2014b) studies, the liver molybdenum level was 4.10–4.92 µg/g in the 17 mg/kg/day groups.

Table A-9. Comparison of Molybdenum Liver Concentrations in Female Rats

Study	Dose Liver molybdenum concentration				
Murray et al. 2019[a] (water exposure, unless noted)	0 mg/kg/day	5 mg/kg/day	17 mg/kg/day	40 mg/kg/day	40 mg/kg/day (dietary exposure)
	2.96 µg/g	3.18 µg/g	4.10 µg/g	6.48 µg/g	7.23 µg/g
Murray et al. 2014a[b] (dietary exposure)	0 mg/kg/day	5 mg/kg/day	17 mg/kg/day	60 mg/kg/day	
	2.46 µg/g	3.51 µg/g	4.92 µg/g	13.0 µg/g	
Fungwe et al. 1989[c] (water exposure)	0 mg/kg/day	0.76 mg/kg/day	1.5 mg/kg/day	7.6 mg/kg/day	15 mg/kg/day
	2.63 µg/g	5.01 µg/g	5.03 µg/g	7.77 µg/g	10.76 µg/g

[a]Parental generation.
[b]Liver concentrations reported in Murray et al. (2019).
[c]Liver concentrations from a study by Fungwe et al. (1990) utilizing the same water concentrations as Fungwe et al. (1989).

The next highest LOAEL is 14 mg molybdenum/kg for decreases in sperm count and motility, increased sperm abnormalities, decreased male fertility, increased post-implantation losses, decreased number of live fetuses, and decreased fetal weight in a study of male rats receiving gavage doses of sodium molybdate 5 days/week during a 60-day period (Pandey and Singh 2002). The reliability of this LOAEL is uncertain due to the lack of information on the copper content of the diet and because decreases in fertility and alterations in sperm parameters have not been observed in other high-quality studies involving exposure to 40 mg molybdenum/kg/day via the diet or drinking water in a 2-generation study (Murray et al. 2019) or 60 mg molybdenum/kg/day via the diet in a 90-day study (Murray et al. 2014a). Additionally, no developmental effects were observed in single-generation (Murray et al. 2014b) or 2-generation (Murray et al. 2019) studies in rats exposed to 37.5–40 mg/kg/day.

As with the reproductive and developmental effects, only one study reported hematological effects. Anemia was reported in rabbits exposed to 54 mg molybdenum/kg/day as sodium molybdate in the diet for 30–84 days (Arrington and Davis 1953). This is considered a low-quality study because the molybdenum was sprayed on the food pellets but there was no measurement of actual dietary

concentrations, only 2–5 animals per group were tested, and no information was provided on which hematological parameters were altered. Additionally, the diet may not have provided adequate copper levels since copper supplementation was administered to the 54 mg/kg/day group to prevent deaths in the 3/5 animals that exhibited "severe toxic symptoms" characteristic of copper deficiency.

If the reproductive, developmental, and hematological effects are excluded because they were reported in lesser-quality studies and were not confirmed in higher-quality studies, then the lowest LOAEL is 60 mg/kg/day for body weight and renal effects (Murray et al. 2014a). A 15% decrease in body weight gain was observed in male rats; no significant alterations were observed in females. Although a decrease in food consumption was also observed at this dose level, decreases in food efficiency observed at this dose suggest that the decrease in body weight was not solely related to the decreased food intake. The renal effects consisted of slight diffuse hyperplasia in the renal proximal tubules of 2/10 female rats. The investigators (Murray et al. 2014a) noted that this effect is an uncommon background finding in rats of this age and considered it to be treatment related; they also suggested that the effect may be due to the high levels of copper in the kidneys. Kidney effects (degeneration followed by regeneration) have been observed in rats exposed to high levels of copper in the diet (Haywood 1985). A second molybdenum study (Bompart et al. 1990) reported diuresis, creatinuria, decreases in creatinine clearance, and increases in daily excretion of immunoreactive kallikrein in rats administered 80 mg molybdenum/kg/day via gavage for 8 weeks. These alterations are suggestive of decreased glomerular function and distal tubule damage; the absence of changes in the brush border enzymes alanine aminopeptidase and γ-glutamyl transpeptidase suggests no damage to the proximal tubule functional capacity. The study did not include histopathological examination of the kidneys. Although the incidence of proximal tubular hyperplasia was not statistically significant in the high-dose females in the Murray et al. (2014a) study, support for identifying this as the critical effect comes from the Bompart et al. (1990) study, which found evidence of impaired renal function in rats exposed to a slightly higher dose.

Several studies have reported decreases in body weight gain; the lowest LOAEL for this effect was 40 mg molybdenum/kg/day as sodium molybdate in the diet in a 2-generation study (Murray et al. 2019). This study reported a 22% decrease in body weight gain on GDs 0–7 in the parental-generation females. The difference in body weight gain over the length of the study was <10% lower than the controls. This was not observed in the F1 generation and was not observed in P or F1 generation rats similarly exposed to 40 mg molybdenum/kg/day as sodium molybdate in the drinking water (Murray et al. 2019). Decreases in body weight have also been observed at higher molybdenum doses (Bompart et al. 1990; Mills et al. 1958; Murray et al. 2014a; Van Reem and Williams 1956). The decrease in body weight gain observed in the Murray et al. (2019) study was not selected as the basis of the MRL because it was not replicated in the F1 generation or in rats exposed via drinking water (Murray et al. 2019).

Selection of the Principal Study: The Murray et al. (2014a) study was selected as the principal study because it identified the lowest LOAEL for renal effects.

Summary of the Principal Study:

Murray FJ, Sullivan FM, Tiwary AK, et al. 2014a. 90-Day subchronic toxicity study of sodium molybdate dihydrate in rats. Regul Toxicol Pharmacol 79:579-588.

Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 5, 17, or 60 mg molybdenum/kg/day (actual concentrations were 0, 4.5, 15.1, and 54.8 mg/kg/day, respectively, in males and 0, 5.4, 19.0, and 65.2 mg/kg/day, respectively, in females and the average overall intakes were 0, 5.0, 17.1, and 60.0 mg/kg/day, respectively) as sodium molybdate dihydrate in the diet for 91 and 92 days; additional groups of rats (10/sex/group) were similarly exposed to 0 or 60 mg/kg/day for 91–92 days and then continued on the basal diet for 60 days. The basal diet contained 906.5 µg/kg molybdenum and

14.23 mg/kg copper; the investigators estimated that the control group received 0.08 mg molybdenum/kg/day. The following parameters were used to assess toxicity: cage-side observations, weekly clinical examinations, ophthalmic examination, weekly body weight measurements, measurement of hematological (hemoglobin, hematocrit, erythrocyte, platelet, mean corpuscular hemoglobin concentration, mean corpuscular volume, red cell distribution width, total and differential leukocyte, reticulocyte, and prothrombin time) and serum chemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, uric acid, total bilirubin, sodium, potassium, chloride, calcium, and inorganic phosphorus) parameters, organ weights (adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate gland and seminal vesicles, spleen, testes, thymus, thyroid/ parathyroid glands, and uterus with cervix), and histopathology examination of major tissues and organs in control and 60 mg/kg/day groups (primary and recovery groups) and the adrenal glands from males and kidneys from females in the 5 and 17 mg/kg/day groups. Additionally, sperm counts and sperm mobility and vaginal cytology and estrus cycles were evaluated.

Significant decreases in body weight gain were observed at 60 mg/kg/day in males starting at week 1 and in females starting at week 6. Terminal body weights were 15.2 and 5.6% less than controls, with only the males being significantly different from controls. At the end of the recovery period, the 60 mg/kg/day males weighed significantly less (9.5%) than controls. Decreases in food consumption were observed on numerous occasions in the males exposed to 60 mg/kg/day; a decrease in food conversion efficiency was also observed in this group. No significant or treatment-related alterations in hematological or serum chemistry parameters were observed. Significant decreases in absolute brain, liver, heart, spleen, and pituitary weights were observed in males exposed to 60 mg/kg/day; however, there were no significant alterations in relative organ weights. Treatment-related histopathological alterations were limited to a slight diffuse hyperplasia in the renal proximal tubules in 2/10 females in the 60 mg/kg/day group; the investigators considered it to be treatment-related because it is an uncommon finding at this age. No significant alterations in vaginal cytology or estrus cycles were observed. Similarly, no significant alterations in spermatid or sperm counts or sperm morphology were observed in males. A slight decrease in sperm motility was observed at 60 mg/kg/day; however, this was likely attributable to the control group having a value that approached the upper limit among historical controls and was not considered treatment related. No alterations in reproductive organ weights or histological alterations were observed.

Selection of the Point of Departure for the MRL: The NOAEL of 17 mg molybdenum/kg/day was selected as the POD for the MRL. BMD modeling was not considered because a response was only observed at the highest dose tested. A dataset exhibiting a response only at the highest dose level would likely provide limited information regarding the shape of a dose-response curve.

Calculations: The investigators estimated doses using body weight and food consumption data.

Intermittent Exposure: Not applicable.

Uncertainty Factor and Modifying Factor: The NOAEL is divided by a total uncertainty factor (UF) of 100 and a modifying factor (MF) of 3

- 10 UF for extrapolation from animals to humans
- 10 UF for human variability
- 3 MF for concern that reproductive and/or developmental effects may be a more sensitive endpoint than kidney effects in populations with marginal copper intakes. The copper content of the Murray et al. (2014b, 2019) reproductive/developmental studies used a commercial diet with a fairly high copper content. In contrast, the Fungwe et al. (1990) study, which reported

reproductive effects, utilized a diet that was slightly higher than the dietary requirement. The differences in the copper contents of the diet may explain differences between the study results.

MRL = NOAEL ÷ (UFs x MF)
0.06 mg molybdenum/kg/day = 17 mg molybdenum/kg/day ÷ ((10 x 10) x 3)

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Selection of the POD is supported by the Bompart et al. (1990) study, which found decreases in kidney function in rats administered sodium molybdate.

The MRL is calculated based on the assumption of healthy dietary levels of molybdenum and copper and represents the level of exposure above and beyond the normal diet.

Agency Contacts (Chemical Managers): G. Daniel Todd

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Molybdenum
CAS Numbers: 7439-98-7
Date: May 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for molybdenum. The only available experimental study was considered a low-quality study that was not considered a suitable basis for an MRL.

Rationale for Not Deriving an MRL: Data on the chronic toxicity of molybdenum come from several population-based studies; most of these studies looked for associations between background exposure to molybdenum and adverse health outcomes. No laboratory animal studies were identified.

Koval'skiy et al. (1961) found increases in blood uric acid and symptoms of gout in residents living in Armenia with high levels of molybdenum in the soil and food; the investigators estimated that the residents were exposed to 10–15 mg/day (0.14–0.21 mg/kg/day). A series of small studies of residents living in areas of Colorado with high levels of molybdenum in the drinking water did not find significant increases in uric acid levels; one study estimated that molybdenum intake was 500 µg/day (0.007 mg/kg/day) (EPA 1979). Other studies have found significant associations between serum or urinary molybdenum levels and the severity of complications from diabetes (Rodriguez Flores et al. 2011), high blood pressure (Yorita Christensen 2013), semen quality (Meeker et al. 2008), testosterone levels (Meeker et al. 2010), and psychomotor index in infants (molybdenum levels were measured in the mothers) (Vazques-Salas et al. 2014). However, none of these studies established causality, and the molybdenum levels accounted for only a small percentage of the variance.

Although the Koval'sky et al. (1961) study provided an estimated dose, the study was not considered suitable for derivation of a chronic-duration oral MRL for molybdenum. The study has a number of deficiencies that limit the interpretation of the results: (1) the control group consisted of 5 individuals compared to 52 subjects in the exposed group; (2) no information was provided on the controls to assess whether they were matched to the exposed group; (3) it does not appear that the study controlled for potential confounders, such as diet and alcohol, which can increase uric acid levels; and (4) NAS (2001) noted that there were potential analytical problems with the measurement of serum and urine copper levels.

Agency Contacts (Chemical Managers): G. Daniel Todd

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR MOLYBDENUM

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to molybdenum.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for molybdenum. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of molybdenum have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of molybdenum are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

- Health Effects
  - Species
    - Human
    - Laboratory mammals
  - Route of exposure
    - Inhalation
    - Oral
    - Dermal (or ocular)
    - Parenteral (these studies will be considered supporting data)
  - Health outcome
    - Death
    - Systemic effects
    - Body weight effects
    - Respiratory effects
    - Cardiovascular effects
    - Gastrointestinal effects
    - Hematological effects
    - Musculoskeletal effects
    - Hepatic effects
    - Renal effects
    - Dermal effects
    - Ocular effects
    - Endocrine effects
    - Immunological effects
    - Neurological effects
    - Reproductive effects
    - Developmental effects
    - Other noncancer effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for molybdenum released for public comment in 2017. The following main databases were searched in January 2018:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for molybdenum. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases

were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to molybdenum were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
01/2018	((("Molybdenum/toxicity"[mh] OR "Molybdenum/adverse effects"[mh] OR "Molybdenum/poisoning"[mh] OR "Molybdenum/pharmacokinetics"[mh]) OR ("Molybdenum"[mh] AND ("environmental exposure"[mh] OR ci[sh])) OR ("Molybdenum"[mh] AND toxicokinetics[mh:noexp]) OR ("Molybdenum/blood"[mh] OR "Molybdenum/cerebrospinal fluid"[mh] OR "Molybdenum/urine"[mh]) OR ("Molybdenum"[mh] AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR ("Molybdenum"[mh] AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR ("Molybdenum/antagonists and inhibitors"[mh]) OR ("Molybdenum/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Molybdenum"[mh] AND cancer[sb]) OR ("Molybdenum/pharmacology"[majr])) AND (2013/12/01 : 3000[dp] OR 2014/12/01 : 3000[mhda])) OR (("1317-33-5"[rn] OR "12033-29-3"[rn] OR "12033-33-9"[rn] OR "11098-99-0"[rn] OR "18868-43-4"[rn] OR "1313-27-5"[rn] OR "1313-29-7"[rn] OR "11098-84-3"[rn] OR "27546-07-2"[rn] OR "12054-85-2"[rn] OR "15060-55-6"[rn] OR "7631-95-0"[rn] OR "10102-40-6"[rn] OR "7789-82-4"[rn] OR "12011-97-1"[rn] OR "11119-46-3"[rn] OR "11062-51-4"[rn] OR "10241-05-1"[rn] OR "1309-56-4"[rn] OR "7783-77-9"[rn] OR "13939-06-5"[rn] OR "14221-06-8"[rn] OR "13814-74-9"[rn] OR "12027-67-7"[rn] OR "13106-76-8"[rn]) AND (("Disulfides/toxicity"[mh] OR "Disulfides/adverse effects"[mh] OR "Disulfides/poisoning"[mh] OR "Disulfides/pharmacokinetics"[mh]) OR ("Disulfides/blood"[mh] OR "Disulfides/cerebrospinal fluid"[mh] OR "Disulfides/urine"[mh]) OR ("Disulfides/antagonists and inhibitors"[mh]) OR ("Disulfides/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Disulfides/pharmacology"[majr]) OR ("Chlorides/toxicity"[mh] OR "Chlorides/adverse effects"[mh] OR "Chlorides/poisoning"[mh] OR "Chlorides/pharmacokinetics"[mh]) OR ("Chlorides/blood"[mh] OR "Chlorides/cerebrospinal fluid"[mh] OR "Chlorides/urine"[mh]) OR ("Chlorides/antagonists and inhibitors"[mh]) OR ("Chlorides/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Chlorides/pharmacology"[majr]) OR ("Oxides/toxicity"[mh] OR "Oxides/adverse effects"[mh] OR "Oxides/poisoning"[mh] OR "Oxides/pharmacokinetics"[mh]) OR ("Oxides/blood"[mh] OR "Oxides/cerebrospinal fluid"[mh] OR "Oxides/urine"[mh]) OR

Table B-2. Database Query Strings

Database search date	Query string
	("Oxides/antagonists and inhibitors"[mh]) OR ("Oxides/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Oxides/pharmacology"[majr]) OR (("disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh]) AND ("environmental exposure"[mh] OR ci[sh])) OR (("disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh]) AND toxicokinetics[mh:noexp]) OR (("disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh]) AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR (("disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh]) AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR (("disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh]) AND cancer[sb])) AND (2013/12/01 : 3000[dp] OR 2014/12/01 : 3000[mhda])) OR "12027-67-7"[rn] OR ((("7439-98-7"[rn] OR "1317-33-5"[rn] OR "12033-29-3"[rn] OR "12033-33-9"[rn] OR "11098-99-0"[rn] OR "18868-43-4"[rn] OR "1313-27-5"[rn] OR "1313-29-7"[rn] OR "11098-84-3"[rn] OR "27546-07-2"[rn] OR "12054-85-2"[rn] OR "15060-55-6"[rn] OR "7631-95-0"[rn] OR "10102-40-6"[rn] OR "7789-82-4"[rn] OR "12011-97-1"[rn] OR "11119-46-3"[rn] OR "11062-51-4"[rn] OR "10241-05-1"[rn] OR "1309-56-4"[rn] OR "7783-77-9"[rn] OR "13939-06-5"[rn] OR "14221-06-8"[rn] OR "13814-74-9"[rn] OR "12027-67-7"[rn] OR "13106-76-8"[rn]) NOT ("molybdenum"[mh] OR "disulfides"[mh] OR "chlorides"[mh] OR "oxides"[mh])) AND (2013/12/01 : 3000[dp] OR 2014/12/01 : 3000[mhda])) OR (("Ammonium molybdenum sulfide"[tw] OR "Ammonium tetrasulfidomolybdate(2-)"[tw] OR "Ammonium tetrathiomolybdate"[tw] OR "Ammonium thiomolybdate(VI)"[tw] OR "ATTM"[tw] OR "Bis(ammonium)tetrathiomolybdate(2-)"[tw] OR "Calcium molybdate"[tw] OR "Calcium molybdate(VI)"[tw] OR "Calcium molybdenate"[tw] OR "Calcium molybdenum oxide"[tw] OR "Coprexa"[tw] OR "Diammonium tetrakis(sulfido)molybdate(2-)"[tw] OR "Diammonium tetrakis(thioxo)molybdate"[tw] OR "Diammonium tetrasulfidomolybdate"[tw] OR "Diammonium tetrathiomolybdate"[tw] OR "Diammonium tetrathiomolybdate(2-)"[tw] OR "Diammonium tetrathiooxomolybdate(2-)"[tw] OR "Diammonium tetrathioxomolybdate(2-)"[tw] OR "Diammonium thiomolybdate"[tw] OR "Dimolybdenum tetraacetate"[tw] OR "Dimolybdenum trioxide"[tw] OR "Dodecachlorohexamolybdenum"[tw] OR "Hexafluoromolybdenum"[tw] OR "Hexamolybdenum dodecachloride"[tw] OR "MC 400WR"[tw] OR "Molybdate, calcium"[tw] OR "Molybdenite"[tw] OR "Molybdenum anhydride"[tw] OR "Molybdenum carbide"[tw] OR "Molybdenum chloride"[tw] OR "Molybdenum chloride oxide"[tw] OR "Molybdenum dioxide"[tw] OR "Molybdenum fluoride"[tw] OR "Molybdenum hexafluoride"[tw] OR "Molybdenum monocarbide"[tw] OR "Molybdenum oxide"[tw] OR "Molybdenum oxychloride"[tw] OR "Molybdenum oxytrichloride"[tw] OR "Molybdenum sesquioxide"[tw] OR "Molybdenum sulfide"[tw] OR "Molybdenum trichloride monoxide"[tw] OR "Molybdenum trichloride oxide"[tw] OR "Molybdenum trisulfide"[tw] OR "Molybdenum(6+) fluoride"[tw] OR "Molybdenum(II) acetate"[tw] OR "Molybdenum(IV) oxide"[tw] OR "Molybdic acid, calcium salt"[tw] OR "Octachlorohexamolybdenum(4+) tetrachloride"[tw] OR "Tetraacetatodimolybdenum"[tw] OR "tetrakis(acetato)di-Molybdenum"[tw] OR "Tetrakis(acetato)dimolybdenum"[tw] OR "Tetrakis(acetato)molybdenum"[tw] OR "Tetrakis(mu-(acetato-O:O'))dimolybdenum"[tw]

Table B-2. Database Query Strings

Database search date	Query string
	OR "tetrakis(mu-acetato)di-Molybdenum"[tw] OR "Tetrakis(mu-acetato)dimolybdenum"[tw] OR "tetrakis[mu-(acetato-O:O')]di-Molybdenum"[tw] OR "Thiomolybdic acid (H2MoS4), diammonium salt"[tw] OR "Thiomolybdic acid, diammonium salt"[tw] OR "Tiomolibdate diammonium"[tw] OR "Trichlorooxomolybdenum"[tw] OR "Trichlorooxomolybdenum(V)"[tw]) AND (2013/12/01 : 3000[dp] OR 2014/12/01 : 3000[crdat] OR 2014/12/01 : 3000[edat])) OR ((("3N5"[tw] OR "A Powder"[tw] OR "Ammonium dimolybdate"[tw] OR "Ammonium heptamolybdate"[tw] OR "Ammonium heptamolybdate tetrahydrate"[tw] OR "Ammonium molibdate"[tw] OR "Ammonium molibdenum oxide"[tw] OR "Ammonium molybdate"[tw] OR "Ammonium molybdate hydrate"[tw] OR "Ammonium molybdate tetrahydrate"[tw] OR "Ammonium molybdate(VI)"[tw] OR "Ammonium molybdenum oxide"[tw] OR "Ammonium molybdenum sulfide"[tw] OR "ammonium paramolybdate"[tw] OR "Ammonium paramolybdate tetrahydrate"[tw] OR "Ammonium tetrasulfidomolybdate(2-)"[tw] OR "Ammonium tetrathiomolybdate"[tw] OR "Ammonium thiomolybdate(VI)"[tw] OR "Amperit 105.054"[tw] OR "Amperit 106.2"[tw] OR "ATTM"[tw] OR "Bis(ammonium)tetrathiomolybdate(2-)"[tw] OR "Bouen SKN 301"[tw] OR "C-Powder"[tw] OR "Calcium molybdate"[tw] OR "Calcium molybdate(VI)"[tw] OR "Calcium molybdenate"[tw] OR "Calcium molybdenum oxide"[tw] OR "Coprexa"[tw] OR "DAG 206"[tw] OR "DAG 325"[tw] OR "DAG-V 657"[tw] OR "Defric coat HMB 2"[tw] OR "Diammonium dimolybdate"[tw] OR "Diammonium tetrakis(sulfido)molybdate(2-)"[tw] OR "Diammonium tetrakis(thioxo)molybdate"[tw] OR "Diammonium tetrasulfidomolybdate"[tw] OR "Diammonium tetrathiomolybdate"[tw] OR "Diammonium tetrathiomolybdate(2-)"[tw] OR "Diammonium tetrathiooxomolybdate(2-)"[tw] OR "Diammonium tetrathioxomolybdate(2-)"[tw] OR "Diammonium thiomolybdate"[tw] OR "Dimolybdenum tetraacetate"[tw] OR "Dimolybdenum trioxide"[tw] OR "dimolybdenum trisulfide "[tw] OR "Disodium molybdate"[tw] OR "Disodium molybdate dihydrate"[tw] OR "Disodium tetraoxomolybdate"[tw] OR "DM 1 (sulfide)"[tw] OR "DMI 7"[tw] OR "Dodecachlorohexamolybdenum"[tw] OR "Hexaammonium heptamolybdate tetrahydrate"[tw] OR "Hexaammonium molybdate tetrahydrate"[tw] OR "Hexacarbonylmolybdenum"[tw] OR "Hexafluoromolybdenum"[tw] OR "Hexamolybdenum dodecachloride"[tw] OR "JCPDS 35-0609"[tw] OR "Liqui-Moly LM 11"[tw] OR "Liqui-Moly LM 2"[tw] OR "Liqui-Moly Z Powder"[tw] OR "LM 13"[tw] OR "MC 400WR"[tw] OR "MChVL"[tw] OR "MD 40"[tw] OR "Metco 63"[tw] OR "MF 000"[tw] OR "MIPO-M 15"[tw] OR "Mo 1202T"[tw] OR "Mo-1202T"[tw] OR "Moly Fine Powder Y"[tw] OR "Moly Powder B"[tw] OR "Moly Powder C"[tw] OR "Moly Powder PA"[tw] OR "Moly Powder PB"[tw] OR "Moly Powder PS"[tw] OR "Molybdate (Mo2O72-), diammonium"[tw] OR "Molybdate (MoO42-), disodium, dihydrate, (T-4)-"[tw] OR "Molybdate, calcium"[tw] OR "Molybdena"[tw] OR "Molybdenite"[tw] OR "Molybdenum"[tw] OR "Molybdenum anhydride"[tw] OR "Molybdenum bisulfide"[tw] OR "Molybdenum carbide"[tw] OR "Molybdenum carbonyl"[tw] OR "Molybdenum chloride"[tw] OR "Molybdenum chloride oxide"[tw] OR "Molybdenum dioxide"[tw] OR "Molybdenum disulfide"[tw] OR "Molybdenum disulphide"[tw] OR "Molybdenum fluoride"[tw] OR "Molybdenum hexacarbonyl"[tw] OR "Molybdenum hexafluoride"[tw] OR "Molybdenum metallicum"[tw] OR "Molybdenum monocarbide"[tw] OR "Molybdenum oxide"[tw] OR "Molybdenum oxychloride"[tw] OR "Molybdenum oxytrichloride"[tw] OR "Molybdenum pentachloride"[tw] OR "Molybdenum peroxide"[tw] OR "Molybdenum sesquioxide"[tw] OR "Molybdenum sesquisulfide"[tw] OR "Molybdenum sodium oxide"[tw] OR "Molybdenum sulfide"[tw] OR "Molybdenum sulphide"[tw] OR "Molybdenum trichloride monoxide"[tw] OR "Molybdenum trichloride oxide"[tw] OR "Molybdenum trioxide"[tw] OR "Molybdenum trioxide pentamer"[tw] OR "Molybdenum trioxide tetramer"[tw] OR "Molybdenum trisulfide"[tw] OR "Molybdenum(6+) fluoride"[tw] OR "Molybdenum(II) acetate"[tw] OR "Molybdenum(II) chloride"[tw] OR "molybdenum(III)

Table B-2. Database Query Strings

Database search date	Query string
	sulfide"[tw] OR "molybdenum(IV) oxide"[tw] OR "Molybdenum(IV) sulfide"[tw] OR "Molybdenum(V) chloride"[tw] OR "Molybdenum(VI) oxide"[tw] OR "Molybdenum(VI) trioxide"[tw] OR "Molybdenumperoxide"[tw] OR "Molybdic acid (H2Mo2O7), diammonium salt"[tw] OR "Molybdic acid (H2MoO4), calcium salt (1:1)"[tw] OR "Molybdic acid anhydride"[tw] OR "Molybdic acid, ammonium salt"[tw] OR "Molybdic acid, calcium salt"[tw] OR "Molybdic acid, disodium salt"[tw] OR "Molybdic acid, disodium salt, dihydrate"[tw] OR "Molybdic anhydride"[tw] OR "Molybdic oxide"[tw] OR "Molybdic trioxide"[tw] OR "Molycolloid CF 626"[tw] OR "Molyform 15"[tw] OR "Molyhibit 100"[tw] OR "Molyka R"[tw] OR "Molyka R-L 3"[tw] OR "Molyke R"[tw] OR "Molykote"[tw] OR "Molykote Microsize Powder"[tw] OR "Molykote Z"[tw] OR "Molykote Z Powder"[tw] OR "Molysulfide"[tw] OR "MOP-P 100"[tw] OR "Mopol M"[tw] OR "Mopol S"[tw] OR "Motimol"[tw] OR "MVCh 1"[tw] OR "Natural molybdenite"[tw] OR "Natural molybdite"[tw] OR "NeoZ"[tw] OR "Nichimoly C"[tw] OR "Octachlorohexamolybdenum(4+) tetrachloride"[tw] OR "OKS 110"[tw] OR "PA Powder"[tw] OR "Pentachloromolybdenum Molybdenite"[tw] OR "Pigment Black 34"[tw] OR "Pol-U"[tw] OR "Powder PA"[tw] OR "RAC 01"[tw] OR "SGC 15"[tw] OR "Sodium molybdate"[tw] OR "Sodium molybdate dihydrate"[tw] OR "Sodium molybdate(VI)"[tw] OR "Sodium molybdate(VI) dihydrate"[tw] OR "Sodium molybdenate"[tw] OR "Sodium molybdenum oxide"[tw] OR "Sodium tetraoxomolybdate(2-)"[tw] OR "Solvest 390A"[tw] OR "Sumipowder PA"[tw] OR "T-Powder"[tw] OR "Tetraacetatodimolybdenum"[tw] OR "tetrakis(acetato)di-Molybdenum"[tw] OR "Tetrakis(acetato)dimolybdenum"[tw] OR "Tetrakis(acetato)molybdenum"[tw] OR "Tetrakis(mu-(acetato-O:O'))dimolybdenum"[tw] OR "tetrakis(mu-acetato)di-Molybdenum"[tw] OR "Tetrakis(mu-acetato)dimolybdenum"[tw] OR "tetrakis[mu-(acetato-O:O')]di-Molybdenum"[tw] OR "Thiomolybdic acid, diammonium salt"[tw] OR "Tiomolibdate diammonium"[tw] OR "TMOIO"[tw] OR "Trichlorooxomolybdenum"[tw] OR "Trichlorooxomolybdenum(V)"[tw] OR "TsM1"[tw] OR "UP 10"[tw] OR "UP 50"[tw] OR ("Hexaammonium heptamolybdate"[tw] OR "Hexammonium heptamolybdat"[tw] OR "Hexammonium tetracosaoxoheptamolybdate"[tw] OR "Molybdate (Mo7O24), hexammonium"[tw] OR "Molybdate (Mo7O246-), ammonium (1:6)"[tw] OR "Molybdate (Mo7O246-), hexaammonium"[tw] OR "Molybdate, hexaammonium"[tw] OR "Molybdic acid (H6Mo7O24), hexaammonium salt"[tw] OR "Molybdic acid, hexaammonium salt"[tw] OR "Diammonium molybdate"[tw] OR "Diammonium tetraoxomolybdate(2-)"[tw] OR "Molybdate (MoO42-), ammonium (1:2), (T-4)-"[tw] OR "Molybdate (MoO42-), diammonium, (beta-4)-"[tw] OR "Molybdate (MoO42-), diammonium, (T-4)-"[tw] OR "Molybdic acid (H2MoO4), diammonium salt"[tw] OR "Molybdic acid, diammonium salt"[tw])) NOT medline[sb]) AND (2013/12/01 : 3000[dp] OR 2014/12/01 : 3000[crdat] OR 2014/12/01 : 3000[edat]))
Toxline	
01/2018	**Date limit 2013 to present:**
	7439-98-7[rn] OR 1317-33-5[rn] OR 12033-29-3[rn] OR 12033-33-9[rn] OR 11098-99-0[rn] OR 18868-43-4[rn] OR 1313-27-5[rn] OR 1313-29-7[rn] OR 11098-84-3[rn] OR 27546-07-2[rn] OR 12054-85-2[rn] OR 15060-55-6[rn] OR 7631-95-0[rn] OR 10102-40-6[rn] OR 7789-82-4[rn] OR 12011-97-1[rn] OR 11119-46-3[rn] OR 11062-51-4[rn] OR 10241-05-1[rn] OR 1309-56-4[rn] OR 7783-77-9[rn] OR 13939-06-5[rn] OR 14221-06-8[rn] OR 13814-74-9[rn]
	"3N5" OR "Ammonium dimolybdate" OR "Ammonium heptamolybdate" OR "Ammonium heptamolybdate tetrahydrate" OR "Ammonium molibdate" OR "Ammonium molibdenum oxide" OR "Ammonium molybdate" OR "Ammonium molybdate hydrate" OR "Ammonium molybdate tetrahydrate" OR "Ammonium molybdate(VI)" OR "Ammonium molybdenum oxide"

Table B-2. Database Query Strings

Database search date	Query string
	"Ammonium molybdenum sulfide" OR "ammonium paramolybdate" OR "Ammonium paramolybdate tetrahydrate" OR "Ammonium tetrasulfidomolybdate(2-)" OR "Ammonium tetrathiomolybdate" OR "Ammonium thiomolybdate(VI)" OR "Amperit 105.054" OR "Amperit 106.2" OR "ATTM" OR "Bis(ammonium)tetrathiomolybdate(2-)" OR "Bouen SKN 301" OR "C-Powder" OR "Calcium molybdate"
	"Calcium molybdate(VI)" OR "Calcium molybdenate" OR "Calcium molybdenum oxide" OR "Coprexa" OR "DAG 206" OR "DAG 325" OR "DAG-V 657" OR "Defric coat HMB 2" OR "Diammonium dimolybdate" OR "Diammonium tetrakis(sulfido)molybdate(2-)" OR "Diammonium tetrakis(thioxo)molybdate" OR "Diammonium tetrasulfidomolybdate" OR "Diammonium tetrathiomolybdate"
	"Diammonium tetrathiomolybdate(2-)" OR "Diammonium tetrathiooxomolybdate(2-)" OR "Diammonium tetrathioxomolybdate(2-)" OR "Diammonium thiomolybdate" OR "Dimolybdenum tetraacetate" OR "Dimolybdenum trioxide" OR "dimolybdenum trisulfide " OR "Disodium molybdate" OR "Disodium molybdate dihydrate" OR "Disodium tetraoxomolybdate" OR "DM 1 (sulfide)" OR "DMI 7"
	"Dodecachlorohexamolybdenum" OR "Hexaammonium heptamolybdate tetrahydrate" OR "Hexaammonium molybdate tetrahydrate" OR "Hexacarbonylmolybdenum" OR "Hexafluoromolybdenum" OR "Hexamolybdenum dodecachloride" OR "JCPDS 35-0609" OR "Liqui-Moly LM 11" OR "Liqui-Moly LM 2" OR "Liqui-Moly Z Powder" OR "LM 13" OR "M 5" OR "MC 400WR"
	"MChVL" OR "MD 40" OR "Metco 63" OR "MF 000" OR "MFR" OR "MIPO-M 15" OR "Mo 1202T" OR "Mo-1202T" OR "Moly Fine Powder Y" OR "Moly Powder B" OR "Moly Powder C" OR "Moly Powder PA" OR "Moly Powder PB" OR "Moly Powder PS" OR "Molybdate (Mo2O72-), diammonium" OR "Molybdate (MoO42-), disodium, dihydrate, (T-4)-" OR "Molybdate, calcium" OR "Molybdena" OR "Molybdenite"
	"Molybdenum" OR "Molybdenum anhydride" OR "Molybdenum bisulfide" OR "Molybdenum carbide" OR "Molybdenum carbonyl" OR "Molybdenum chloride" OR "Molybdenum chloride oxide" OR "Molybdenum dioxide" OR "Molybdenum disulfide" OR "Molybdenum disulphide" OR "Molybdenum fluoride" OR "Molybdenum hexacarbonyl" OR "Molybdenum hexafluoride"
	"Molybdenum metallicum" OR "Molybdenum monocarbide" OR "Molybdenum oxide" OR "Molybdenum oxychloride" OR "Molybdenum oxytrichloride" OR "Molybdenum pentachloride" OR "Molybdenum peroxide" OR "Molybdenum sesquioxide" OR "Molybdenum sesquisulfide" OR "Molybdenum sodium oxide" OR "Molybdenum sulfide" OR "Molybdenum sulphide" OR "Molybdenum trichloride monoxide"
	"Molybdenum trichloride oxide" OR "Molybdenum trioxide" OR "Molybdenum trioxide pentamer" OR "Molybdenum trioxide tetramer" OR "Molybdenum trisulfide" OR "Molybdenum(6+) fluoride" OR "Molybdenum(II) acetate" OR "Molybdenum(II) chloride" OR "molybdenum(III) sulfide" OR "molybdenum(IV) oxide" OR "Molybdenum(IV) sulfide" OR "Molybdenum(V) chloride"
	"Molybdenum(VI) oxide" OR "Molybdenum(VI) trioxide" OR "Molybdenumperoxide" OR "Molybdic acid (H2Mo2O7), diammonium salt" OR "Molybdic acid (H2MoO4), calcium salt (1:1)" OR "Molybdic acid anhydride" OR "Molybdic acid, ammonium salt" OR "Molybdic acid, calcium salt" OR "Molybdic acid, disodium salt" OR "Molybdic acid, disodium salt, dihydrate" OR "Molybdic anhydride"
	"Molybdic oxide" OR "Molybdic trioxide" OR "Molycolloid CF 626" OR "Molyform 15" OR "Molyhibit 100" OR "Molyka R" OR "Molyka R-L 3" OR "Molyke R" OR "Molykote" OR "Molykote Microsize Powder" OR "Molykote Z" OR "Molykote Z Powder" OR "Molysulfide"

Table B-2. Database Query Strings

Database search date	Query string
	OR "MOP-P 100" OR "Mopol M" OR "Mopol S" OR "Motimol" OR "MVCh 1" OR "Natural molybdenite" OR "Natural molybdite" OR "NeoZ"
	"Nichimoly C" OR "Octachlorohexamolybdenum(4+) tetrachloride" OR "OKS 110" OR "PA Powder" OR "Pentachloromolybdenum Molybdenite" OR "Pigment Black 34" OR "Pol-U" OR "Powder PA" OR "RAC 01" OR "SGC 15" OR "Sodium molybdate" OR "Sodium molybdate dihydrate" OR "Sodium molybdate(VI)" OR "Sodium molybdate(VI) dihydrate" OR "Sodium molybdenate"
	"Sodium molybdenum oxide" OR "Sodium tetraoxomolybdate(2-)" OR "Solvest 390A" OR "Sumipowder PA" OR "T-Powder" OR "Tetraacetatodimolybdenum" OR "tetrakis(acetato)di-Molybdenum" OR "Tetrakis(acetato)dimolybdenum" OR "Tetrakis(acetato)molybdenum" OR "Tetrakis(mu-(acetato-O:O'))dimolybdenum" OR "tetrakis(μ-acetato)di-Molybdenum" OR "Tetrakis(μ-acetato)dimolybdenum"
	"tetrakis[μ-(acetato-O:O')]di-Molybdenum" OR "Thiomolybdic acid, diammonium salt" OR "Tiomolibdate diammonium" OR "TMOIO" OR "Trichlorooxomolybdenum" OR "Trichlorooxomolybdenum(V)" OR "TsM1"
	No date limit:
	"Hexaammonium heptamolybdate" OR "Hexammonium heptamolybdat" OR "Hexammonium tetracosaoxoheptamolybdate" OR "Molybdate (Mo7O24), hexammonium" OR "Molybdate (Mo7O246-), ammonium (1:6)" OR "Molybdate (Mo7O246-), hexaammonium" OR "Molybdate, hexaammonium" OR "Molybdic acid (H6Mo7O24), hexaammonium salt" OR "Molybdic acid, hexaammonium salt" OR 12027-67-7[rn]
	"Diammonium molybdate" OR "Diammonium tetraoxomolybdate(2-)" OR "Molybdate (MoO42-), ammonium (1:2), (T-4)-" OR "Molybdate (MoO42-), diammonium, (beta-4)-" OR "Molybdate (MoO42-), diammonium, (T-4)-" OR "Molybdic acid (H2MoO4), diammonium salt" OR "Molybdic acid, diammonium salt" OR 13106-76-8[rn]
Toxcenter	
01/2018	(FILE 'HOME' ENTERED AT 14:16:56 ON 12 JAN 2018) FILE 'TOXCENTER' ENTERED AT 14:17:07 ON 12 JAN 2018 CHARGED TO COST=EH011.05.LB.02.05 L1 34132 SEA FILE=TOXCENTER 7439-98-7 OR 1317-33-5 OR 12033-29-3 OR 12033-33-9 OR 11098-99-0 OR 18868-43-4 OR 1313-27-5 OR 1313-29-7 OR 11098-84-3 OR 27546-07-2 OR 12054-85-2 OR 15060-55-6 OR 7631-95-0 OR 10102-40-6 L2 523 SEA FILE=TOXCENTER 7789-82-4 OR 12011-97-1 OR 11119-46-3 OR 11062-51-4 OR 10241-05-1 OR 1309-56-4 OR 7783-77-9 OR 13939-06-5 OR 14221-06-8 OR 13814-74-9 L3 1148 SEA FILE=TOXCENTER 12027-67-7 OR 13106-76-8 L4 34486 SEA FILE=TOXCENTER L1 OR L2 L5 636 SEA FILE=TOXCENTER L3 NOT L4 L6 35122 SEA FILE=TOXCENTER L1 OR L2 OR L3 L7 22953 SEA FILE=TOXCENTER L6 NOT PATENT/DT L8 22927 SEA FILE=TOXCENTER L7 NOT TSCATS/FS ACT TOXQUERY/Q --------- L9 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L10 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT,

Table B-2. Database Query Strings

Database search date	Query string
	IT)
	L11 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L12 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L13 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L14 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L15 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L16 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L17 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L18 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L19 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L20 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L21 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L22 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L23 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L24 QUE (ENDOCRIN? AND DISRUPT?)
	L25 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L26 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L27 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L28 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L29 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L30 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L31 QUE (NEPHROTOX? OR HEPATOTOX?)
	L32 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L33 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L34 QUE L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33
	L35 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)

Table B-2. Database Query Strings

Database search date	Query string
	L36 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L37 QUE L34 OR L35 OR L36
	L38 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L39 QUE L37 OR L38

	L40 8795 SEA FILE=TOXCENTER L8 AND L39
	L41 1436 SEA FILE=TOXCENTER L40 AND ED>=20141201
	L42 1422 SEA FILE=TOXCENTER L41 AND PY>2012
	L43 184 SEA FILE=TOXCENTER L40 AND L3
	L44 1597 SEA FILE=TOXCENTER L41 OR L43
	L45 0 SEA FILE=TOXCENTER L44 AND MEDLINE/SB
	L46 269 SEA FILE=TOXCENTER L44 AND MEDLINE/FS
	L47 263 SEA FILE=TOXCENTER L44 AND BIOSIS/FS
	L48 1050 SEA FILE=TOXCENTER L44 AND CAPLUS/FS
	L49 15 SEA FILE=TOXCENTER L44 NOT (L46 OR L47 OR L48)
	L50 1425 DUP REM L46 L47 L49 L48 (172 DUPLICATES REMOVED) ANSWERS '1-1425' FROM FILE TOXCENTER
	L*** DEL 269 S L44 AND MEDLINE/FS
	L*** DEL 269 S L44 AND MEDLINE/FS
	L51 269 SEA FILE=TOXCENTER L50
	L*** DEL 263 S L44 AND BIOSIS/FS
	L*** DEL 263 S L44 AND BIOSIS/FS
	L52 234 SEA FILE=TOXCENTER L50
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L53 908 SEA FILE=TOXCENTER L50
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L54 14 SEA FILE=TOXCENTER L50
	L55 211 SEA FILE=TOXCENTER (L51 OR L52 OR L53 OR L54) AND BIOSIS/FS AND ED>=20141201
	L*** DEL 269 S L44 AND MEDLINE/FS
	L*** DEL 269 S L44 AND MEDLINE/FS
	L56 269 SEA FILE=TOXCENTER L50
	L*** DEL 263 S L44 AND BIOSIS/FS
	L*** DEL 263 S L44 AND BIOSIS/FS
	L57 234 SEA FILE=TOXCENTER L50
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L58 908 SEA FILE=TOXCENTER L50
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L59 14 SEA FILE=TOXCENTER L50
	L60 826 SEA FILE=TOXCENTER (L56 OR L57 OR L58 OR L59) AND CAPLUS/FS AND ED>=20141201
	L*** DEL 269 S L44 AND MEDLINE/FS

Table B-2. Database Query Strings

Database search date	Query string
	L*** DEL 269 S L44 AND MEDLINE/FS
	L61 269 SEA FILE=TOXCENTER L50
	L*** DEL 263 S L44 AND BIOSIS/FS
	L*** DEL 263 S L44 AND BIOSIS/FS
	L62 234 SEA FILE=TOXCENTER L50
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L63 908 SEA FILE=TOXCENTER L50
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L64 14 SEA FILE=TOXCENTER L50
	L65 0 SEA FILE=TOXCENTER (L61 OR L62 OR L63 OR L64) NOT (CAPLUS/FS OR MEDLINE/FS OR BIOSIS/FS) AND ED>=20141201
	L*** DEL 269 S L44 AND MEDLINE/FS
	L*** DEL 269 S L44 AND MEDLINE/FS
	L66 269 SEA FILE=TOXCENTER L50
	L*** DEL 263 S L44 AND BIOSIS/FS
	L*** DEL 263 S L44 AND BIOSIS/FS
	L67 234 SEA FILE=TOXCENTER L50
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L68 908 SEA FILE=TOXCENTER L50
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L69 14 SEA FILE=TOXCENTER L50
	L70 150 SEA FILE=TOXCENTER (L66 OR L67 OR L68 OR L69) NOT ED>=20141201
	L71 23 SEA FILE=TOXCENTER L70 AND BIOSIS/FS
	L72 14 SEA FILE=TOXCENTER L70 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L73 82 SEA FILE=TOXCENTER L70 AND CAPLUS/FS
	L74 52 SEA FILE=TOXCENTER L60 AND ?MOLYB?/TI
	L75 29 SEA FILE=TOXCENTER L73 AND ?MOLYB?/TI
	D SCAN L55
	L76 37 SEA FILE=TOXCENTER L71 OR L72
	D SCAN L76
	D SCAN L74
	D SCAN L75
	L*** DEL 269 S L44 AND MEDLINE/FS
	L*** DEL 269 S L44 AND MEDLINE/FS
	L77 269 SEA FILE=TOXCENTER L50
	L*** DEL 263 S L44 AND BIOSIS/FS
	L*** DEL 263 S L44 AND BIOSIS/FS
	L78 234 SEA FILE=TOXCENTER L50
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L*** DEL 1050 S L44 AND CAPLUS/FS
	L79 908 SEA FILE=TOXCENTER L50
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L*** DEL 15 S L44 NOT (L46 OR L47 OR L48)
	L80 14 SEA FILE=TOXCENTER L50

Table B-2. Database Query Strings

Database search date	Query string
	L81 1275 SEA FILE=TOXCENTER (L77 OR L78 OR L79 OR L80) AND ED>=20141201 L82 1265 SEA FILE=TOXCENTER L81 AND (L1 OR L2) L83 1012 SEA FILE=TOXCENTER L81 NOT (L55 OR L74) L84 84 SEA FILE=TOXCENTER L70 NOT (L76 OR L75) D SCAN L84 D SCAN L83 (FILE 'HOME' ENTERED AT 20:36:59 ON 14 JAN 2018) FILE 'TOXCENTER' ENTERED AT 20:37:09 ON 14 JAN 2018 CHARGED TO COST=EH011.05.LB.02.05 ACT MOLY1/A --------- L1 (34132)SEA FILE=TOXCENTER 7439-98-7 OR 1317-33-5 OR 12033-29-3 OR 12033-33-9 OR 11098-99-0 OR 18868-43-4 OR 1313-27-5 OR 1313-29-7 OR 11098-84-3 OR 27546-07-2 OR 12054-85-2 OR 15060-55-6 OR 7631-95-0 OR 10102-40-6 L2 (523)SEA FILE=TOXCENTER 7789-82-4 OR 12011-97-1 OR 11119-46-3 OR 11062-51-4 OR 10241-05-1 OR 1309-56-4 OR 7783-77-9 OR 13939-06-5 OR 14221-06-8 OR 13814-74-9 L3 (1148)SEA FILE=TOXCENTER 12027-67-7 OR 13106-76-8 L4 (35122)SEA FILE=TOXCENTER L1 OR L2 OR L3 L5 (22953)SEA FILE=TOXCENTER L4 NOT PATENT/DT L6 (22927)SEA FILE=TOXCENTER L5 NOT TSCATS/FS L7 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L8 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L9 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L10 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L11 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L12 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L13 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L14 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L15 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L16 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L17 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L18 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L19 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L20 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR

Table B-2. Database Query Strings

Database search date	Query string
	SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L21 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L22 QUE (ENDOCRIN? AND DISRUPT?)
	L23 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L24 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L25 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L26 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR
	NEOPLAS?)
	L27 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L28 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L29 QUE (NEPHROTOX? OR HEPATOTOX?)
	L30 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L31 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L32 QUE L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31
	L33 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE
	OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE
	OR PORCINE OR MONKEY? OR MACAQUE?)
	L34 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA
	OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L35 QUE L32 OR L33 OR L34
	L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR
	PRIMATES OR PRIMATE?)
	L37 QUE L35 OR L36
	L38 (8795)SEA FILE=TOXCENTER L6 AND L37
	L39 (1436)SEA FILE=TOXCENTER L38 AND ED>=20141201
	L40 (184)SEA FILE=TOXCENTER L38 AND L3
	L41 (1597)SEA FILE=TOXCENTER L39 OR L40
	L42 (269)SEA FILE=TOXCENTER L41 AND MEDLINE/FS
	L43 (263)SEA FILE=TOXCENTER L41 AND BIOSIS/FS
	L44 (1050)SEA FILE=TOXCENTER L41 AND CAPLUS/FS
	L45 (15)SEA FILE=TOXCENTER L41 NOT (L42 OR L43 OR L44)
	L46 (1425)DUP REM L42 L43 L45 L44 (172 DUPLICATES REMOVED)
	L47 (269)SEA FILE=TOXCENTER L46
	L48 (234)SEA FILE=TOXCENTER L46
	L49 (908)SEA FILE=TOXCENTER L46
	L50 (14)SEA FILE=TOXCENTER L46
	L51 (211)SEA FILE=TOXCENTER (L47 OR L48 OR L49 OR L50) AND BIOSIS/FS AND ED>=20141201
	L52 (269)SEA FILE=TOXCENTER L46

Table B-2. Database Query Strings

Database search date	Query string
	L53 (234)SEA FILE=TOXCENTER L46 L54 (908)SEA FILE=TOXCENTER L46 L55 (14)SEA FILE=TOXCENTER L46 L56 (826)SEA FILE=TOXCENTER (L52 OR L53 OR L54 OR L55) AND CAPLUS/FS AND ED>=20141201 L57 (52)SEA FILE=TOXCENTER L56 AND ?MOLYB?/TI L58 (269)SEA FILE=TOXCENTER L46 L59 (234)SEA FILE=TOXCENTER L46 L60 (908)SEA FILE=TOXCENTER L46 L61 (14)SEA FILE=TOXCENTER L46 L62 (1275)SEA FILE=TOXCENTER (L58 OR L59 OR L60 OR L61) AND ED>=20141201 L63 1012 SEA FILE=TOXCENTER L62 NOT (L51 OR L57) --------- L64 97 SEA FILE=TOXCENTER L63 AND (MOLYB?/TI OR DIMOLYB?/TI OR DODECACHLOROHEXAMOLYB?/TI OR HEPTAMOLYB?/TI OR HEXACARBONYLMOLY B?/TI OR HEXAFLUOROMOLYB?/TI OR HEXAMOLYB?/TI OR OCTACHLOROHEXA MOLYB?/TI OR PARAMOLYB?/TI) L65 5 SEA FILE=TOXCENTER L63 AND (PENTACHLOROMOLYB?/TI OR TETRAACETAT ODIMOLYB?/TI OR TETRAOXOMOLYB?/TI OR TETRASULFIDOMOLYB?/TI OR TETRATHIOMOLYB?/TI OR TETRATHIOOXOMOLYB?/TI OR THIOMOLYB?/TI OR TRICHLOROOXOMOLYB?/TI) L66 1 SEA FILE=TOXCENTER L63 AND ("3N5"/TI OR "AMMONIUM MOLIBDATE"/TI OR "AMMONIUM MOLIBDENUM OXIDE"/TI OR "AMPERIT 105.054"/TI OR "AMPERIT 106.2"/TI OR "ATTM"/TI OR "BIS(AMMONIUM)TETRATHIOMOLYB DATE(2-)"/TI OR "BOUEN SKN 301"/TI) L67 0 SEA FILE=TOXCENTER L63 AND ("C-POWDER"/TI OR "COPREXA"/TI OR "DAG 206"/TI OR "DAG 325"/TI OR "DAG-V 657"/TI OR "DEFRIC COAT HMB 2"/TI OR "DM 1 (SULFIDE)"/TI OR "DMI 7"/TI OR "JCPDS 35-0609"/TI OR "LIQUI-MOLY LM 11"/TI OR "LIQUI-MOLY LM 2"/TI) L68 0 SEA FILE=TOXCENTER L63 AND ("LIQUI-MOLY Z POWDER"/TI OR "LM 13"/TI OR "MC 400WR"/TI OR "MCHVL"/TI OR "MD 40"/TI OR "METCO 63"/TI OR "MF 000"/TI OR "MIPO-M 15"/TI OR "MO 1202T"/TI OR "MO-1202T"/TI OR "MOLY FINE POWDER Y"/TI) L69 0 SEA FILE=TOXCENTER L63 AND ("MOLY POWDER B"/TI OR "MOLY POWDER C"/TI OR "MOLY POWDER PA"/TI OR "MOLY POWDER PB"/TI OR "MOLY POWDER PS"/TI OR "MOLYCOLLOID CF 626"/TI OR "MOLYFORM 15"/TI OR "MOLYHIBIT 100"/TI OR "MOLYKA R"/TI OR "MOLYKA R-L 3"/TI) L70 0 SEA FILE=TOXCENTER L63 AND ("MOLYKE R"/TI OR "MOLYKOTE"/TI OR "MOLYKOTE MICROSIZE POWDER"/TI OR "MOLYKOTE Z"/TI OR "MOLYKOTE Z POWDER"/TI OR "MOLYSULFIDE"/TI OR "MOP-P 100"/TI OR "MOPOL M"/TI OR "MOPOL S"/TI OR "MOTIMOL"/TI OR "MVCH 1"/TI) L71 0 SEA FILE=TOXCENTER L63 AND ("NEOZ"/TI OR "NICHIMOLY C"/TI OR

Table B-2. Database Query Strings

Database search date	Query string
	"OKS 110"/TI OR "PA POWDER"/TI OR "PIGMENT BLACK 34"/TI OR "POL-U"/TI OR "POWDER PA"/TI OR "RAC 01"/TI OR "SGC 15"/TI OR "SOLVEST 390A"/TI OR "SUMIPOWDER PA"/TI OR "T-POWDER"/TI OR "TIOMOLIBDATE DIAMMONIUM"/TI OR "TMOIO"/TI OR "TSM1"/TI)
	L72 103 SEA FILE=TOXCENTER L64 OR L65 OR L66
	D SCAN L72
	ACT MOLY2/A

	L76 (34132)SEA FILE=TOXCENTER 7439-98-7 OR 1317-33-5 OR 12033-29-3 OR 12033-33-9 OR 11098-99-0 OR 18868-43-4 OR 1313-27-5 OR 1313-29-7 OR 11098-84-3 OR 27546-07-2 OR 12054-85-2 OR 15060-55-6 OR 7631-95-0 OR 10102-40-6
	L77 (523)SEA FILE=TOXCENTER 7789-82-4 OR 12011-97-1 OR 11119-46-3 OR 11062-51-4 OR 10241-05-1 OR 1309-56-4 OR 7783-77-9 OR 13939-06-5 OR 14221-06-8 OR 13814-74-9
	L78 (1148)SEA FILE=TOXCENTER 12027-67-7 OR 13106-76-8
	L79 (35122)SEA FILE=TOXCENTER L76 OR L77 OR L78
	L80 (22953)SEA FILE=TOXCENTER L79 NOT PATENT/DT
	L81 (22927)SEA FILE=TOXCENTER L80 NOT TSCATS/FS
	L82 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L83 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)
	L84 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L85 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L86 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L87 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L88 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L89 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L90 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L91 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L92 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L93 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L94 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L95 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L96 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L97 QUE (ENDOCRIN? AND DISRUPT?)

Table B-2. Database Query Strings

Database search date	Query string
	L98 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L99 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L100 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L101 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L102 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L103 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L104 QUE (NEPHROTOX? OR HEPATOTOX?)
	L105 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L106 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L107 QUE L82 OR L83 OR L84 OR L85 OR L86 OR L87 OR L88 OR L89 OR L90 OR L91 OR L92 OR L93 OR L94 OR L95 OR L96 OR L97 OR L98 OR L99 OR L100 OR L101 OR L102 OR L103 OR L104 OR L105 OR L106
	L108 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L109 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L110 QUE L107 OR L108 OR L109
	L111 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L112 QUE L110 OR L111
	L113(8795)SEA FILE=TOXCENTER L81 AND L112
	L114(1436)SEA FILE=TOXCENTER L113 AND ED>=20141201
	L115(184)SEA FILE=TOXCENTER L113 AND L78
	L116(1597)SEA FILE=TOXCENTER L114 OR L115
	L117(269)SEA FILE=TOXCENTER L116 AND MEDLINE/FS
	L118(263)SEA FILE=TOXCENTER L116 AND BIOSIS/FS
	L119(1050)SEA FILE=TOXCENTER L116 AND CAPLUS/FS
	L120(15)SEA FILE=TOXCENTER L116 NOT (L117 OR L118 OR L119)
	L121(1425)DUP REM L117 L118 L120 L119 (172 DUPLICATES REMOVED)
	L122(269)SEA FILE=TOXCENTER L121
	L123(234)SEA FILE=TOXCENTER L121
	L124(908)SEA FILE=TOXCENTER L121
	L125(14)SEA FILE=TOXCENTER L121
	L126(150)SEA FILE=TOXCENTER (L122 OR L123 OR L124 OR L125) NOT ED>=20141
	L127(23)SEA FILE=TOXCENTER L126 AND BIOSIS/FS
	L128(14)SEA FILE=TOXCENTER L126 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/
	L129(82)SEA FILE=TOXCENTER L126 AND CAPLUS/FS
	L130(29)SEA FILE=TOXCENTER L129 AND ?MOLYB?/TI

Table B-2. Database Query Strings

Database search date	Query string
	L131(37)SEA FILE=TOXCENTER L127 OR L128 L132 84 SEA FILE=TOXCENTER L126 NOT (L131 OR L130) --------- L133 15 SEA FILE=TOXCENTER L132 AND (MOLYB?/TI OR DIMOLYB?/TI OR DODECACHLOROHEXAMOLYB?/TI OR HEPTAMOLYB?/TI OR HEXACARBONYLMOLYB?/TI OR HEXAFLUOROMOLYB?/TI OR HEXAMOLYB?/TI OR OCTACHLOROHEXAMOLYB?/TI OR PARAMOLYB?/TI) L134 0 SEA FILE=TOXCENTER L1L32 AND (PENTACHLOROMOLYB?/TI OR TETRAACETATODIMOLYB?/TI OR TETRAOXOMOLYB?/TI OR TETRASULFIDOMOLYB?/TI OR TETRATHIOMOLYB?/TI OR TETRATHIOOXOMOLYB?/TI OR THIOMOLYB?/TI OR TRICHLOROOXOMOLYB?/TI) L135 0 SEA FILE=TOXCENTER L132 AND ("3N5"/TI OR "AMMONIUM MOLIBDATE"/TI OR "AMMONIUM MOLIBDENUM OXIDE"/TI OR "AMPERIT 105.054"/TI OR "AMPERIT 106.2"/TI OR "ATTM"/TI OR "BIS(AMMONIUM)TETRATHIOMOLYBDATE(2-)"/TI OR "BOUEN SKN 301"/TI) L136 0 SEA FILE=TOXCENTER L132 AND ("C-POWDER"/TI OR "COPREXA"/TI OR "DAG 206"/TI OR "DAG 325"/TI OR "DAG-V 657"/TI OR "DEFRIC COAT HMB 2"/TI OR "DM 1 (SULFIDE)"/TI OR "DMI 7"/TI OR "JCPDS 35-0609"/TI OR "LIQUI-MOLY LM 11"/TI OR "LIQUI-MOLY LM 2"/TI) L137 0 SEA FILE=TOXCENTER L132 AND ("LIQUI-MOLY Z POWDER"/TI OR "LM 13"/TI OR "MC 400WR"/TI OR "MCHVL"/TI OR "MD 40"/TI OR "METCO 63"/TI OR "MF 000"/TI OR "MIPO-M 15"/TI OR "MO 1202T"/TI OR "MO-1202T"/TI OR "MOLY FINE POWDER Y"/TI) L138 0 SEA FILE=TOXCENTER L132 AND ("MOLY POWDER B"/TI OR "MOLY POWDER C"/TI OR "MOLY POWDER PA"/TI OR "MOLY POWDER PB"/TI OR "MOLY POWDER PS"/TI OR "MOLYCOLLOID CF 626"/TI OR "MOLYFORM 15"/TI OR "MOLYHIBIT 100"/TI OR "MOLYKA R"/TI OR "MOLYKA R-L 3"/TI) L139 0 SEA FILE=TOXCENTER L132 AND ("MOLYKE R"/TI OR "MOLYKOTE"/TI OR "MOLYKOTE MICROSIZE POWDER"/TI OR "MOLYKOTE Z"/TI OR "MOLYKOTE Z POWDER"/TI OR "MOLYSULFIDE"/TI OR "MOP-P 100"/TI OR "MOPOL M"/TI OR "MOPOL S"/TI OR "MOTIMOL"/TI OR "MVCH 1"/TI) L140 0 SEA FILE=TOXCENTER L132 AND ("NEOZ"/TI OR "NICHIMOLY C"/TI OR "OKS 110"/TI OR "PA POWDER"/TI OR "PIGMENT BLACK 34"/TI OR "POL-U"/TI OR "POWDER PA"/TI OR "RAC 01"/TI OR "SGC 15"/TI OR "SOLVEST 390A"/TI OR "SUMIPOWDER PA"/TI OR "T-POWDER"/TI OR "TIOMOLIBDATE DIAMMONIUM"/TI OR "TMOIO"/TI OR "TSM1"/TI) D SCAN L133

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS[a]	
01/2018	Compounds searched: 7439-98-7, 1317-33-5, 12033-29-3, 12033-33-9, 11098-99-0, 18868-43-4, 1313-27-5, 1313-29-7, 11098-84-3, 27546-07-2, 12054-85-2, 15060-55-6, 7631-95-0, 10102-40-6, 7789-82-4, 12011-97-1, 11119-46-3, 11062-51-4, 10241-05-1, 1309-56-4, 7783-77-9, 13939-06-5, 14221-06-8, 13814-74-9, 12027-67-7, 13106-76-8
NTP	
01/2018	**14th ROC (https://ntp.niehs.nih.gov/pubhealth/roc/index-1.html):** 7439-98-7 OR 1317-33-5 OR 12033-29-3 OR 12033-33-9 OR 11098-99-0 OR 18868-43-4 OR 1313-27-5 OR 1313-29-7 OR 11098-84-3 OR 27546-07-2 OR 12054-85-2 OR 15060-55-6 OR 7631-95-0 OR 10102-40-6 OR 7789-82-4 OR 12011-97-1 OR 11119-46-3 OR 11062-51-4 OR 10241-05-1 OR 1309-56-4 OR 7783-77-9 OR 13939-06-5 OR 14221-06-8 OR 13814-74-9 OR 12027-67-7 OR 13106-76-8 molybdenum OR molybdate OR molybdic OR dimolybdate OR dimolybdenum OR dodecachlorohexamolybdenum OR heptamolybdate OR hexacarbonylmolybdenum OR hexafluoromolybdenum OR hexamolybdenum OR molibdate OR molibdenum OR octachlorohexamolybdenum OR paramolybdate OR pentachloromolybdenum OR tetraacetatodimolybdenum OR tetraoxomolybdate OR tetrasulfidomolybdate OR tetrathiomolybdate OR tetrathiooxomolybdate OR thiomolybdate OR thiomolybdic OR tiomolibdate OR trichlorooxomolybdenum OR pigment black 34 **NTP Site Search (http://ntpsearch.niehs.nih.gov/home) with Content Types "Reports & Publications", "Systematic Review" or "Testing Status":** 7439-98-7 OR 1317-33-5 OR 12033-29-3 OR 12033-33-9 OR 11098-99-0 OR 18868-43-4 OR 1313-27-5 OR 1313-29-7 OR 11098-84-3 OR 27546-07-2 OR 12054-85-2 OR 15060-55-6 OR 7631-95-0 OR 10102-40-6 OR 7789-82-4 OR 12011-97-1 OR 11119-46-3 OR 11062-51-4 OR 10241-05-1 OR 1309-56-4 OR 7783-77-9 OR 13939-06-5 OR 14221-06-8 OR 13814-74-9 OR 12027-67-7 OR 13106-76-8 molybdenum OR molybdate OR molybdic OR dimolybdate OR dimolybdenum OR dodecachlorohexamolybdenum OR heptamolybdate OR hexacarbonylmolybdenum OR hexafluoromolybdenum OR hexamolybdenum OR molibdate OR molibdenum OR octachlorohexamolybdenum OR paramolybdate OR pentachloromolybdenum OR tetraacetatodimolybdenum OR tetraoxomolybdate OR tetrasulfidomolybdate OR tetrathiomolybdate OR tetrathiooxomolybdate OR thiomolybdate OR thiomolybdic OR tiomolibdate OR trichlorooxomolybdenum OR pigment black 34
Regulations.gov	
01/2018	**Notices or rules:** 7439-98-7, 1317-33-5, 12033-29-3, 12033-33-9, 11098-99-0, 18868-43-4, 1313-27-5, 1313-29-7, 11098-84-3, 27546-07-2, 12054-85-2, 15060-55-6, 7631-95-0, 10102-40-6, 7789-82-4, 12011-97-1, 11119-46-3, 11062-51-4, 10241-05-1, 1309-56-4, 7783-77-9, 13939-06-5, 14221-06-8, 13814-74-9, 12027-67-7, 13106-76-8
NIH RePORTER	
04/2019	**Text Search:** "3N5" OR "Ammonium dimolybdate" OR "Ammonium heptamolybdate" OR "Ammonium heptamolybdate tetrahydrate" OR "Ammonium molibdate" OR "Ammonium molibdenum oxide" OR "Ammonium molybdate" OR "Ammonium molybdate hydrate" OR "Ammonium molybdate tetrahydrate" OR "Ammonium molybdate(VI)" OR "Ammonium molybdenum oxide" OR "Ammonium molybdenum sulfide" OR "ammonium paramolybdate" OR "Ammonium paramolybdate tetrahydrate" OR "Ammonium tetrasulfidomolybdate(2-)" OR "Ammonium tetrathiomolybdate" OR "Ammonium thiomolybdate(VI)" OR "Amperit 105.054" OR "Amperit 106.2" OR

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"ATTM" OR "Bis(ammonium)tetrathiomolybdate(2-)" OR "Bouen SKN 301" OR "C-Powder" OR "Calcium molybdate" OR "Calcium molybdate(VI)" OR "Calcium molybdenate" OR "Calcium molybdenum oxide" OR "Coprexa" OR "DAG 206" OR "DAG 325" OR "DAG-V 657" OR "Defric coat HMB 2" OR "Diammonium dimolybdate" OR "Diammonium tetrakis(sulfido)molybdate(2-)" OR "Diammonium tetrakis(thioxo)molybdate" OR "Diammonium tetrasulfidomolybdate" OR "Diammonium tetrathiomolybdate" OR "Diammonium tetrathiomolybdate(2-)" OR "Diammonium tetrathiooxomolybdate(2-)" OR "Diammonium tetrathioxomolybdate(2-)" OR "Diammonium thiomolybdate" OR "Dimolybdenum tetraacetate" OR "Dimolybdenum trioxide" OR "dimolybdenum trisulfide " OR "Disodium molybdate" OR "Disodium molybdate dihydrate" OR "Disodium tetraoxomolybdate" OR "DM 1 (sulfide)" OR "DMI 7" OR "Dodecachlorohexamolybdenum" OR "Hexaammonium heptamolybdate tetrahydrate" OR "Hexaammonium molybdate tetrahydrate" OR "Hexacarbonylmolybdenum" OR "Hexafluoromolybdenum" OR "Hexamolybdenum dodecachloride" OR "JCPDS 35-0609" OR "Liqui-Moly LM 11" OR "Liqui-Moly LM 2" OR "Liqui-Moly Z Powder" OR "LM 13" OR "MC 400WR" OR "MChVL" OR "MD 40" OR "Metco 63" OR "MF 000" OR "MIPO-M 15" OR "Mo 1202T" OR "Mo-1202T" OR "Moly Fine Powder Y" OR "Moly Powder B" OR "Moly Powder C" OR "Moly Powder PA" OR "Moly Powder PB" OR "Moly Powder PS" OR "Molybdate (Mo2O72-), diammonium" OR "Molybdate (MoO42-), disodium, dihydrate, (T-4)-" OR "Molybdate, calcium" OR "Molybdena" OR "Molybdenite" OR "Molybdenum" OR "Molybdenum anhydride" OR "Molybdenum bisulfide" OR "Molybdenum carbide" OR "Molybdenum carbonyl" OR "Molybdenum chloride" OR "Molybdenum chloride oxide" OR "Molybdenum dioxide" OR "Molybdenum disulfide" OR "Molybdenum disulphide" OR "Molybdenum fluoride" OR "Molybdenum hexacarbonyl" OR "Molybdenum hexafluoride" OR "Molybdenum metallicum" OR "Molybdenum monocarbide" OR "Molybdenum oxide" OR "Molybdenum oxychloride" **(Advanced), Search in: Projects AdminIC: All, Fiscal Year: Active Projects** **Text Search:** "Molybdenum oxytrichloride" OR "Molybdenum pentachloride" OR "Molybdenum peroxide" OR "Molybdenum sesquioxide" OR "Molybdenum sesquisulfide" OR "Molybdenum sodium oxide" OR "Molybdenum sulfide" OR "Molybdenum sulphide" OR "Molybdenum trichloride monoxide" OR "Molybdenum trichloride oxide" OR "Molybdenum trioxide" OR "Molybdenum trioxide pentamer" OR "Molybdenum trioxide tetramer" OR "Molybdenum trisulfide" OR "Molybdenum(6) fluoride" OR "Molybdenum(II) acetate" OR "Molybdenum(II) chloride" OR "molybdenum(III) sulfide" OR "molybdenum(IV) oxide" OR "Molybdenum(IV) sulfide" OR "Molybdenum(V) chloride" OR "Molybdenum(VI) oxide" OR "Molybdenum(VI) trioxide" OR "Molybdenumperoxide" OR "Molybdic acid (H2Mo2O7), diammonium salt" OR "Molybdic acid (H2MoO4), calcium salt (1:1)" OR "Molybdic acid anhydride" OR "Molybdic acid, ammonium salt" OR "Molybdic acid, calcium salt" OR "Molybdic acid, disodium salt" OR "Molybdic acid, disodium salt, dihydrate" OR "Molybdic anhydride" OR "Molybdic oxide" OR "Molybdic trioxide" OR "Molycolloid CF 626" OR "Molyform 15" OR "Molyhibit 100" OR "Molyka R" OR "Molyka R-L 3" OR "Molyke R" OR "Molykote" OR "Molykote Microsize Powder" OR "Molykote Z" OR "Molykote Z Powder" OR "Molysulfide" OR "MOP-P 100" OR "Mopol M" OR "Mopol S" OR "Motimol" OR "MVCh 1" OR "Natural molybdenite" OR "Natural molybdite" OR "NeoZ" OR "Nichimoly C" OR "Octachlorohexamolybdenum(4) tetrachloride" OR "OKS 110" OR "PA Powder" OR "Pentachloromolybdenum Molybdenite" OR "Pigment Black 34" OR "Pol-U" OR "Powder PA" OR "RAC 01" OR "SGC 15" OR "Sodium molybdate" OR "Sodium molybdate dihydrate" OR "Sodium molybdate(VI)" OR "Sodium molybdate(VI) dihydrate" OR "Sodium molybdenate" OR "Sodium molybdenum oxide" OR "Sodium tetraoxomolybdate(2-)" OR "Solvest 390A" OR "Sumipowder PA" OR

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"Tetraacetatodimolybdenum" OR "tetrakis(acetato)di-Molybdenum" OR "Tetrakis(acetato)dimolybdenum" OR "Tetrakis(acetato)molybdenum" OR "Tetrakis(mu-(acetato-O:O'))dimolybdenum" OR "tetrakis(mu-acetato)di-Molybdenum" OR "Tetrakis(mu-acetato)dimolybdenum" OR "tetrakis[mu-(acetato-O:O')]di-Molybdenum" OR "Thiomolybdic acid, diammonium salt" OR "Tiomolibdate diammonium" OR "TMOIO" OR "Trichlorooxomolybdenum" OR "Trichlorooxomolybdenum(V)" OR "TsM1" OR "Hexaammonium heptamolybdate" OR "Hexammonium heptamolybdat" OR "Hexammonium tetracosaoxoheptamolybdate" OR "Molybdate (Mo7O24), hexammonium" OR "Molybdate (Mo7O246-), ammonium (1:6)" OR "Molybdate (Mo7O246-), hexaammonium" **(Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects** **Text Search:** "Molybdate, hexaammonium" OR "Molybdic acid (H6Mo7O24), hexaammonium salt" OR "Molybdic acid, hexaammonium salt" OR "Diammonium molybdate" OR "Diammonium tetraoxomolybdate(2-)" OR "Molybdate (MoO42-), ammonium (1:2), (T-4)-" OR "Molybdate (MoO42-), diammonium, (beta-4)-" OR "Molybdate (MoO42-), diammonium, (T-4)-" OR "Molybdic acid (H2MoO4), diammonium salt" OR "Molybdic acid, diammonium salt" (Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects Text Search: molybdenum OR molybdate OR molybdic OR dimolybdate OR dimolybdenum OR dodecachlorohexamolybdenum OR heptamolybdate OR hexacarbonylmolybdenum OR hexafluoromolybdenum OR hexamolybdenum OR molibdate OR molibdenum OR octachlorohexamolybdenum OR paramolybdate OR pentachloromolybdenum OR tetraacetatodimolybdenum OR tetraoxomolybdate OR tetrasulfidomolybdate OR tetrathiomolybdate OR tetrathiooxomolybdate OR thiomolybdate OR thiomolybdic OR tiomolibdate OR trichlorooxomolybdenum OR pigment black 34 **(Advanced), Search in: Projects AdminIC: All, Fiscal Year: Active Projects**
Other	Identified throughout the assessment process

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The 2018 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 2,394
- Number of records identified from other strategies: 114
- Total number of records to undergo literature screening: 2,508

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on molybdenum:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the

second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

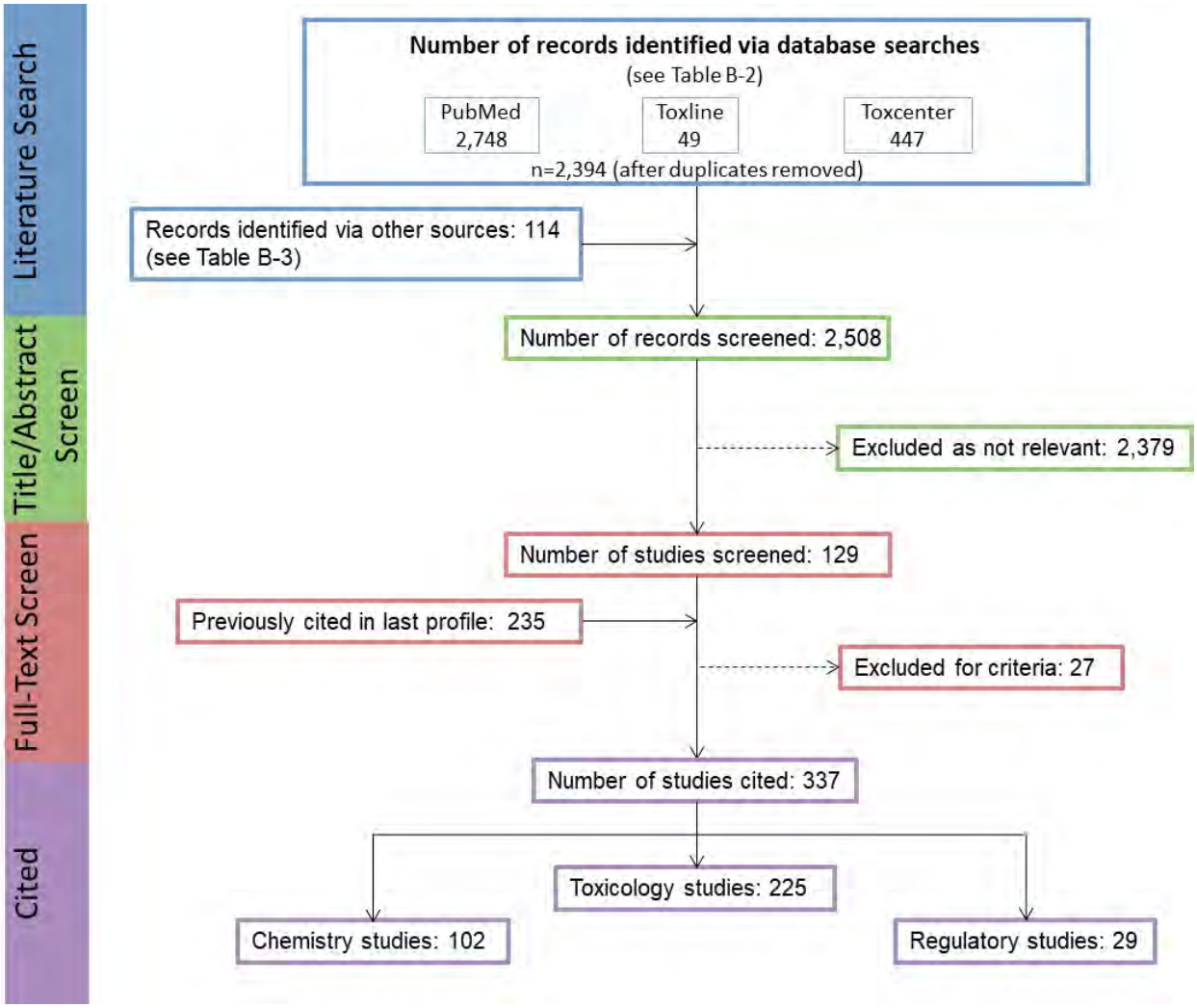
- Number of titles and abstracts screened: 2,508
- Number of studies considered relevant and moved to the next step: 129

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 129
- Number of studies cited in the pre-public draft of the toxicological profile: 235
- Total number of studies cited in the profile: 337

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. January 2018 Literature Search Results and Screen for Molybdenum



APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR MOLYBDENUM

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to molybdenum, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to molybdenum:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to molybdenum. The inclusion criteria used to identify relevant studies examining the health effects of molybdenum are presented in Table C-1.

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects
Cancer

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen was conducted to identify studies examining the health effects of molybdenum. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

C.2.1 Literature Search

As noted in Appendix B, the current literature search was intended to update the draft toxicological profile for molybdenum released for public comment in 2017. See Appendix B for the databases searched and the search strategy.

A total of 2,508 records relevant to all sections of the toxicological profile were identified (after duplicate removal).

C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of molybdenum.

Title and Abstract Screen. In the Title and Abstract Screen step, 2,508 records were reviewed; 71 documents were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

Full Text Screen. In the second step in the literature screening process for the systematic review, a full text review of 92 health effects documents (documents identified in the update literature search and documents cited in older versions of the profile) was performed. From those 92 documents, 115 studies were included in the qualitative review.

C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

Table C-2. Data Extracted From Individual Studies

Citation
Chemical form
Route of exposure (e.g., inhalation, oral, dermal)
Specific route (e.g., gavage in oil, drinking water)
Species
Strain
Exposure duration category (e.g., acute, intermediate, chronic)
Exposure duration
Frequency of exposure (e.g., 6 hours/day, 5 days/week)
Exposure length
Number of animals or subjects per sex per group
Dose/exposure levels
Parameters monitored
Description of the study design and method
Summary of calculations used to estimate doses (if applicable)
Summary of the study results
Reviewer's comments on the study
Outcome summary (one entry for each examined outcome)
No-observed-adverse-effect level (NOAEL) value
Lowest-observed-adverse-effect level (LOAEL) value
Effect observed at the LOAEL value

A summary of the extracted data for each study is presented in the Supplemental Document for Molybdenum and overviews of the results of the studies are presented in Sections 2.2–2.18 of the profile and in the Levels of Significant Exposures tables in Section 2.1 of the profile (Tables 2-1–2-3).

C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for molybdenum identified in human and animal studies are presented in Tables C-3 and C-4, respectively. The available human studies examined a limited number of endpoints and reported respiratory, hepatic, endocrine, other systemic (alterations in uric acid levels), reproductive, and developmental effects. Animal studies examined a number of endpoints following inhalation and oral exposure; no dermal exposure studies were identified. These studies examined most systemic endpoints and reported respiratory, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, and body weight effects. Additionally, animal studies

Table C-3. Overview of the Health Outcomes for Molybdenum Evaluated In Human Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological	Neurological	Reproductive	Developmental	Other Noncancer	Caner
Inhalation studies																	
Cohort		2 1														1 1	1 1
Case control																	
Population																	
Case series																	
Oral studies																	
Cohort														2 2	2 1	1 0	
Case control																	
Population	1 0	1 0	3 2			1 0	1 1			1 1	3 2						2 0
Case series																1 0	
Dermal studies																	
Cohort																	
Case control																	
Population																	
Case series																	

Number of studies examining endpoint	0	1	2	3	4	5-9	≥10	
Number of studies reporting outcome	0	1	2	3	4	5-9	≥10	

Table C-4. Overview of the Health Outcomes for Molybdenum Evaluated in Experimental Animal Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological[a]	Neurological[a]	Reproductive[a]	Developmental	Other Noncancer	Caner
Inhalation studies																	
Acute-duration	5 5	5 0															
Intermediate-duration		2 0	2 0	2 0	2 0	2 0	2 0	2 0			2 0		2 0	2 0			
Chronic-duration	2 0	2 2	2 0	2 0		2 0	2 0	2 0			2 0		2 0	2 0			2 2
Oral studies																	
Acute-duration	6 2			1 1	1 0	5 4	2 1	2 0						4 3			
Intermediate-duration	41 28	3 0	2 0	3 1	19 6	13 10	8 6	9 6	3 3		8 5		1 0	12 8	12 5	2 0	
Chronic-duration																	
Dermal studies																	
Acute-duration									7 0	4 4		4 0					
Intermediate-duration																	
Chronic-duration																	

Number of studies examining endpoint	0	1	2	3	4	5-9	≥10
Number of studies reporting outcome	0	1	2	3	4	5-9	≥10

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

have reported neurological, reproductive, and developmental effects. Although animal studies have identified a number of affected tissues and systems, interpretation of much of the data is limited by an inadequate amount of copper in the diet. Studies in which the diet did not contain adequate levels of copper or administered ammonium tetrathiomolybdate were carried through Step 3 of the systematic review, but were not considered in the identification of potential health effect outcomes of concern. Additionally, body weight effects were not considered a primary effect especially since most studies did not provide data on food intake; thus, this endpoint was not considered in the assessment of potential human hazards. Studies examining the respiratory, hepatic, renal, uric acid, reproductive, and developmental outcomes were carried through to Steps 4–8 of the systematic review. There were 115 studies (published in 92 documents) examining these potential outcomes were carried through to Steps 4–8 of the systematic review.

C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed using OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-5, C-6, and C-7, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies

Selection bias
Were the comparison groups appropriate?
Confounding bias
Did the study design or analysis account for important confounding and modifying variables?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were the research personnel and human subjects blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were experimental conditions identical across study groups?
Were the research personnel blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

First Tier. Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

Second Tier. A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

Third Tier. Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of molybdenum health effects studies (observational epidemiology, human-controlled exposure studies, and animal experimental studies) are presented in Tables C-8, C-9, and C-10, respectively.

C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including DHHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to molybdenum and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

Table C-8. Summary of Risk of Bias Assessment for Molybdenum—Observational Epidemiological Studies

Reference	Risk of bias criteria and ratings: Selection bias — Were the comparison groups appropriate?	Confounding bias — Did the study design or analysis account for important confounding and modifying variables?*	Attrition / exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?*	Detection bias — Is there confidence in the outcome assessment?*	Selective reporting bias — Were all measured outcomes reported?	Risk of bias tier
Outcome: Respiratory effects							
Cohort studies							
Ott et al. 2004	–	–	+	na	+	++	Second
Walravens et al. 1979	–	–	+	+	–	+	Second
Outcome: Hepatic effects							
Cross-sectional studies							
Mendy et al. 2012	+	+	+	+	–	+	Second
Outcome: Alterations in uric acid levels							
Cross-sectional studies							
Koval'sky et al. 1961	–	–	+	–	+	+	Second
Cohort studies							
Walravens et al. 1979	–	–	+	+	–	+	Second
Outcome: Reproductive effects							
Cross-sectional studies							
Lewis and Meeker 2015	na	-	+	+	+	+	First
Meeker et al. 2008	+	+	+	++	++	++	First
Meeker et al. 2010	+	+	++	+	++	++	First

Table C-8. Summary of Risk of Bias Assessment for Molybdenum—Observational Epidemiological Studies

Reference	Risk of bias criteria and ratings: Selection bias — Were the comparison groups appropriate?	Confounding bias — Did the study design or analysis account for important confounding and modifying variables?*	Attrition / exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?*	Detection bias — Is there confidence in the outcome assessment?*	Selective reporting bias — Were all measured outcomes reported?	Risk of bias tier
Outcome: Developmental effects							
Cross-sectional studies							
Vazquez-Salas et al. 2014	+	+	+	+	++	+	First
Shirai et al. 2010	na	–	+	+	+	+	Second

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; na = not applicable

*Key question used to assign risk of bias tier.

Table C-9. Summary of Risk of Bias Assessment for Molybdenum—Human-Controlled Exposure Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Risk of bias tier
Outcome: Alterations in uric acid levels								
Oral acute exposure								
Deosthale and Gopalan 1974	na	+	+	+	+	+	++	First

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; na = not applicable

*Key question used to assign risk of bias tier.

Table C-10. Summary of Risk of Bias Assessment for Molybdenum—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Other bias: Did the study design or analysis account for important confounding and modifying variables?	Risk of bias tier
Outcome: Respiratory effects										
Inhalation acute exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Inhalation intermediate exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Inhalation chronic exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Outcome: Hepatic effects										
Inhalation intermediate exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Inhalation chronic exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First

Table C-10. Summary of Risk of Bias Assessment for Molybdenum—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Other bias: Did the study design or analysis account for important confounding and modifying variables?	Risk of bias tier
Oral acute exposure										
Bersenyi et al. 2008 (rabbit)	–	+	+	–	++	–	+	+	+	First
Bersenyi et al. 2008 (rabbit)	–	+	+	–	++	–	+	+	+	First
Oral intermediate exposure										
Murray et al. 2014a (rat)	++	+	++	–	++	++	++	++	++	First
Rana and Chauhan 2000 (rat)	–	+	+	–	++	+	–	++	–	Second
Rana and Kumar 1980b (rat)	–	+	+	–	++	–	–	+	–	Third
Rana and Kumar 1980c (rat)	+	+	–	–	++	–	+	++	–	First
Rana and Kumar 1983 (rat)	+	+	–	–	++	+	+	++	–	First
Rana and Prakash 1986 (rat)	–	+	+	–	++	–	+	+	+	First
Rana et al. 1980 (rat)	–	+	+	–	+	–	+	+	+	First
Rana et al. 1985 (rat)	+	+	+	–	++	+	+	+	+	First
Outcome: Renal effects										
Inhalation intermediate exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First

Table C-10. Summary of Risk of Bias Assessment for Molybdenum—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Other bias: Did the study design or analysis account for important confounding and modifying variables?	Risk of bias tier
Inhalation chronic exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Oral acute exposure										
Bersenyi et al. 2008 (rabbit, males)	–	+	+	–	++	–	+	+	+	First
Bersenyi et al. 2008 (rabbit, females)	–	+	+	–	++	–	+	+	+	First
Oral intermediate exposure										
Bandyopadhyay et al. 1981 (rat)	–	+	+	–	++	–	+	++	++	First
Bompart et al. 1990 (rat)	+	+	+	–	++	+	+	++	+	First
Murray et al. 2014a (rat)	++	+	++	–	++	++	++	++	++	First
Rana et al. 1980 (rat)	–	+	+	–	+	–	+	+	+	First
Rana and Kumar 1980c	+	+	–	–	++	–	+	++	–	First
Rana and Kumar 1983 (rat)	+	+	–	–	++	+	+	++	–	First

Table C-10. Summary of Risk of Bias Assessment for Molybdenum—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias — Was administered dose or exposure level adequately randomized?	Selection bias — Was the allocation to study groups adequately concealed?	Performance bias — Were experimental conditions identical across study groups?	Performance bias — Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?	Detection bias — Is there confidence in the outcome assessment?*	Selective reporting bias — Were all measured outcomes reported?	Other bias — Did the study design or analysis account for important confounding and modifying variables?	Risk of bias tier
Outcome: Alterations in uric acid levels										
Oral intermediate exposure										
Murray et al. 2014a (rat)	++	+	++	-	++	++	++	++	++	First
Outcome: Reproductive effects										
Inhalation intermediate exposure										
NTP 1997 (rat)	++	+	++	+	++	++	++	++	+	First
NTP 1997 (mouse)	++	+	++	+	++	++	++	++	+	First
Oral acute exposure										
Zhang et al. 2013 (mouse)	–	+	++	–	++	– –	+	++	–	First
Zhai et al. 2013 (mouse)	–	+	++	–	++	– –	+	++	+	First
Bersenyi et al. 2008 (rabbit, males)	–	+	+	–	++	–	+	+	+	First
Bersenyi et al. 2008 (rabbit, females)	–	+	+	–	++	–	+	+	+	First
Oral intermediate exposure										
Fungwe et al. 1990 (rat)	+	+	+	–	++	–	+	+	– –	First
Jeter and Davis 1954 (rat, adults)	–	+	+	–	++	–	+	+	–	First

Table C-10. Summary of Risk of Bias Assessment for Molybdenum—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias — Was administered dose or exposure level adequately randomized?	Selection bias — Was the allocation to study groups adequately concealed?	Performance bias — Were experimental conditions identical across study groups?	Performance bias — Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?	Detection bias — Is there confidence in the outcome assessment?*	Selective reporting bias — Were all measured outcomes reported?	Other bias — Did the study design or analysis account for important confounding and modifying variables?	Risk of bias tier
Jeter and Davis 1954 (rat, weanling)	–	+	+	–	++	–	+	+	– –	First
Murray et al. 2014a (rat)	++	+	++	–	++	++	++	++	++	First
Murray et al. 2019 (rat)	++	+	++	–	++	++	++	++	++	First
Pandey and Singh 2002 (rat)	–	+	++	–	++	+	+	++	–	First
Pandey and Singh 2002 (rat fertility study)	–	+	++	–	++	+	+	++	–	First
Outcome: Developmental effects										
Oral intermediate exposure										
Jeter and Davis 1954 (rat, weanling)	–	+	+	–	++	–	+	+	– –	First
Murray et al. 2014b (rat)	++	+	+	–	++	++	+	++	+	First
Pandey and Singh 2002 (rat)	–	+	++	–	++	+	+	++	–	First

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question used to assign risk of bias tier.

C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to molybdenum and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-11, C-12, and C-13, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".
- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".
- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".
- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

Table C-11. Key Features of Study Design for Observational Epidemiology Studies

Exposure was experimentally controlled
Exposure occurred prior to the outcome
Outcome was assessed on individual level rather than at the population level
A comparison group was used

Table C-12. Key Features of Study Design for Human-Controlled Exposure Studies

A comparison group was used or the subjects served as their own control
A sufficient number of subjects were tested
Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

Table C-13. Key Features of Study Design for Experimental Animal Studies

A concurrent control group was used
A sufficient number of animals per group were tested
Appropriate parameters were used to assess a potential adverse effect
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

The presence or absence of the key features and the initial confidence levels for studies examining The presence or absence of the key features and the initial confidence levels for studies examining respiratory, gastrointestinal, renal, dermal, and ocular effects observed in the observational epidemiology, human-controlled exposure, and animal experimental studies are presented in Tables C-14, C-15, and C-16, respectively.

Table C-14. Presence of Key Features of Study Design for Molybdenum—Observational Epidemiology Studies

Reference	Key features: Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	Initial study confidence
Outcome: Respiratory effects					
Cohort studies					
Ott et al. 2004	No	Yes	Yes	No	Low
Walravens et al. 1979	No	No	No	No	Very Low
Outcome: Hepatic effects					
Cross-sectional studies					
Mendy et al. 2012	No	No	Yes	Yes	Low
Outcome: Alterations in uric acid levels					
Cross-sectional studies					
Koval'sky et al. 1961	No	Yes	Yes	No	Low
Cohort studies					
Walravens et al. 1979	No	No	No	No	Very Low
Outcome: Reproductive effects					
Cross-sectional studies					
Lewis and Meeker 2015	No	No	Yes	Yes	Low
Meeker et al. 2008	No	No	Yes	Yes	Low
Meeker et al. 2010	No	No	Yes	Yes	Low

Table C-14. Presence of Key Features of Study Design for Molybdenum—Observational Epidemiology Studies

Reference	Key features				Initial study confidence
	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	
Outcome: Developmental effects					
Cross-sectional studies					
Vazquez-Salas et al. 2014	No	No	Yes	Yes	Low
Shirai et al. 2010	No	No	Yes	Yes	Low

Table C-15. Presence of Key Features of Study Design for Molybdenum—Human-Controlled Exposure Studies

Reference	Key feature				Initial study confidence
	Concurrent control group or self-control	Sufficient number of subjects tested	Appropriate methods to measure outcome	Adequate data for statistical analysis	
Outcome: Alterations in uric acid levels					
Oral acute exposure					
Deosthale and Gopalan 1974	Yes	No	Yes	No	Low

Table C-16. Presence of Key Features of Study Design for Molybdenum—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Outcome: Respiratory effects					
Inhalation acute exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Inhalation intermediate exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Inhalation chronic exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Outcome: Hepatic effects					
Inhalation intermediate exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Inhalation chronic exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Oral acute exposure					
Bersenyi et al. 2008 (rabbit, males)	Yes	No	Yes	Yes	Moderate
Bersenyi et al. 2008 (rabbit, females)	Yes	No	Yes	Yes	Moderate
Oral intermediate exposure					
Murray et al. 2014a (rat)	Yes	Yes	Yes	Yes	High
Rana and Chauhan 2000 (rat)	Yes	Yes	No	Yes	Moderate
Rana and Kumar 1980b (rat)	Yes	Yes	No	Yes	Moderate
Rana and Kumar 1980c (rat)	Yes	Yes	No	Yes	Moderate
Rana and Kumar 1983 (rat)	Yes	Yes	No	Yes	Moderate
Rana and Prakash 1986 (rat)	Yes	Yes	No	Yes	Moderate
Rana et al. 1980 (rat)	Yes	Yes	No	No	Low
Rana et al. 1985 (rat)	Yes	Yes	No	Yes	Moderate
Outcome: Renal effects					
Inhalation intermediate exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High

Table C-16. Presence of Key Features of Study Design for Molybdenum—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Inhalation chronic exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Oral acute exposure					
Bersenyi et al. 2008 (rabbit, males)	Yes	No	Yes	Yes	Moderate
Bersenyi et al. 2008 (rabbit, females)	Yes	No	Yes	Yes	Moderate
Oral intermediate exposure					
Bandyopadhyay et al. 1981 (rat)	Yes	No	Yes	No	Low
Bompart et al. 1990 (rat)	Yes	No	Yes	Yes	Moderate
Murray et al. 2014a (rat)	Yes	Yes	Yes	Yes	High
Murray et al. 2019 (rat)	Yes	Yes	Yes	Yes	High
Rana et al. 1980 (rat)	Yes	Yes	No	No	Low
Rana and Kumar 1980c	Yes	Yes	No	Yes	Moderate
Rana and Kumar 1983 (rat)	Yes	Yes	No	Yes	Moderate
Outcome: Alterations in uric acid levels					
Oral intermediate exposure					
Murray et al. 2014a (rat)	Yes	Yes	Yes	Yes	High
Outcome: Reproductive effects					
Inhalation intermediate exposure					
NTP 1997 (rat)	Yes	Yes	Yes	Yes	High
NTP 1997 (mouse)	Yes	Yes	Yes	Yes	High
Oral acute exposure					
Zhang et al. 2013 (mouse)	Yes	Yes	No	Yes	Moderate
Zhai et al. 2013 (mouse)	Yes	Yes	No	Yes	Moderate
Bersenyi et al. 2008 (rabbit, males)	Yes	No	No	Yes	Low
Bersenyi et al. 2008 (rabbit, females)	Yes	No	No	No	Very Low
Oral intermediate exposure					
Fungwe et al. 1990 (rat)	Yes	No	Yes	Yes	Moderate
Jeter and Davis 1954 (rat, adult)	Yes	No	No	No	Very Low
Murray et al. 2014a (rat)	Yes	Yes	Yes	Yes	High
Murray et al. 2019 (rat)	Yes	Yes	Yes	Yes	High
Pandey and Singh 2002 (rat)	Yes	Yes	No	Yes	Moderate
Pandey and Singh 2002 (rat, fertility study)	Yes	Yes	Yes	Yes	High

Table C-16. Presence of Key Features of Study Design for Molybdenum—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Key feature: Sufficient number of animals per group	Key feature: Appropriate parameters to assess potential effect	Key feature: Adequate data for statistical analysis	Initial study confidence
Outcome: Developmental effects					
Oral intermediate exposure					
Jeter and Davis 1954 (rat, weanling)	Yes	No	No	No	Very Low
Murray et al. 2014b (rat)	Yes	Yes	Yes	Yes	High
Murray et al. 2019 (rat)	Yes	Yes	Yes	Yes	High
Pandey and Singh 2002 (rat)	Yes	Yes	Yes	Yes	High

A summary of the initial confidence ratings for each outcome is presented in Table C-17. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-17.

Table C-17. Initial Confidence Rating for Molybdenum Health Effects Studies

	Finding	Initial study confidence	Initial confidence rating
Outcome: Respiratory effects (inhalation only)			
Inhalation acute exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Inhalation intermediate exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Inhalation chronic exposure			
Human studies			
Observational studies			
Ott et al. 2004	Effect	Low	Low
Walravens et al. 1979	Effect	Very Low	

Table C-17. Initial Confidence Rating for Molybdenum Health Effects Studies

	Finding	Initial study confidence	Initial confidence rating
Animal studies			
NTP 1997 (rat)	Effect	High	High
NTP 1997 (mouse)	Effect	High	
Outcome: Hepatic effects			
Inhalation intermediate exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Inhalation chronic exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Oral acute exposure			
Animal studies			
Bersenyi et al. 2008 (rabbit, males)	Effect	Moderate	Moderate
Bersenyi et al. 2008 (rabbit, females)	Effect	Moderate	
Oral intermediate exposure			
Animal studies			
Murray et al. 2014a (rat)	No effect	High	High
Rana and Chauhan 2000 (rat)	Effect	Moderate	
Rana and Kumar 1980b (rat)	Effect	Moderate	
Rana and Kumar 1980c (rat)	Effect	Moderate	
Rana and Kumar 1983 (rat)	Effect	Moderate	Low
Rana and Prakash 1986 (rat)	Effect	Moderate	
Rana et al. 1980 (rat)	Effect	Low	
Rana et al. 1985 (rat)	Effect	Moderate	
Oral chronic exposure			
Human studies			
Observational studies			
Mendy et al. 2012	Effect	Low	Low
Outcome: Renal effects			
Inhalation intermediate exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Inhalation chronic exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Oral acute exposure			
Animal studies			
Bersenyi et al. 2008 (rabbit, males)	No effect	Moderate	Moderate
Bersenyi et al. 2008 (rabbit, females)	No effect	Moderate	

Table C-17. Initial Confidence Rating for Molybdenum Health Effects Studies

	Finding	Initial study confidence	Initial confidence rating
Oral intermediate exposure			
Animal studies			
Bandyopadhyay et al. 1981 (rat)	Effect	Low	High
Bompart et al. 1990 (rat)	Effect	Moderate	
Murray et al. 2014a (rat)	Effect	High	
Rana et al. 1980 (rat)	Effect	Low	
Rana and Kumar 1980c	Effect	Moderate	
Rana and Kumar 1983 (rat)	Effect	Moderate	
Murray et al. 2019 (rat)	No effect	High	High
Outcome: Alterations in uric acid levels			
Inhalation chronic exposure			
Human studies			
Observational studies			
Walravens et al. 1979	Effect	Very Low	Very Low
Oral acute exposure			
Human studies			
Controlled exposure			
Deosthale and Gopalan 1974	No Effect	Low	Low
Oral intermediate exposure			
Animal studies			
Murray et al. 2014a (rat)	No effect	High	High
Oral chronic exposure			
Human studies			
Observational studies			
Koval'sky et al. 1961	Effect	Low	Low
Outcome: Reproductive effects			
Inhalation intermediate exposure			
Animal studies			
NTP 1997 (rat)	No effect	High	High
NTP 1997 (mouse)	No effect	High	
Oral acute exposure			
Animal studies			
Zhang et al. 2013 (mouse)	Effect	Moderate	Moderate
Zhai et al. 2013 (mouse)	Effect	Moderate	
Bersenyi et al. 2008 (male, rabbit)	Effect	Low	
Bersenyi et al. 2008 (female, rabbit)	No effect	Very Low	Very low
Oral intermediate exposure			
Animal studies			
Fungwe et al. 1990 (rat)	Effect	Moderate	High
Jeter and Davis 1954 (rat, adult)	Effect	Very Low	
Jeter and Davis 1954 (rat, weanling)	Effect	Very Low	
Pandey and Singh 2002 (rat)	Effect	Moderate	

Table C-17. Initial Confidence Rating for Molybdenum Health Effects Studies

	Finding	Initial study confidence	Initial confidence rating
Pandey and Singh 2002 (rat, fertility study)	Effect	High	
Murray et al. 2014a (rat)	No effect	High	High
Murray et al. 2019 (rat)	No effect	High	
Oral chronic exposure			
Human studies			
Observational studies			
Lewis and Meeker 2015	Effect	Low	
Meeker et al. 2008	Effect	Low	Low
Meeker et al. 2010	Effect	Low	
Outcome: Developmental effects			
Oral intermediate exposure			
Animal studies			
Pandey and Singh 2002 (rat)	Effect	High	High
Jeter and Davis 1954 (rat, weanling)	No effect	Very Low	
Murray et al. 2014b (rat)	No effect	High	High
Murray et al. 2019 (rat)	No effect	High	
Oral chronic exposure			
Human studies			
Observational studies			
Vazquez-Salas et al. 2014	Effect	Low	Low
Shirai et al. 2010	No effect	Low	Low

C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for respiratory, hepatic, renal, alterations in uric acid levels, reproductive, and developmental effects are presented in Table C-18. If the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. An overview of the confidence in the body of evidence for all health effects associated with molybdenum exposure is presented in Table C-19.

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-14, C-15, and C-16). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
  - No downgrade if most studies are in the risk of bias first tier
  - Downgrade one confidence level if most studies are in the risk of bias second tier
  - Downgrade two confidence levels if most studies are in the risk of bias third tier

Table C-18. Adjustments to the Initial Confidence in the Body of Evidence

	Initial confidence	Adjustments to the initial confidence rating	Final confidence
Outcome: Respiratory effects			
Observational studies (effect)	Low	-1 risk of bias; -1 imprecision	Very low
Animal studies (effect)	High	None	High
Animal studies (no effect)	High	+1 magnitude	High
Outcome: Hepatic effects			
Observational studies (effect)	Low	-1 risk of bias	Very low
Animal studies (effect)	Moderate	-1 indirectness (secondary outcomes);	Moderate
Animal studies (no effect)	High	None	High
Outcome: Renal effects			
Animal studies	High	None	High
Animal studies	High	None	High
Outcome: Alterations in uric acid levels			
Observational studies (effect)	Low	-1 risk of bias	Very low
Controlled exposure studies (no effect)	Low	None	Low
Animal studies (no effect)	High	None	High
Outcome: Reproductive effects			
Observational studies (effect)	Low	None	Low
Animal studies (effect)	High	-1 inconsistency	Moderate
Animal studies (no effect)	High	None	High
Outcome: Developmental effects			
Observational studies (effect)	Low	None	Low
Observational studies (no effect)	Low	None	Low
Animal studies	High	-1 inconsistency	Moderate
Animal studies	High	None	High

Table C-19. Confidence in the Body of Evidence for Molybdenum

	Confidence in body of evidence	
Outcome	Human studies	Animal studies
Respiratory effects	Very low (effect)	High (effect) High (no effect)
Hepatic effects	Very low (effect)	Moderate (effect) High (no effect)
Renal effects	No data	High (effect) High (no effect)
Alterations in uric acid levels	Very low (effect) Low (no effect)	High (effect)
Reproductive Effects	Low (effect)	Moderate (effect) High (no effect)
Developmental effects	Low (effect) Low (no effect)	Moderate (effect) High (no effect)

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
    - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
    - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
    - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
    - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
    - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
    - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
    - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

  Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:
    - No downgrade if none of the factors are considered indirect
    - Downgrade one confidence level if one of the factors is considered indirect
    - Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
    - No downgrade if there are no serious imprecisions
    - Downgrade one confidence level for serious imprecisions
    - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
    - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
    - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence of a monotonic dose-response gradient
    - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level if there is a high degree of consistency in the database

C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for molybdenum, the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for molybdenum is presented in Table C-20.

Table C-20. Level of Evidence of Health Effects for Molybdenum

Outcome	Confidence in body of evidence	Direction of health effect	Level of evidence for health effect
Human studies			
Respiratory effects (inhalation only)	Very low	Health effect	Inadequate
Hepatic effects	Very low	Health effect	Inadequate
Renal effects	No data	No data	No data
Alterations in uric acid levels	Low	Health effect	Inadequate
Reproductive effects	Low	Health effect	Low
Developmental effects	Low	Health effect	Low
Animal studies			
Respiratory effects (inhalation only)	High	Health effect No health effect	High High
Hepatic effects	Moderate	Health effect No health effect	Moderate High
Renal effects	High	Health effect	High
Alterations in uric acid levels	High	No effect	Evidence of no health effect

Table C-20. Level of Evidence of Health Effects for Molybdenum

Outcome	Confidence in body of evidence	Direction of health effect	Level of evidence for health effect
Reproductive effects	Moderate	Health effect No health effect	Moderate High
Developmental effects[a]	Moderate	Health effect No health effect	High Evidence of no health effect

C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

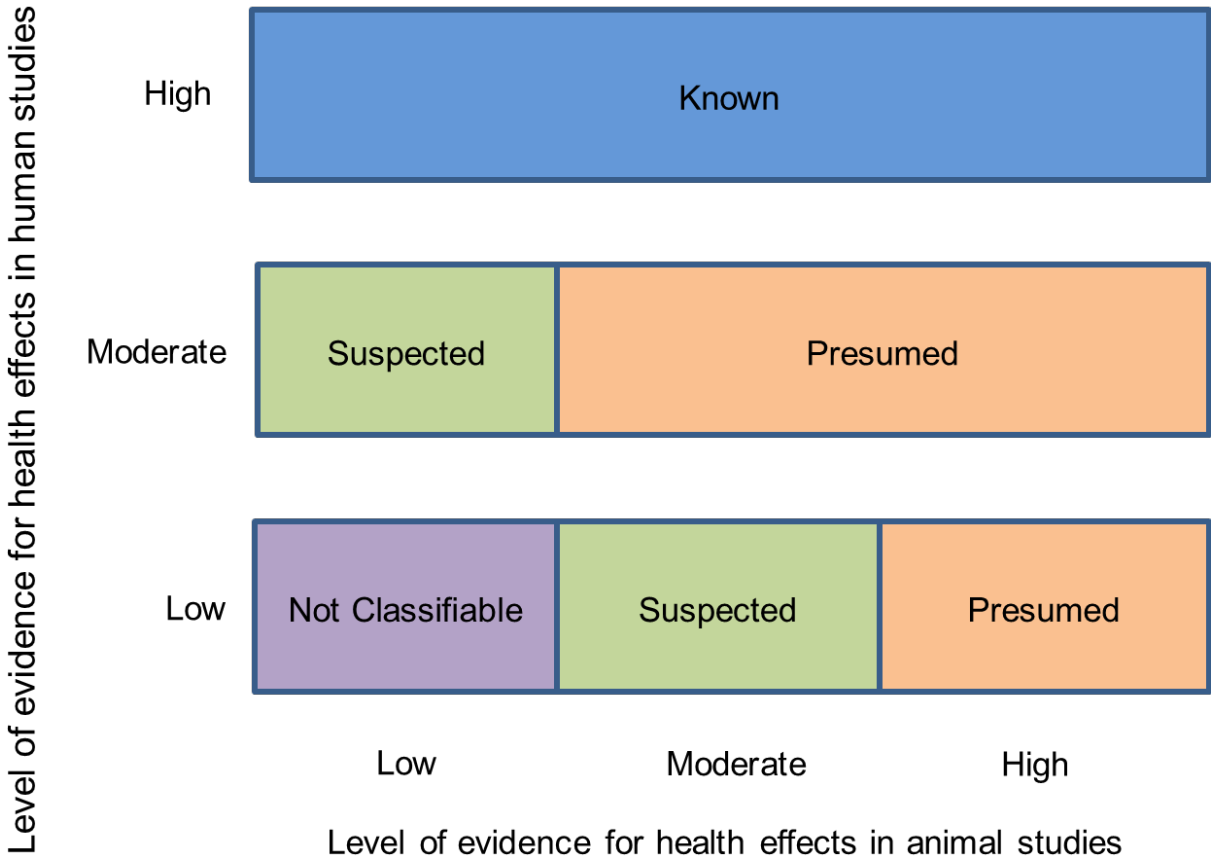
The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in and described below:

- **Known:** A health effect in this category would have:
  - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** moderate level of evidence in animal studies
- **Not classifiable:** A health effect in this category would have:
  - Low level of evidence in human studies **AND** low level of evidence in animal studies

Figure C-1. Hazard Identification Scheme



Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for molybdenum are listed below and summarized in Table C-21.

Table C-21. Hazard Identification Conclusions for Molybdenum

Outcome	Hazard identification
Respiratory effects	Presumed health effect following long-term inhalation exposure
Hepatic effects	Not classifiable as a hazard to humans
Renal effects	Presumed health effect
Alterations in uric acid levels	Not classifiable as a hazard to humans
Reproductive effects	Suspected health effect
Developmental effects	Not classifiable as a hazard to humans

Presumed Health Effects

- Respiratory effects following long-term inhalation exposure to molybdenum oxides
  - Inadequate evidence from studies of molybdenum oxide workers (Ott et al. 2004; Walravens et al. 1979).
  - High level of evidence from chronic studies in rats and mice exposed to molybdenum trioxide (NTP 1997). Respiratory effects were not observed following acute- or intermediate-duration inhalation exposure.
- Renal effects
  - No data in humans.
  - High level of evidence of histological alterations in kidneys, alterations in renal function, and/or increased lipid levels in the kidneys in orally exposed rats (Bandyopadhyay et al. 1981; Bompart et al. 1990; Murray et al. 2014a; Rana and Kumar 1980c, 1983; Rana et al. 1980).

Not Classifiable as a Hazard to Humans

- Hepatic effects
  - Inadequate evidence of increased risk of self-reported liver conditions from a cross-sectional study (Mendy et al. 2012).
  - High evidence of no histological alterations following intermediate or chronic inhalation exposure of rats and mice to molybdenum trioxide (NTP 1997), acute oral exposure of rabbits to ammonium heptamolybdate (Bersenyi et al. 2008), or intermediate oral exposure of rats to sodium molybdate (Murray et al. 2014a;).
  - Moderate evidence of increases in clinical chemistry parameters and/or liver lipid levels in rabbits following acute oral exposure (Bersenyi et al. 2008) or rats exposed orally exposed to high doses (Rana and Chauhan 2000; Rana and Kumar 1980b, 1980c, 1983; Rana and Prakash 1986; Rana et al. 1980, 1985).
  - The hazard identification for hepatic effects was downgraded to Not Classifiable because the toxicological significance of the alterations in serum enzyme levels and lipid levels were not known and well-designed inhalation and oral laboratory animal studies have not reported histological alterations.
- Alterations in uric acid levels
  - Low evidence of an effect in cross-sectional studies (Koval'skiy et al. 1961; Walravens et al. 1979).
  - High confidence in an animal study not finding an effect (Murray et al. 2014a).
- Reproductive effects
  - Low level of evidence of male reproductive effects in cross-sectional studies (Lewis and Meeker 2015; Meeker et al. 2008, 2010).

- Two high-quality, intermediate-duration (Murray et al. 2014a) and 2-generation (Murray et al. 2019) studies have not reported reproductive effects.
- There is a moderate level of evidence of male and/or female reproductive effects in orally exposed rats (Fungwe et al. 1990; Pandey and Singh 2002), mice (Zhai et al. 2013; Zhang et al. 2013), and rabbits (Bersenyi et al. 2008).

- Developmental effects
  - Low evidence of an effect in a cross-sectional study. Two cross-sectional studies reported no alterations in newborn body weight (Shirai et al. 2010; Vazquez-Salas et al. 2014); one study reported decreases in psychomotor development indices (Vazquez-Salas et al. 2014).
  - Three studies in rats did not find alterations in resorptions, post-implantation losses, or fetal body weights (Jeter and Davis 1954; Murray et al. 2014b, 2019); the initial confidence levels for two of these studies were high and the third study was very low. A fourth study (initial high confidence level) involving male-only exposure found decreases in number of live fetuses and fetal body weights (Pandey and Singh 2002). The animal studies had different study designs (male only, female only, male and female exposure) making a comparison across studies difficult. Additionally, none of the animal studies evaluated potential neurodevelopmental effects, which were observed in an epidemiology study. Thus, the available data were not considered adequate for drawing a conclusion on the potential developmental toxicity of molybdenum in humans.

APPENDIX D. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page D-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page D-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX D

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (3)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

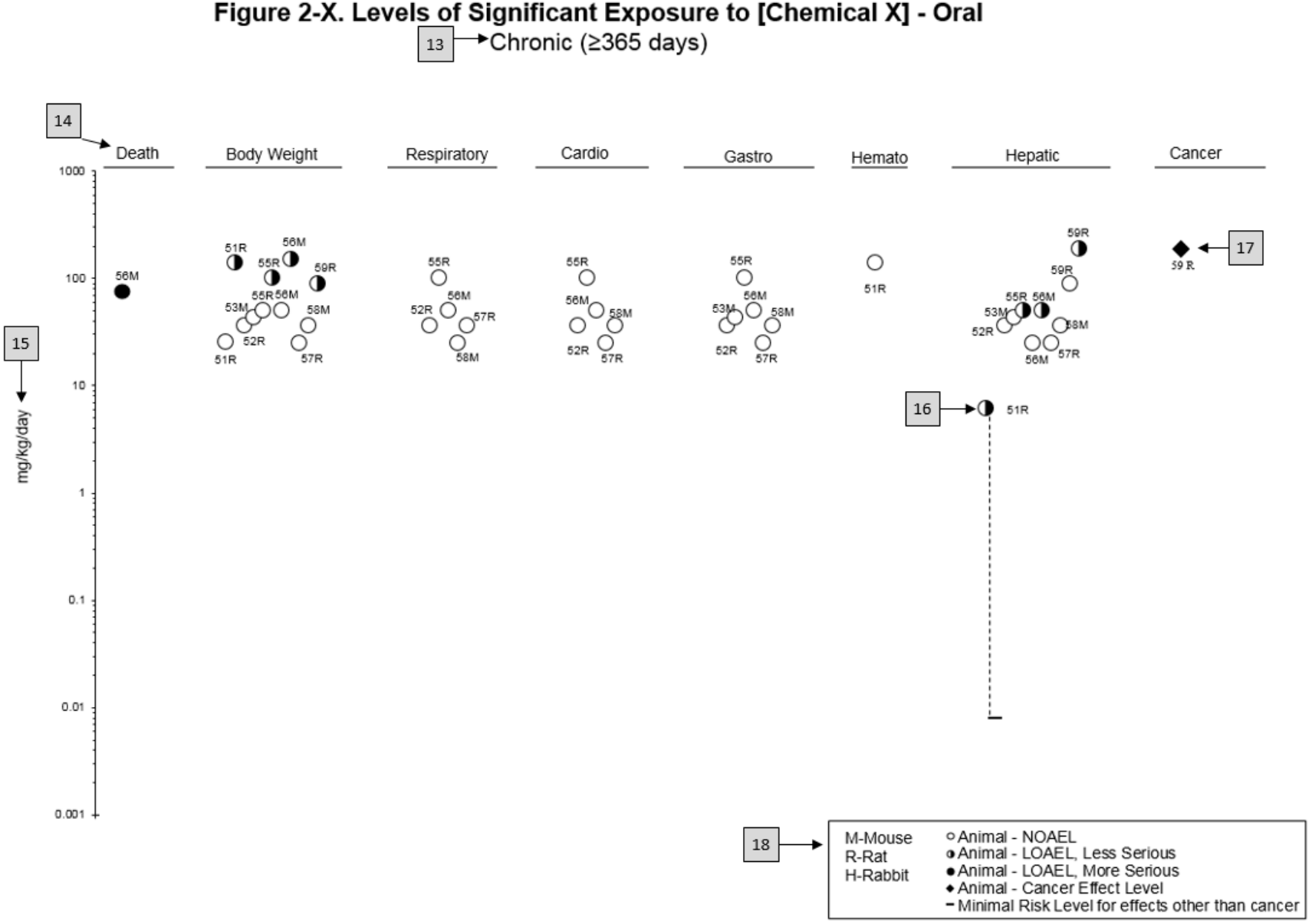
[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

1

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



1

APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX F. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	Celsius
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram

NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States

USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

Attachment 2

United States Environmental Protection Agency

Office of Water
EPA 822-F-22-002
June 2022

Technical Fact Sheet: Drinking Water Health Advisories for Four PFAS (PFOA, PFOS, GenX chemicals, and PFBS)

Summary

As part of EPA's commitment to safeguard communities from per- and polyfluoroalkyl substances (PFAS), EPA has issued interim updated drinking water health advisories for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), and final health advisories for hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (together referred to as "GenX chemicals") and perfluorobutane sulfonic acid and its related compound potassium perfluorobutane sulfonate (together referred to as "PFBS"). The interim health advisories for PFOA and PFOS are intended to provide information to states and public water systems until the National Primary Drinking Water regulation for PFAS takes effect. All four of these health advisories provide drinking water system operators, and state, tribal, and local officials who have the primary responsibility for overseeing these systems, with information on the health risks of these chemicals, so they can take the appropriate actions to protect their residents.

Background

What Are PFAS?

PFAS are synthetic chemicals that have been manufactured and used by a broad range of industries since the 1940s. PFAS are used in many applications because of their unique physical properties such as resistance to high and low temperatures, resistance to degradation, and nonstick characteristics. PFAS have been detected worldwide in the air, soil, and water. Due to their widespread use and persistence in the environment, most people in the United States have been exposed to PFAS. There is evidence that exposure above specific levels to certain PFAS may cause adverse health effects.

What Are Drinking Water Health Advisories?

Drinking water health advisories (HAs) provide information on contaminants that can cause human health effects and are known or anticipated to occur in drinking water. EPA's HAs are non-enforceable and non-regulatory and provide technical information to drinking water system operators, as well as federal, state, tribal, and local officials on health effects, analytical methods, and treatment technologies associated with drinking water contamination.

Why is EPA Issuing These HAs?

In 2016, EPA published HAs for PFOA and PFOS based on the evidence available at that time (U.S. EPA 2016, a,b). The science has evolved since then and EPA is now replacing the 2016 advisories with interim updated lifetime HAs for PFOA and PFOS that are based on new studies and draft toxicity values from EPA's 2021 draft PFOA and PFOS health effects documents. Fulfilling EPA's commitment in its October 2021 PFAS Strategic Roadmap, EPA has issued final lifetime HAs for GenX chemicals and PFBS.

How Does EPA Calculate HAs?

The following equation is used to derive a lifetime noncancer health advisory. A lifetime noncancer health advisory is designed to be protective of noncancer effects over a lifetime of exposure, including sensitive populations and life stages, and is typically based on data from experimental animal toxicity and/or human studies.

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC}$$

Where:

RfD = chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime.

DWI-BW = drinking water intake rate adjusted for body weight—the 90th percentile DWI for the selected population or life stage, adjusted for body weight (BW), in units of L/kg bw-day. The DWI-BW considers both direct and indirect consumption of tap water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee).

RSC = relative source contribution—the percentage of the total oral exposure attributed to drinking water sources (U.S. EPA, 2000) where the remainder of the exposure is allocated to all other routes or sources.

What Types of Health Outcomes are Associated with Exposure to These Four PFAS, and How Did EPA Develop the HAs?

PFOA and PFOS

EPA is conducting extensive evaluations of human epidemiological and experimental animal study data to support the Safe Drinking Water Act (SDWA) National Primary Drinking Water Regulation for PFOA and PFOS. In November 2021, EPA released draft documents that summarize the updated health effects analyses for EPA Science Advisory Board (SAB) review (U.S. EPA, 2021a, b). EPA evaluated over 400 studies published since 2016 and used new human health risk assessment approaches, tools, and models. Human studies have found associations between PFOA and/or PFOS exposure and effects on the immune system, the cardiovascular system, development (e.g., decreased birth weight), and cancer. The new published peer-reviewed data and draft EPA analyses (U.S. EPA, 2021a, b) indicate that the levels at which negative health outcomes could occur are much lower than previously understood when the agency issued its 2016 HAs for PFOA and PFOS (70 parts per trillion or ppt). EPA's 2021 draft non-cancer reference doses (RfDs) based on human epidemiology studies for various effects (e.g., developmental/growth, cardiovascular health outcomes, immune health) range from ~10^{-7} to 10^{-9} mg/kg/day. These draft RfDs are two to four orders of magnitude lower than EPA's 2016 RfDs of 2 x 10^{-5} mg/kg/day (U.S. EPA, 2021a, b).

The most sensitive non-cancer effect based on the draft EPA analyses, decreased immunity (i.e., decreased serum antibody concentrations after vaccination) in children in a human epidemiology study, was selected as the basis for the draft RfD (toxicity value) in the PFOA and PFOS health effects draft documents (U.S. EPA, 2021a, b). EPA used the draft RfD to derive the interim updated HAs for PFOA and PFOS. In the critical study, EPA selected the critical effect of decreased serum antibody concentration in children associated with increased serum PFOA and/or PFOS concentrations. EPA expects this critical effect to be protective of all other adverse health effects observed in humans because this adverse effect can reduce the protection afforded by vaccines after exposure to PFOA/PFOS during a sensitive developmental life stage and it yields the lowest point of departure (POD) (U.S. EPA, 2021a, b). For both PFOA and PFOS, an intraspecies uncertainty factor

(UF_H) of 10 was applied to account for variability in the response within the human population (U.S. EPA, 2002). EPA identified children ages 0-5 years as a sensitive life stage, based on the critical study, and selected the corresponding DWI-BW. Based on a literature search of the available information on exposure sources and routes, EPA calculated the interim HAs for PFOA and PFOS using an RSC of 0.20, meaning that 20% of the exposure – equal to the RfD – is allocated to drinking water, and the remaining 80% is attributed to all other potential exposure sources (U.S. EPA, 2022a, b; U.S. EPA, 2000).

While there is evidence that PFOA is likely to be carcinogenic to humans, EPA has not derived a cancer risk concentration in water for PFOA at this time. For PFOS, there is suggestive evidence of carcinogenic potential in humans. Additional analyses of the cancer study data are ongoing for both PFOA and PFOS.

The underlying science that EPA used to develop the interim health advisories is currently undergoing SAB review, and therefore, these interim health advisories are subject to change. After receiving the SAB's final report, EPA will complete its revisions to address their feedback and recommendations, which could lead the agency to draw different conclusions than are reflected in the draft health effects analyses (U.S. EPA, 2021a, b). As a result, the interim health advisory levels for PFOA and PFOS (U.S. EPA, 2022a, b) could change. EPA may update or remove the interim health advisories for PFOA and PFOS upon finalization of the National Primary Drinking Water Regulation.

GenX Chemicals and PFBS

EPA's final health advisories for GenX chemicals and PFBS are based on animal toxicity studies following oral exposure to these chemicals. Studies of exposure to GenX chemicals have reported health effects in the liver, kidney, immune system, development, as well as cancer. The most sensitive non-cancer effect among the available data was an adverse liver effect (constellation of liver lesions) (U.S. EPA, 2021c). This critical effect was the basis for the final chronic RfD which EPA used to derive the final HA for GenX chemicals. To develop the final chronic RfD for GenX chemicals, EPA applied a composite UF of 3,000 (i.e., 10X for intraspecies variability (UF_H), 3X for interspecies differences (UF_A), 10X for extrapolation from a subchronic to a chronic dosing duration (UF_S), and 10X for database deficiencies (UF_D)) (U.S. EPA, 2021c). EPA identified lactating women as an adult life stage with the greatest potential exposure from drinking water, based on the critical study, and selected the corresponding DWI-BW. EPA calculated the final HA for GenX chemicals using an RSC of 0.20, meaning that 20% of the exposure -- equal to the RfD -- is allocated to drinking water, and the remaining 80% is attributed to all other potential exposure sources (U.S. EPA, 2022c). There is suggestive evidence of carcinogenic potential of oral exposure to GenX chemicals in humans and the available data are insufficient to derive a cancer risk concentration in water for GenX chemicals.

For PFBS, animal studies have reported health effects on the thyroid, reproductive system, development, and kidney following oral exposure. The most sensitive non-cancer effect was an adverse effect on the thyroid (i.e., decreased serum total thyroxine) in newborn mice in a study with exposure throughout gestation in the mothers. This critical effect was the basis for the final chronic RfD which EPA used to derive the final HA for PFBS (U.S. EPA, 2021d; U.S. EPA, 2022d). EPA applied a composite UF of 300 (i.e., 10X for intraspecies variability (UF_H), 3X for interspecies differences (UF_A), and 10X for database deficiencies (UF_D)) (U.S. EPA, 2021d). EPA identified women of child-bearing age as a sensitive life stage, based on the critical study, and selected the corresponding DWI-BW. EPA calculated the final HA for PFBS using an RSC of 0.20, meaning that 20% of the exposure – equal to the RfD – is allocated to drinking water, and the remaining 80% is attributed to all other potential exposure sources (U.S. EPA, 2022d). There were no studies identified that evaluated potential cancer effects after PFBS exposure so the potential for cancer effects after PFBS exposure could not be evaluated.

What are the HAs for the four PFAS?

PFOA Interim Updated Health Advisory – Input Parameters and HA Value			
Parameter	**Value**	**Units**	**Source**
Chronic RfD	1.5E-9	mg/kg/day	U.S. EPA, 2021a. *Draft* RfD based on developmental immune health outcome (suppression of tetanus vaccine response in 7-year-old children). Human epidemiological studies.
DWI-BW	0.0701	L/kg-day	U.S. EPA, 2019. 90th percentile direct and indirect consumption of community water, consumers-only population, two-day average, for children ages 0 to <5 years based on 2005−2010 National Health and Nutrition Examination Survey (NHANES).
RSC	0.2	N/A	U.S. EPA, 2021a. RSC based on a review of the current scientific literature.
PFOA Interim Updated Lifetime Health Advisory = 4E-09 mg/L or 0.004 ppt **(EPA 2022a)**			

PFOS Interim Updated Health Advisory – Input Parameters and HA Value			
Parameter	**Value**	**Units**	**Source**
Chronic RfD	7.9E-09	mg/kg/day	U.S. EPA, 2021b. *Draft* RfD based on developmental immune health outcome (suppression of diphtheria vaccine response in 7-year-old children). Human epidemiological studies.
DWI-BW	0.0701	L/kg-day	U.S. EPA, 2019. 90th percentile direct and indirect consumption of community water, consumers-only population, two-day average, for children ages 0 to <5 years based on 2005−2010 NHANES.
RSC	0.2	N/A	U.S. EPA, 2021b. RSC based on a review of the current scientific literature.
PFOS Interim Updated Lifetime Health Advisory = 2E-08 mg/L or 0.02 ppt **(EPA 2022b)**			

GenX Chemicals Final Health Advisory – Input Parameters and HA Value			
Parameter	**Value**	**Units**	**Source**
Chronic RfD	3E-06	mg/kg/day	U.S. EPA, 2021c. Final RfD based on critical liver effects (constellation of liver lesions as defined by the National Toxicology Program Pathology Working Group) in parental female mice exposed to HFPO dimer acid ammonium salt by gavage for 53–64 days.
DWI-BW	0.0469	L/kg-day	U.S. EPA, 2019. 90th percentile two-day average, consumer only estimate of combined direct and indirect community water ingestion for lactating women (13 to <50 years) based on 2005−2010 NHANES.
RSC	0.2	N/A	U.S. EPA, 2021c. Based on a review of the current scientific literature.
GenX Chemicals Final Lifetime Health Advisory = 0.00001 mg/L or 10 ppt **(EPA 2022c)**			

PFBS Final Health Advisory – Input Parameters and HA Value			
Parameter	**Value**	**Units**	**Source**
Chronic RfD	3E-04	mg/kg/day	U.S. EPA, 2021d: Final RfD based on critical effect of decreased serum total thyroxine (T4) in newborn (postnatal day (PND) 1) mice after gestational exposure to the mother.
DWI-BW	0.0354	L/kg-day	U.S. EPA, 2019. 90th percentile two-day average, consumer only estimate of combined direct and indirect community water ingestion for women of childbearing age (13 to <50 years) based on 2005−2010 NHANES.
RSC	0.2	N/A	U.S. EPA, 2021d. Based on a review of the current scientific literature.
PFBS Final Lifetime Health Advisory = 0.002 mg/L or 2,000 ppt **(EPA 2022d)**			

Application of Health Advisories to Different Exposure Scenarios

Because the critical effects identified for PFOA, PFOS, and PFBS are developmental effects that can potentially result from short-term exposure to these PFAS during a critical period of development, EPA guidelines support applying the lifetime health advisories for these three PFAS to both short-term and chronic risk assessment scenarios (U.S. EPA, 1991).

The lifetime health advisory for GenX chemicals used a chronic RfD from the final EPA toxicity assessment (U.S. EPA, 2021c) based on the critical effect of adverse liver effects in adults (parental females) from a subchronic study (53–64 day exposure). In the assessment, a 10X UF_S for subchronic to chronic exposure was applied to derive the chronic RfD (U.S. EPA, 2021c). Because the critical effect identified for GenX chemicals is in adults, the HA applies to chronic exposure scenarios. The HA was based on exposure to lactating women, an adult life stage with the greatest drinking water intake rate. Application of the GenX chemicals HA to a shorter-term risk assessment scenario would provide a conservative, health protective approach in the absence of other information.

Consideration of Noncancer Health Risks from PFAS Mixtures

EPA recently released a *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* that is currently undergoing SAB review (U.S. EPA, 2021e). That draft document provides a flexible, data-driven framework that facilitates practical evaluation of two or more PFAS based on current, available EPA chemical mixtures approaches and methods. Examples are presented for three approaches—Hazard Index (HI), Relative Potency Factor (RPF), and Mixture BMD—to demonstrate application to PFAS mixtures. To use these approaches, specific input values and information for each PFAS are needed or can be developed.

The health advisory documents provide an example of how to use the HI approach to assess the potential noncancer risk of a mixture of PFOA, PFOS, GenX chemicals, and PFBS (U.S. EPA, 2022 a-d). A mixture PFAS HI can be calculated when health-based water concentrations (e.g., HAs, MCLGs) for a set of PFAS are available or can be calculated. In the example, hazard quotients (HQs) are calculated by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HA (e.g., expressed as ng/L), as shown in the equation below. Component HQs are then summed across the PFAS mixture to yield the mixture PFAS HI. A mixture PFAS HI greater than 1 indicates an exceedance of the health protective level and indicates potential human health risk for noncancer effects from the PFAS mixture in water. When component health-based water concentrations (in this case, HAs) are below the analytical method detection limit, as is the case for PFOA and PFOS, such individual component HQs exceed 1, meaning that any detectable level of PFOA or PFOS will result in an HI greater than 1 for the whole mixture. Further analysis could provide a refined assessment of the potential for health effects associated with the individual PFAS and their contributions to the potential joint toxicity associated with the mixture. For more details, please see U.S. EPA (2021e).

$$HI = \left(\frac{[PFOA_{water}]}{[PFOA_{HA}]}\right) + \left(\frac{[PFOS_{water}]}{[PFOS_{HA}]}\right) + \left(\frac{[GenX_{water}]}{[GenX_{HA}]}\right) + \left(\frac{[PFBS_{water}]}{[PFBS_{HA}]}\right)$$

Where:

HI = hazard index;

[$PFAS_{water}$] = concentration for a given PFAS in water;

[$PFAS_{HA}$] = the HA value for a given PFAS

Where can I find more information?

To view the HA documents, go to: https://www.epa.gov/sdwa/drinking-water-health-advisories-has

To view the PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024, go to: https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

For information on drinking water, go to: www.epa.gov/safewater

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Attachment 3

United States Environmental Protection Agency
Office of Water
Office of Science and Technology
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EPA-822-B-00-004
October 2000

Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)

EPA 822-B-00-004
October 2000

Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)

Final

Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
Washington, DC 20460

NOTICE

The policies and procedures set forth in this document are intended solely to describe EPA methods for developing or revising ambient water quality criteria to protect human health, pursuant to Section 304(a) of the Clean Water Act, and to serve as guidance to States and authorized Tribes for developing their own water quality criteria. This guidance does not substitute for the Clean Water Act or EPA's regulations; nor is it a regulation itself. Thus, it does not impose legally-binding requirements on EPA, States, Tribes or the regulated community, and may not apply to a particular situation based upon the circumstances.

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

This document presents EPA's recommended Methodology for developing ambient water quality criteria as required under Section 304(a) of the Clean Water Act (CWA). The Methodology is guidance for scientific human health assessments used by EPA to develop, publish, and from time to time revise, recommended criteria for water quality accurately reflecting the latest scientific knowledge. The recommended criteria serve States and Tribes' needs in their development of water quality standards under Section 303(c) of the CWA.

The term "water quality criteria" is used in two sections of the Clean Water Act, Section 304(a)(1) and Section 303(c)(2). The term has a different program impact in each section. In Section 304, the term represents a scientific assessment of ecological and human health effects that EPA recommends to States and authorized Tribes for establishing water quality standards that ultimately provide a basis for controlling discharges or releases of pollutants. Ambient water quality criteria associated with specific stream uses when adopted as State or Tribal water quality standards under Section 303 define the maximum levels of a pollutant necessary to protect designated uses in ambient waters. The water quality criteria adopted in the State or Tribal water quality standards could have the same numerical limits as the criteria developed under Section 304. However, in many situations States and authorized Tribes may want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. When adopting their water quality criteria, States and authorized Tribes have four options: (1) adopt EPA's 304(a) recommendations; (2) adopt 304(a) criteria modified to reflect site-specific conditions; (3) develop criteria based on other scientifically defensible methods; or (4) establish narrative criteria where numeric criteria cannot be determined.

EPA will use this Methodology to develop new ambient water quality criteria and to revise existing recommended water quality criteria. It also provides States and authorized Tribes the necessary guidance to adjust water quality criteria developed under Section 304 to reflect local conditions or to develop their own water quality criteria using scientifically defensible methods consistent with this Methodology. EPA encourages States and authorized Tribes to use this Methodology to develop or revise water quality criteria to appropriately reflect local conditions. EPA believes that ambient water quality criteria inherently require several risk management decisions that are, in many cases, better made at the State, Tribal, or regional level. Additional guidance to assist States and authorized Tribes in the modification of criteria based on the Methodology will accompany this document in the form of three companion Technical Support Documents on Risk Assessment, Exposure Assessment, and Bioaccumulation Assessment.

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Geoffrey H. Grubbs
Director
Office of Science and Technology

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ACKNOWLEDGMENTS

Project Leader

Denis Borum	U.S. EPA Office of Science and Technology

Coauthors

Risk Assessment

Joyce M. Donohue, Ph.D.*	U.S. EPA Office of Science and Technology
Julie T. Du, Ph.D.*	U.S. EPA Office of Science and Technology
Charles O. Abernathy, Ph.D.	U.S. EPA Office of Science and Technology

Exposure

Denis Borum *	U.S. EPA Office of Science and Technology
Helen Jacobs, M.S.	U.S. EPA Office of Science and Technology
Henry Kahn, D.Sc.	U.S. EPA Office of Science and Technology

Bioaccumulation

Keith G. Sappington, M.S.*	U.S. EPA Office of Science and Technology
Lawrence P. Burkhard, Ph.D.	U.S. EPA Office of Research and Development
Philip M. Cook, Ph.D.	U.S. EPA Office of Research and Development
Erik L. Winchester, M.S.	U.S. EPA Office of Science and Technology

U.S. EPA Technical Reviewers

William Beckwith	U.S. EPA Region 1
Jeff Bigler	U.S. EPA Office of Science and Technology
Sally Brough	U.S. EPA Region 10
Karen Clark	U.S. EPA Office of General Counsel
Gregory Currey	U.S. EPA Office of Wastewater Management
Vicki Dellarco	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances
Charles Delos	U.S. EPA Office of Science and Technology
Arnold Den	U.S. EPA Region 9
Catherine Eiden	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances
Michael Firestone	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances
Steven Galson	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances
Sue Gilbertson	U.S. EPA Office of Science and Technology
Denise Hakowski	U.S. EPA Region 3
Joel Hansel	U.S. EPA Region 4
Wayne Jackson	U.S. EPA Region 2
Annie Jarabek	U.S. EPA Office of Research and Development
William Jordan	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances

Margaret Kelly	U.S. EPA Office of Children's Health Protection
Henry Lee	U.S. EPA Office of Research and Development
Sharon Lin	U.S. EPA Office of Wetlands, Oceans, and Watersheds
Roseanne Lorenzana	U.S. EPA Region 10
Gregory McCabe	U.S. EPA Region 7
Jennifer Mclain	U.S. EPA Office of Ground Water and Drinking Water
Bruce Mintz	U.S. EPA Office of Research and Development
Dave Moon	U.S. EPA Region 8
William Morrow	U.S. EPA Office of Science and Technology
Jacqueline Moya	U.S. EPA Office of Research and Development
Deirdre Murphy	U.S. EPA Office of Air Quality Planning and Standards
Joseph Nabholz	U.S. EPA Office of Prevention, Pesticides, and Toxic Substances
Russell Nelson	U.S. EPA Region 6
Jennifer Orme-Zavaleta	U.S. EPA Office of Research and Development
Lynn Papa	U.S. EPA Office of Research and Development
Robert Pepin	U.S. EPA Region 5
David Pfeifer	U.S. EPA Region 5
Rita Schoeny	U.S. EPA Office of Science and Technology
Charles Stephan	U.S. EPA Office of Research and Development
Linda Teuschler	U.S. EPA Office of Research and Development
David Tomey	U.S. EPA Region 1
Fritz Wagener	U.S. EPA Region 4
Jennifer Wigal	U.S. EPA Office of Science and Technology
Jeanette Wiltse	U.S. EPA Office of Scence and Technology
Gary Wolinsky	U.S. EPA Region 9
Philip Woods	U.S. EPA Region 9
William Wuerthele	U.S. EPA Region 8

* Principal U.S. EPA Author and Contact

EXTERNAL PEER REVIEW WORKGROUP

The following professionals were part of the External Peer Review Workgroup that provided technical and scientific review regarding the content and technical approach in the July 1998 *Draft Ambient Water Quality Criteria Derivation Methodology: Human Health*. Their comments were reviewed and incorporated where appropriate to develop this final document.

Kenneth T. Bogen, Ph.D.	Lawrence Livermore National Laboratory
Paul E. Brubaker, Ph.D.	P.E. Brubaker Associates
Peter L. DeFur, Ph.D.	Virginia Commonwealth University
Karen Erstfeld, Ph.D.	Rutgers University
Bob Fares, Ph.D.	Environmental Standards, Inc.
Laura Green, Ph.D.	Cambridge Environmental, Inc.
Robert Hales, Ph.D.	Virginia Institute of Marine Science
Brendan Hickie, Ph.D.	Trent University
Ernest Hodgson, Ph.D.	North Carolina State University
Paul Locke, Ph.D.	Johns Hopkins University
Lynn S. McCarty, Ph.D.	LS McCarty Scientific Research and Consulting
Erik Rifkin, Ph.D.	Rifkin and Associates, Inc.
Damian Shea, Ph.D.	North Carolina State University
Nga Tran, Ph.D.	Johns Hopkins University
Curtis Travis, Ph.D.	Project Performance Corp.

Potential areas for conflict of interest were investigated via direct inquiry with the peer reviews and review of their current affiliations. No conflicts of interest were identified.

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LIST OF ACRONYMS

ADI	Acceptable Daily Intake
ARAR	Applicable or Relevant and Appropriate Requirements
ASTM	American Society of Testing and Materials
AWQC	Ambient Water Quality Criteria
BAF	Bioaccumulation Factor
BAF_{ℓ}^{fd}	Baseline Bioaccumulation Factor
BCF	Bioconcentration Factor
BCF_{ℓ}^{fd}	Baseline Bioconcentration Factor
BCF_{T}^{t}	Bioconcentration Factor Based on Total Concentrations in Tissue and Water
BMD	Benchmark Dose
BMDL	Lower-Bound Confidence Limit on the BMD
BMF	Biomagnification Factor
BMR	Benchmark Response
BSAF	Biota-Sediment Accumulation Factors
BW	Body Weight
C_{ℓ}	Lipid-normalized Concentration
C_{soc}	Organic Carbon-normalized Concentration
C_t	Concentration of the Chemical in the Specified Wet Tissue
C_w	Concentration of the Chemical in Water
CDC	U.S. Centers for Disease Control and Prevention
CSFII	Continuing Survey of Food Intake by Individuals
CWA	Clean Water Act
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DI	Drinking Water Intake
DNA	Deoxyribonucleic Acid
DNOC	2,4-dinitro-o-cresol
DOC	Dissolved Organic Carbon
ED_{10}	Dose Associated with a 10 Percent Extra Risk
EPA	Environmental Protection Agency
f_{fd}	Fraction Freely Dissolved
f_{ℓ}	Fraction Lipid
FCM	Food Chain Multiplier
FEL	Frank Effect Level
FI	Fish Intake
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLI	Great Lakes Water Quality Initiative
HCBD	Hexachlorobutadiene
IARC	International Agency for Research on Cancer
II	Incidental Ingestion
ILSI	International Life Sciences Institute

IRIS	Integration Risk Information System
kg	kilogram
K_{ow}	Octanol-Water Partition Coefficient
L	Liter
LAS	Linear Alkylbenzesulfonate
LED_{10}	The Lower 95 Percent Confidence Limit on a Dose Associated with a 10 Percent Extra Risk
LMS	Linear Multistage Model
LOAEL	Lowest Observed Adverse Effect Level
M_l	Mass of Lipid in Specified Tissue
M_t	Mass of Specified Tissue (Wet Weight)
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MF	Modifying Factor
mg	Milligrams
ml	Milliliters
MOA	Mode of Action
MOE	Margin of Exposure
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NFCS	Nationwide Food Consumption Survey
NHANES	National Health and Nutrition Examination Survey
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NPDES	National Pollutant Discharge Elimination System
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyls
POD	Point of Departure
POC	Particulate Organic Carbon
RDA	Recommended Daily Allowance
RfC	Reference Concentration
RfD	Reference Dose
RfD_{DT}	Reference Dose for Developmental Effects
RPF	Relative Potency Factor
RSC	Relative Source Contribution
RSD	Risk-Specific Dose
SAB	Science Advisory Board
SDWA	Safe Drinking Water Act
SF	Safety Factor
STORET	Storage Retrieval
TEAM	Total Exposure Assessment Methodology
TEF	Toxicity Equivalency Factor
TMDL	Total Maximum Daily Load
TSD	Technical Support Document
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency

UF	Uncertainty Factor
WQBEL	Water Quality-Based Effluent Limits

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1. INTRODUCTION

1.1 WATER QUALITY CRITERIA AND STANDARDS

Pursuant to Section 304(a)(1) of the Clean Water Act (CWA), the U.S. Environmental Protection Agency (EPA) is required to publish, and from time to time thereafter revise, criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on human health which may be expected from the presence of pollutants in any body of water.

Historically, the ambient water quality criteria (AWQC or 304(a) criteria) provided two essential types of information: (1) discussions of available scientific data on the effects of the pollutants on public health and welfare, aquatic life, and recreation; and (2) quantitative concentrations or qualitative assessments of the levels of pollutants in water which, if not exceeded, will generally ensure adequate water quality for a specified water use. Water quality criteria developed under Section 304(a) are based solely on data and scientific judgments on the relationship between pollutant concentrations and environmental and human health effects. The 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting the criteria in ambient water. These 304(a) criteria may be used as guidance by States and authorized Tribes to establish water quality standards, which ultimately provide a basis for controlling discharges or releases of pollutants into ambient waters.

In 1980, AWQC were derived for 64 pollutants using guidelines developed by the Agency for calculating the impact of waterborne pollutants on aquatic organisms and on human health. Those guidelines consisted of systematic procedures for assessing valid and appropriate data concerning a pollutant's acute and chronic adverse effects on aquatic organisms, nonhuman mammals, and humans.

1.2 PURPOSE OF THIS DOCUMENT

The *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (hereafter the "2000 Human Health Methodology") addresses the development of AWQC to protect human health. The Agency intends to use the 2000 Human Health Methodology both to develop new AWQC for additional pollutants and to revise existing AWQC. Within the next several years, EPA intends to focus on deriving AWQC for chemicals of high priority (including, but not limited to, mercury, arsenic, PCBs, and dioxin). Furthermore, EPA anticipates that 304(a) criteria development in the future will be for bioaccumulative chemicals and pollutants considered highest priority by the Agency. The 2000 Human Health Methodology is also intended to provide States and authorized Tribes flexibility in establishing water quality standards by providing scientifically valid options for developing their own water quality criteria that consider local conditions. States and authorized Tribes are strongly encouraged to use this Methodology to derive their own AWQC. However, the 2000 Human Health Methodology also defines the default factors EPA intends to use in evaluating and determining consistency of State water quality standards with the requirements of the CWA. The Agency intends to use these default factors to calculate national water quality criteria under

Section 304(a) of the Act. EPA will also use this Methodology as guidance when promulgating water quality standards for a State or Tribe under Section 303(c) of the CWA.

This Methodology does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, the 2000 Human Health Methodology cannot impose legally-binding requirements on EPA, States, Tribes or the regulated community, and may not apply to a particular situation based upon the circumstances. EPA and State/Tribal decision-makers retain the discretion to use different, scientifically defensible, methodologies to develop human health criteria on a case-by-case basis that differ from this Methodology where appropriate. EPA may change the Methodology in the future through intermittent refinements as advances in science or changes in Agency policy occur.

The 2000 Human Health Methodology incorporates scientific advancements made over the past two decades. The use of this Methodology is an important component of the Agency's efforts to improve the quality of the Nation's waters. EPA believes the Methodology will enhance the overall scientific basis of water quality criteria. Further, the Methodology should help States and Tribes address their unique water quality issues and risk management decisions, and afford them greater flexibility in developing their water quality programs.

There are three companion Technical Support Document (TSD) volumes for the 2000 Human Health Methodology: a Risk Assessment TSD; an Exposure Assessment TSD; and a Bioaccumulation TSD. These documents are intended to further support States and Tribes in developing AWQC to reflect local conditions. The Risk Assessment TSD (USEPA, 2000) is being published concurrently with this Methodology. Publication of the Exposure Assessment and Bioaccumulation TSDs are anticipated in 2001.

1.3 HISTORY OF THE AMBIENT WATER QUALITY CRITERIA (AWQC) METHODOLOGY

In 1980, EPA published AWQC for 64 pollutants/pollutant classes identified in Section 307(a) of the CWA and provided a methodology for deriving the criteria (USEPA, 1980). These 1980 AWQC National Guidelines (or the "1980 Methodology") for developing AWQC for the protection of human health addressed three types of endpoints: noncancer, cancer, and organoleptic (taste and odor) effects. Criteria for protection against noncancer and cancer effects were estimated by using risk assessment-based procedures, including extrapolation from animal toxicity or human epidemiological studies. Basic human exposure assumptions were applied to the criterion equation.

The risk assessment-based procedures used to derive the AWQC to protect human health were specific to whether the endpoint was cancer or noncancer. When using cancer as the critical risk assessment endpoint (which had been assumed not to have a threshold), the AWQC were presented as a range of concentrations associated with specified incremental lifetime risk

levels[1]. When using noncancer effects as the critical endpoint, the AWQC reflected an assessment of a "no-effect" level, since noncancer effects were assumed to have a threshold. The key features of each procedure are described briefly in the following paragraphs.

Cancer effects. If human or animal studies on a contaminant indicated that it induced a statistically significant carcinogenic response, the 1980 AWQC National Guidelines treated the contaminant as a carcinogen and derived a low-dose cancer potency factor from available animal data using the linearized multistage model (LMS). The LMS, which uses a linear, nonthreshold assumption for low-dose risk, was used by the Agency as a science policy choice in protecting public health, and represented a plausible upper limit for low-dose risk. The cancer potency factor, which expresses incremental, lifetime risk as a function of the rate of intake of the contaminant, was then combined with exposure assumptions to express that risk in terms of an ambient water concentration. In the 1980 AWQC National Guidelines, the Agency presented a range of contaminant concentrations corresponding to incremental cancer risks of 10^{-7} to 10^{-5} (that is, a risk of one additional case of cancer in a population of ten million to one additional cancer case in a population of one hundred thousand, respectively).

Noncancer effects. If the pollutant was not considered to have the potential for causing cancer in humans (later defined as a known, probable, or possible human carcinogen by the 1986 *Guidelines for Carcinogen Risk Assessment,* USEPA, 1986d), the 1980 AWQC National Guidelines treated the contaminant as a noncarcinogen; a criterion was derived using a threshold concentration for noncancer adverse effects. The criteria derived from noncancer data were based on the Acceptable Daily Intake (ADI) (now termed the reference dose [RfD]). ADI values were generally derived using a no-observed-adverse-effect level (NOAEL) from animal studies, although human data were used whenever available. The ADI was calculated by dividing the NOAEL by an uncertainty factor to account for uncertainties inherent in extrapolating limited toxicological data to humans. In accordance with the National Research Council recommendations of 1977 (NRC, 1977), safety factors (SFs) (later redefined as uncertainty factors) of 10, 100, or 1,000 were used, depending on the quality of the data.

Organoleptic effects. Organoleptic characteristics were also used in developing criteria for some contaminants to control undesirable taste and/or odor imparted by them to ambient water. In some cases, a water quality criterion based on organoleptic effects would be more stringent than a criterion based on toxicologic endpoints. The 1980 AWQC National Guidelines emphasized that criteria derived for organoleptic endpoints are not based on toxicological information, have no direct relationship to adverse human health effects and, therefore, do not necessarily represent approximations of acceptable risk levels for humans.

[1]Throughout this document, the term "risk level" regarding a cancer assessment using linear approach refers to an upper-bound estimate of excess lifetime cancer risk.

1.4 RELATIONSHIP OF WATER QUALITY STANDARDS TO AWQC

Under Section 303(c) of the CWA, States have the primary responsibility for establishing water quality standards, defined under the Act as designated beneficial uses of a water segment and the water quality criteria necessary to support those uses. Additionally, Native American Tribes authorized to administer the water quality standards program under 40 CFR 131.8 establish water quality standards for waters within their jurisdictions. This statutory framework allows States and authorized Tribes to work with local communities to adopt appropriate designated uses and to adopt criteria to protect those designated uses. Section 303(c) provides for EPA review of water quality standards and for promulgation of a superseding Federal rule in cases where State or Tribal standards are not consistent with the applicable requirements of the CWA and the implementing Federal regulations, or where the Agency determines Federal standards are necessary to meet the requirements of the Act. Section 303(c)(2)(B) specifically requires States and authorized Tribes to adopt water quality criteria for toxics for which EPA has published criteria under Section 304(a) and for which the discharge or presence could reasonably be expected to interfere with the designated use adopted by the State or Tribe. In adopting such criteria, States and authorized Tribes must establish numerical values based on one of the following: (1) 304(a) criteria; (2) 304(a) criteria modified to reflect site-specific conditions; or, (3) other scientifically defensible methods. In addition, States and authorized Tribes can establish narrative criteria where numeric criteria cannot be determined.

It must be recognized that the Act uses the term "criteria" in two different ways. In Section 303(c), the term is part of the definition of a water quality standard. Specifically, a water quality standard is composed of designated uses and the criteria necessary to protect those uses. Thus, States and authorized Tribes are required to adopt regulations which contain legally enforceable criteria. However, in Section 304(a) the term criteria is used to describe the scientific information that EPA develops to be used as guidance by States, authorized Tribes and EPA when establishing water quality standards pursuant to 303(c). Thus, two distinct purposes are served by the 304(a) criteria. The first is as guidance to the States and authorized Tribes in the development and adoption of water quality criteria which will protect designated uses, and the second is as the basis for promulgation of a superseding Federal rule when such action is necessary.

1.5 NEED FOR THE AWQC METHODOLOGY REVISIONS

Since 1980, EPA risk assessment practices have evolved significantly in all of the major Methodology areas: that is, cancer and noncancer risk assessments, exposure assessments, and bioaccumulation. When the 1980 Methodology was developed, EPA had not yet developed formal cancer or noncancer risk assessment guidelines. Since then, EPA has published several risk assessment guidelines. In cancer risk assessment, there have been advances in the use of mode of action (MOA) information to support both the identification of potential human carcinogens and the selection of procedures to characterize risk at low, environmentally relevant exposure levels. EPA published *Proposed Guidelines for Carcinogen Risk Assessment* (USEPA, 1996a, hereafter the "1996 proposed cancer guidelines"). These guidelines presented revised procedures to quantify cancer risk at low doses, replacing the current default use of the LMS model. Following review by the Agency's Science Advisory Board (SAB), EPA published the

revised *Guidelines for Carcinogen Risk Assessment–Review Draft* in July 1999 (USEPA, 1999a, hereafter the "1999 draft revised cancer guidelines"). In noncancer risk assessment, the Agency is moving toward the use of the benchmark dose (BMD) and other dose-response approaches in place of the traditional NOAEL approach to estimate an RfD or Reference Concentration (RfC). *Guidelines for Mutagenicity Risk Assessment* were published in 1986 (USEPA, 1986b). In 1991, the Agency published *Guidelines for Developmental Toxicity Risk Assessment* (USEPA, 1991), and it issued *Guidelines for Reproductive Toxicity Risk Assessment* in 1996 (USEPA, 1996b). In 1998, EPA published final *Guidelines for Neurotoxicity Risk Assessment* (USEPA, 1998), and in 1999 it issued the draft *Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 1999b).

In 1986, the Agency made available to the public the Integrated Risk Information System (IRIS). IRIS is a database that contains risk information on the cancer and noncancer effects of chemicals. The IRIS assessments are peer reviewed and represent EPA consensus positions across the Agency's program and regional offices.

New studies have addressed water consumption and fish tissue consumption. These studies provide a more current and comprehensive description of national, regional, and special-population consumption patterns that EPA has reflected in the 2000 Human Health Methodology. In addition, more formalized procedures are now available to account for human exposure from multiple sources when setting health goals such as AWQC that address only one exposure source. In 1986, the Agency published the *Total Exposure Assessment Methodology (TEAM) Study: Summary and Analysis, Volume I, Final Report* (USEPA, 1986c), which presents a process for conducting comprehensive evaluation of human exposures. In 1992, EPA published the revised *Guidelines for Exposure Assessment* (USEPA, 1992), which describe general concepts of exposure assessment, including definitions and associated units, and provide guidance on planning and conducting an exposure assessment. The *Exposure Factors Handbook* was updated in 1997 (USEPA, 1997a). Also in 1997, EPA developed *Guiding Principles for Monte Carlo Analysis* (USEPA, 1997b) and published its *Policy for Use of Probabilistic Analysis in Risk Assessment* (see http://www.epa.gov/ncea/mcpolicy.htm). The Monte Carlo guidance can be applied to exposure assessments and risk assessments. The Agency has recently developed the Relative Source Contribution (RSC) Policy for assessing total human exposure to a contaminant and apportioning the RfD among the media of concern, published for the first time in this Methodology.

The Agency has moved toward the use of a bioaccumulation factor (BAF) to reflect the uptake of a contaminant from all sources (e.g., ingestion, sediment) by fish and shellfish, rather than just from the water column as reflected by the use of a bioconcentration factor (BCF) in the 1980 Methodology. The Agency has also developed detailed procedures and guidelines for estimating BAF values.

Another reason for the 2000 Human Health Methodology is the need to bridge the gap between the differences in the risk assessment and risk management approaches used by EPA's Office of Water for the derivation of AWQC under the authority of the CWA and Maximum Contaminant Level Goals (MCLGs) under the Safe Drinking Water Act (SDWA). Three notable differences are the treatment of chemicals designated as Group C, possible human carcinogens

under the 1996 proposed cancer guidelines, the consideration of non-water sources of exposure when setting an AWQC or MCLG for a noncarcinogen, and cancer risk ranges. Those three differences are described in the three subsections below, respectively.

1.5.1 Group C Chemicals

Chemicals were typically classified as Group C–i.e., possible human carcinogens–under the existing (1986) EPA cancer classification scheme for any of the following reasons:

1) Carcinogenicity has been documented in only one test species and/or only one cancer bioassay and the results do not meet the requirements of "sufficient evidence."

2) Tumor response is of marginal statistical significance due to inadequate design or reporting.

3) Benign, but not malignant, tumors occur with an agent showing no response in a variety of short-term tests for mutagenicity.

4) There are responses of marginal statistical significance in a tissue known to have a high or variable background rate.

The 1986 *Guidelines for Carcinogen Risk Assessment* (hereafter the "1986 cancer guidelines") specifically recognized the need for flexibility with respect to quantifying the risk of Group C, possible human carcinogens. The 1986 cancer guidelines noted that agents judged to be in Group C, possible human carcinogens, may generally be regarded as suitable for quantitative risk assessment, but that case-by-case judgments may be made in this regard.

The EPA Office of Water has historically treated Group C chemicals differently under the CWA and the SDWA. It is important to note that the 1980 AWQC National Guidelines for setting AWQC under the CWA predated EPA's carcinogen classification system, which was proposed in 1984 (USEPA, 1984) and finalized in 1986 (USEPA, 1986a). The 1980 AWQC National Guidelines did not explicitly differentiate among agents with respect to the weight of evidence for characterizing them as likely to be carcinogenic to humans. For all pollutants judged as having adequate data for quantifying carcinogenic risk–including those now classified as Group C–AWQC were derived based on data on cancer incidence. In the1980 AWQC National Guidelines, EPA emphasized that the AWQC for carcinogens should state that the recommended concentration for maximum protection of human health is zero. At the same time, the criteria published for specific carcinogens presented water concentrations for these pollutants corresponding to individual lifetime excess cancer risk levels in the range of 10^{-7} to 10^{-5}.

In the development of national primary drinking water regulations under the SDWA, EPA is required to promulgate a health-based MCLG for each contaminant. The Agency policy has been to set the MCLG at zero for chemicals with strong evidence of carcinogenicity associated with exposure from water. For chemicals with limited evidence of carcinogenicity, including many Group C agents, the MCLG was usually obtained using an RfD based on the

pollutant's noncancer effects with the application of an additional uncertainty factor of 1 to 10 to account for carcinogenic potential of the chemical. If valid noncancer data for a Group C agent were not available to establish an RfD but adequate data are available to quantify the cancer risk, then the MCLG was based upon a nominal lifetime excess cancer risk in the range of 10^{-6} to10^{-5} (ranging from one case in a population of one million to one case in a population of one hundred thousand). Even in those cases where the RfD approach has been used for the derivation of the MCLG for a Group C agent, the drinking water concentrations associated with excess cancer risks in the range of 10^{-6} to 10^{-5} were also provided for comparison.

It should also be noted that EPA's pesticides program has applied both of the previously described methods for addressing Group C chemicals in actions taken under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and finds both methods applicable on a case-by-case basis. Unlike the drinking water program, however, the pesticides program does not add an extra uncertainty factor to account for potential carcinogenicity when using the RfD approach.

In the 1999 draft revised cancer guidelines, there are no more alphanumeric categories. Instead, there will be longer narratives for hazard characterization that will use consistent descriptive terms when assessing cancer risk.

1.5.2 Consideration of Non-water Sources of Exposure

The 1980 AWQC National Guidelines recommended that contributions from non-water sources, namely air and non-fish dietary intake, be subtracted from the Acceptable Daily Intake (ADI), thus reducing the amount of the ADI "available" for water-related sources of intake. In practice, however, when calculating human health criteria, these other exposures were generally not considered because reliable data on these exposure pathways were not available. Consequently, the AWQC were usually derived such that drinking water and fish ingestion accounted for the entire ADI (now called RfD).

In the drinking water program, a similar "subtraction" method was used in the derivation of MCLGs proposed and promulgated in drinking water regulations through the mid-1980s. More recently, the drinking water program has used a "percentage" method in the derivation of MCLGs for noncarcinogens. In this approach, the percentage of total exposure typically accounted for by drinking water, referred to as the relative source contribution (RSC), is applied to the RfD to determine the maximum amount of the RfD "apportioned" to drinking water reflected by the MCLG value. In using this percentage procedure, the drinking water program also applies a ceiling level of 80 percent of the RfD and a floor level of 20 percent of the RfD. That is, the MCLG cannot account for more than 80 percent of the RfD, nor less than 20 percent of the RfD.

The drinking water program usually takes a conservative approach to public health by applying an RSC factor of 20 percent to the RfD when adequate exposure data do not exist, assuming that the major portion (80 percent) of the total exposure comes from other sources, such as diet.

In the 2000 Human Health Methodology, guidance for the routine consideration of non-water sources of exposure [both ingestion exposures (e.g., food) and exposures other than the oral route (e.g., inhalation)] is presented. The approach is called the Exposure Decision Tree. Relative source contribution estimates will be made by EPA using this approach, which allows for use of either the subtraction or percentage methods, depending on chemical-specific circumstances, within the 20 to 80 percent range described above.

1.5.3 Cancer Risk Ranges

In addition to the different risk assessment approaches discussed above for deriving AWQC and MCLGs for Group C agents, there have been different risk management approaches by the drinking water and surface water programs on lifetime excess risk values when setting health-based criteria for carcinogens. The surface water program has derived AWQC for carcinogens that generally corresponded to lifetime excess cancer risk levels of 10^{-7} to 10^{-5}. The drinking water program has set MCLGs for Group C agents based on a slightly less stringent risk range of 10^{-6} to 10^{-5}, while MCLGs for chemicals with strong evidence of carcinogenicity (that is, classified as Group A, known, or B probable, human carcinogen) are set at zero. The drinking water program is now following the principles of the 1999 draft revised cancer guidelines to determine the type of low-dose extrapolation based on mode of action.

It is also important to note that under the drinking water program, for those substances having an MCLG of zero, enforceable Maximum Contaminant Levels (MCLs) have generally been promulgated to correspond with cancer risk levels ranging from 10^{-6} to 10^{-4}. Unlike AWQC and MCLGs which are strictly health-based criteria, MCLs are developed with consideration given to the costs and technological feasibility of reducing contaminant levels in water to meet those standards.

With the 2000 Human Health Methodology, EPA will publish its national 304(a) water quality criteria at a 10^{-6} risk level, which EPA considers appropriate for the general population. EPA is increasing the degree of consistency between the drinking water and ambient water programs, given the somewhat different requirements of the CWA and SDWA.

1.6 OVERVIEW OF THE AWQC METHODOLOGY REVISIONS

The following equations for deriving AWQC include toxicological and exposure assessment parameters which are derived from scientific analysis, science policy, and risk management decisions. For example, values for parameters such as a field-measured BAF or a point of departure from an animal study [in the form of a lowest-observed-adverse-effect level (LOAEL)/no-observed -adverse-effect level (NOAEL)/lower 95 percent confidence limit on a dose associated with a 10 percent extra risk (LED_{10})] are empirically measured using scientific methods. By contrast, the decision to use animal effects as surrogates for human effects involves judgment on the part of the EPA (and similarly, by other agencies) as to the best practice to follow when human data are lacking. Such a decision is, therefore, a matter of science policy. The choice of default fish consumption rates for protection of a certain percentage (i.e., the 90th percentile) of the general population is clearly a risk management decision. In many cases, the Agency has selected parameter values using its best judgment regarding the overall protection afforded by the resulting AWQC when all parameters are combined. For a longer discussion of the differences between science, science policy, and risk management, please refer to Section 2 of this document. Section 2 also provides further details with regard to risk characterization for this Methodology, with emphasis placed on explaining the uncertainties in the overall risk assessment.

The generalized equations for deriving AWQC based on noncancer effects are:

Noncancer Effects[2]

$$AWQC = RfD \cdot RSC \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \cdot BAF_i)} \right) \qquad \text{(Equation 1-1)}$$

Cancer Effects: Nonlinear Low-Dose Extrapolation

$$AWQC = \frac{POD}{UF} \cdot RSC \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \cdot BAF_i)} \right) \qquad \text{(Equation 1-2)}$$

[2] Although appearing in this equation as a factor to be multiplied, the RSC can also be an amount subtracted. Refer to the explanation key below the equations.

Cancer Effects: Linear Low-Dose Extrapolation

$$AWQC = RSD \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \cdot BAF_i)} \right) \qquad \text{(Equation 1-3)}$$

where:

AWQC	=	Ambient Water Quality Criterion (mg/L)
RfD	=	Reference dose for noncancer effects (mg/kg-day)
POD	=	Point of departure for carcinogens based on a nonlinear low-dose extrapolation (mg/kg-day), usually a LOAEL, NOAEL, or LED_{10}
UF	=	Uncertainty Factor for carcinogens based on a nonlinear low-dose extrapolation (unitless)
RSD	=	Risk-specific dose for carcinogens based on a linear low-dose extrapolation (mg/kg-day) (dose associated with a target risk, such as 10^{-6})
RSC	=	Relative source contribution factor to account for non-water sources of exposure. (Not used for linear carcinogens.) May be either a percentage (multiplied) or amount subtracted, depending on whether multiple criteria are relevant to the chemical.
BW	=	Human body weight (default = 70 kg for adults)
DI	=	Drinking water intake (default = 2 L/day for adults)
FI_i	=	Fish intake at trophic level (TL) I (I = 2, 3, and 4) (defaults for total intake = 0.0175 kg/day for general adult population and sport anglers, and 0.1424 kg/day for subsistence fishers). Trophic level breakouts for the general adult population and sport anglers are: TL2 = 0.0038 kg/day; TL3 = 0.0080 kg/day; and TL4 = 0.0057 kg/day.
BAF_i	=	Bioaccumulation factor at trophic level I (I=2, 3 and 4), lipid normalized (L/kg)

For highly bioaccumulative chemicals where ingestion from water might be considered negligible, EPA is currently evaluating the feasibility of developing and implementing AWQCs that are expressed in terms of concentrations in tissues of aquatic organisms. Such tissue residue criteria might be used as an alternative to AWQCs which are expressed as concentrations in water, particularly in situations where AWQCs are at or below the practical limits for quantifying a chemical in water. Even though tissue residue criteria would not require the use of a BAF in their derivation, implementing such criteria would still require a mechanism for relating chemical loads and concentrations in water and sediment to concentrations in tissues of appropriate fish and shellfish (e.g., a BAF or bioaccumulation model). At this time, no revisions are planned to the Methodology to provide specific guidance on developing fish tissue-based water quality criteria. However, guidance may be provided in the future either as a separate document or integrated in a specific 304(a) water quality criteria document for a chemical that warrants such an approach.

AWQC for the protection of human health are designed to minimize the risk of adverse effects occurring to humans from chronic (lifetime) exposure to substances through the ingestion of drinking water and consumption of fish obtained from surface waters. The Agency is not recommending the development of additional water quality criteria similar to the "drinking water health advisories" that focus on acute or short-term effects; these are not seen as routinely having a meaningful role in the water quality criteria and standards program. However, as discussed below, there may be some instances where the consideration of acute or short-term toxicity and exposure in the derivation of AWQC is warranted.

Although the AWQC are based on chronic health effects data (both cancer and noncancer effects), the criteria are intended to also be protective against adverse effects that may reasonably be expected to occur as a result of elevated acute or short-term exposures. That is, through the use of conservative assumptions with respect to both toxicity and exposure parameters, the resulting AWQC should provide adequate protection not only for the general population over a lifetime of exposure, but also for special subpopulations who, because of high water- or fish-intake rates, or because of biological sensitivities, have an increased risk of receiving a dose that would elicit adverse effects. The Agency recognizes that there may be some cases where the AWQC based on chronic toxicity may not provide adequate protection for a subpopulation at special risk from shorter-term exposures. The Agency encourages States, Tribes, and others employing the 2000 Human Health Methodology to give consideration to such circumstances in deriving criteria to ensure that adequate protection is afforded to all identifiable subpopulations. (See Section 4.3, Factors Used in the AWQC Computation, for additional discussion of these subpopulations.)

The EPA is in the process of revising its cancer guidelines, including its descriptions of human carcinogenic potential. Once final guidelines are published, they will be the basis for assessment under this methodology. In the meanwhile, the 1986 guidelines are used and extended with principles discussed in EPA's 1999 *Guidelines for Carcinogen Risk Assessment - Review Draft* (hereafter "1999 draft revised cancer guidelines"). These principles arise from new science about cancer discovered in the last 15 years and from EPA policy of recent years supporting full characterization of hazard and risk both for the general population and potentially sensitive groups such as children. These principles are incorporated in recent and ongoing assessments such as the reassessment of dioxin, consistent with the 1986 guidelines. Until final guidelines are published, information is presented to describe risk under both the old guidelines and draft revisions. Dose-response assessment under the 1986 guidelines employs a linearized multistage model to extrapolate tumor dose-response observed in animal or human studies down to zero dose, zero extra risk. The dose-response assessment under EPA's 1999 draft revised cancer guidelines is a two-step process. In the first step, the response data are modeled in the range of empirical observation. Modeling in the observed range is done with biologically based or appropriate curve-fitting modeling. In the second step, extrapolation below the range of observation is accomplished by biologically based modeling if there are sufficient data or by a default procedure (linear, nonlinear, or both). A point of departure (POD) for extrapolation is estimated from modeling observed data. The lower 95 percent confidence limit on a dose associated with 10 percent extra risk (LED_{10}) is the standard POD for low-dose extrapolation. The linear default procedure is a straight line extrapolation to the origin (i.e., zero dose, zero extra risk) from the POD, which is the LED_{10} identified in the observable response

range. The result of this procedure is generally comparable (within 2-fold) to that of using a linearized multistage model under existing, 1986 guidelines. The linear low-dose extrapolation applies to agents that are best characterized by the assumption of linearity (e.g., direct DNA reactive mutagens) for their MOA. A linear approach would also be applied when inadequate or no information is available to explain the carcinogenic MOA; this is a science policy choice in the interest of public health. If it is determined that the MOA understanding fully supports a nonlinear extrapolation, the AWQC is derived using the nonlinear default which is based on a margin of exposure (MOE) analysis using the LED_{10} as the POD and applying uncertainty factors (UFs) to arrive at an acceptable MOE. There may be situations where it is appropriate to apply both the linear and nonlinear default procedures (e.g., for an agent that is both DNA reactive and active as a promoter at higher doses).

For substances that are carcinogenic, particularly those for which the MOA suggests nonlinearity at low doses, the Agency recommends that an integrated approach be taken in looking at cancer and noncancer effects. If one effect does not predominate, AWQC values should be determined for both carcinogenic and noncarcinogenic endpoints. The lower of the resulting values should be used for the AWQC.

When deriving AWQC for noncarcinogens and carcinogens based on a nonlinear low-dose extrapolation, a factor is included to account for other non-water exposure sources [both ingestion exposures (e.g., food) and exposures other than the oral route (e.g., inhalation)] so that the entire RfD, or POD/UF, is not apportioned to drinking water and fish consumption alone. Guidance is provided in the 2000 Human Health Methodology for determining the factor (i.e., the RSC) to be used for a particular chemical. The Agency is recommending the use of an Exposure Decision Tree procedure to support the determination of the appropriate RSC value for a given water contaminant. In the absence of data, the Agency intends to use 20 percent of the RfD (or POD/UF) as the default RSC in calculating 304(a) criteria or promulgating State or Tribal water quality standards under Section 303(c).

With AWQC derived for carcinogens based on a linear low-dose extrapolation, the Agency will publish recommended criteria values at a 10^{-6} risk level. States and authorized Tribes can always choose a more stringent risk level, such as 10^{-7}. EPA also believes that criteria based on a 10^{-5} risk level are acceptable for the general population as long as States and authorized Tribes ensure that the risk to more highly exposed subgroups (sportfishers or subsistence fishers) does not exceed the 10^{-4} level. Clarification on this risk management decision is provided in Section 2 of this document.

The default fish consumption value for the general adult population in the 2000 Human Health Methodology is 17.5 grams/day, which represents an estimate of the 90th percentile consumption rate for the U.S. adult population based on the U.S. Department of Agriculture's (USDA's) Continuing Survey of Food Intake by Individuals (CSFII) 1994-96 data (USDA, 1998). EPA will use this default intake rate with future national 304(a) criteria derivations or revisions. This default value is chosen to be protective of the majority of the general population. However, States and authorized Tribes are urged to use a fish intake level derived from local data on fish consumption in place of this default value when deriving AWQC, ensuring that the fish intake level chosen is protective of highly exposed individuals in the population. EPA has

provided default values for States and authorized Tribes that do not have adequate information on local or regional consumption patterns, based on numerous studies that EPA has reviewed on sport anglers and subsistence fishers. EPA's defaults for these population groups are estimates of their average consumption. EPA recommends a default of 17.5 grams/day for sport anglers as an approximation of their average consumption and 142.4 grams/day for subsistence fishers, which falls within the range of averages for this group. Consumption rates for women of childbearing age and children younger than 14 are also provided to maximize protection in those cases where these subpopulations may be at greatest risk.

In the 2000 Human Health Methodology, criteria are derived using a BAF rather than a BCF. To derive the BAF, States and authorized Tribes may use EPA's Methodology or any method consistent with this Methodology. EPA's highest preference in developing BAFs are BAFs based on field-measured data from local/regional fish.

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USEPA (U.S. Environmental Protection Agency). 2000. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000). Technical Support Document Volume 1: Risk Assessment.* Office of Science and Technology, Office of Water. Washington, DC. EPA-822-B-00-005. August.

2. CLARIFICATIONS ON THE METHODOLOGY, RISK CHARACTERIZATION, AND OTHER ISSUES FOR DEVELOPING CRITERIA

2.1 IDENTIFYING THE POPULATION SUBGROUP THAT THE AWQC SHOULD PROTECT

Water quality criteria are derived to establish ambient concentrations of pollutants which, if not exceeded, will protect the general population from adverse health impacts from those pollutants due to consumption of aquatic organisms and water, including incidental water consumption related to recreational activities. For each pollutant, chronic criteria are derived to reflect long-term consumption of food and water. An important decision to make when setting AWQC is the choice of the particular population to protect. For instance, criteria could be set to protect those individuals who have average or "typical" exposures, or the criteria could be set so that they offer greater protection to those individuals who are more highly exposed. EPA has selected default parameter values that are representative of several defined populations: adults in the general population; sport (recreational) fishers; subsistence fishers; women of childbearing age (defined as ages 15-44); and children (up to the age of 14). In deciding on default parameter values, EPA is aware that multiple parameters are used in combination when calculating AWQC (e.g., intake rates and body weight). EPA describes the estimated population percentiles that are represented by each of the default exposure parameter values in Section 4.

EPA's national 304(a) criteria are usually derived to protect the majority of the general population from chronic adverse health effects. EPA has used a combination of median values, mean values, and percentile estimates for the parameter value defaults to calculate its national 304(a) criteria. EPA believes that its assumptions afford an overall level of protection targeted at the high end of the general population (i.e., the target population or the criteria-basis population). EPA also believes that this is reasonably conservative and appropriate to meet the goals of the CWA and the 304(a) criteria program. EPA considers that its target protection goal is satisfied if the population as a whole will be adequately protected by the human health criteria when the criteria are met in ambient water. However, associating the derived criteria with a specific population percentile is far more difficult, and such a quantitative descriptor typically requires detailed distributional exposure and dose information. EPA's *Guidelines For Exposure Assessment* (USEPA, 1992) describes the extreme difficulty in making accurate estimates of exposures and indicates that uncertainties at the more extreme ends of the distribution increase greatly. On quantifying population exposures/risks, the guidelines specifically state:

> *In practice, it is difficult even to establish an accurate mean health effect risk for a population. This is due to many complications, including uncertainties in using animal data for human dose-response relationships, nonlinearities in the dose-response curve, projecting incidence data from one group to another dissimilar group, etc. Although it has been common practice to estimate the number of cases of disease, especially cancer, for populations exposed to chemicals, it should be understood that these estimates are not meant to be accurate estimates of real (or actuarial) cases of disease. The estimate's value lies in framing*

hypothetical risk in an understandable way rather than in any literal interpretation of the term "cases."

Although it is not possible to subject the estimates to such a rigorous analysis (say, for example, to determine what criterion value provides protection of exactly the 90th percentile of the population), EPA believes that the combination of parameter value assumptions achieves its target goal, without being inordinately conservative. The standard assumptions made for the national 304(a) criteria are as follows. The assumed body weight value used is an arithmetic mean, as are the RSC intake estimates of other exposures (e.g., non-fish dietary), when data are available. The BAF component data (e.g., for lipid values, for particulate and dissolved organic carbon) are based on median (i.e., 50th percentile) values. The drinking water intake values are approximately 90th percentile estimates and fish intake values are 90th percentile estimates. EPA believes the use of these values will result in 304(a) criteria that are protective of a majority of the population; this is EPA's goal.

However, EPA also strongly believes that States and authorized Tribes should have the flexibility to develop criteria, on a site-specific basis, that provide additional protection appropriate for highly exposed populations. EPA is aware that exposure patterns in general, and fish consumption in particular, vary substantially. EPA understands that highly exposed populations may be widely distributed geographically throughout a given State or Tribal area. EPA recommends that priority be given to identifying and adequately protecting the most highly exposed population. Thus, if the State or Tribe determines that a highly exposed population is at greater risk and would not be adequately protected by criteria based on the general population, and by the national 304(a) criteria in particular, EPA recommends that the State or Tribe adopt more stringent criteria using alternative exposure assumptions.

EPA has provided recommended default intake rates for various population groups for State and Tribal consideration. EPA does not intend for these alternative default values to be prescriptive. EPA strongly emphasizes its preference that States and Tribes use local or regional data over EPA's defaults, if they so choose, as being more representative of their population groups of concern.

In the course of updating the 2000 Human Health Methodology, EPA received some questions regarding the population groups for which the criteria would be developed. EPA does not intend to derive multiple 304(a) criteria for all subpopulation groups for every chemical. As stated above, criteria that address chronic adverse health effects are most applicable to the CWA Section 304(a) criteria program and the chemicals evaluated for this program. If EPA determined that pregnant women/fetuses or young children were the target population (or criteria basis population) of a chemical's RfD or POD/UF, then the 304(a) criteria would be developed using exposure parameters for that subgroup. This would only be relevant for acute or subchronic toxicity situations. This does not conflict with the fact that chronic health effects potentially reflect a person's exposure during both childhood and adult years.

For RfD-based and POD/UF-based chemicals, EPA's policy is that, in general, the RfD (or POD/UF) should not be exceeded and the exposure assumptions used should reflect the population of concern. It is recommended that when a State or authorized Tribe sets a

waterbody-specific AWQC, they consider the populations most exposed via water and fish. EPA's policy on cancer risk management goals is discussed in Section 2.4.

Health Risks to Children

In recognition that children have a special vulnerability to many toxic substances, EPA's Administrator directed the Agency in 1995 to explicitly and consistently take into account environmental health risks to infants and children in all risk assessments, risk characterizations, and public health standards set for the United States. In April 1997, President Clinton signed Executive Order 13045 on the protection of children from environmental health risks, which assigned a high priority to addressing risks to children. In May 1997, EPA established the Office of Children's Health Protection to ensure the implementation of the President's Executive Order. EPA has increased efforts to ensure its guidance and regulations take into account risks to children. Circumstances where risks to children should be considered in the context of the 2000 Human Health Methodology are discussed in the Section 3.2, Noncancer Effects (in terms of developmental and reproductive toxicity) and in Section 4, Exposure (for appropriate exposure intake parameters).

Details on risk characterization and the guiding principles stated above are included in EPA's March 21, 1995 policy statement and the discussion of risk characterization (USEPA, 1995) and the 1999 *Guidelines for Carcinogen Risk Assessment. Review Draft* (USEPA, 1999a) and the *Reproductive and Toxicity Risk Assessment Guidelines* of 1996 (USEPA, 1996b).

2.2 SCIENCE, SCIENCE POLICY, AND RISK MANAGEMENT

An important part of risk characterization, as described later in Section 2.7, is to make risk assessments transparent. This means that conclusions drawn from the science are identified separately from policy judgments and risk management decisions, and that the use of default values or methods, as well as the use of assumptions in risk assessments, are clearly articulated. In this Methodology, EPA has attempted to separate scientific analysis from science policy and risk management decisions for clarity. This should allow States and Tribes (who are also prospective users of this Methodology) to understand the elements of the Methodology accurately and clearly, and to easily separate out the scientific decisions from the science policy and risk management decisions. This is important so that when questions are asked regarding the scientific merit, validity, or apparent stringency or leniency of AWQC, the implementer of the criteria can clearly explain what judgments were made to develop the criterion in question and to what degree these judgments were based on science, science policy, or risk management. To some extent this process will also be displayed in future AWQC documents.

When EPA speaks of science or scientific analysis, it is referring to the extraction of data from toxicological or exposure studies and surveys with a minimum of judgment being used to make inferences from the available evidence. For example, if EPA is describing a POD from an animal study (e.g., a LOAEL), this is usually determined as a lowest dose that produces an observable adverse effect. This would constitute a scientific determination. Judgments applying science policy, however, may enter this determination. For example, several scientists may differ in their opinion of what is adverse, and this in turn can influence the selection of a LOAEL

in a given study. The use of an animal study to predict effects in a human in the absence of human data is an inherent science policy decision. The selection of specific UFs when developing an RfD is another example of science policy. In any risk assessment, a number of decision points occur where risk to humans can only be inferred from the available evidence. Both scientific judgments and policy choices may be involved in selecting from among several possible inferences when conducting a risk assessment.

Risk management is the process of selecting the most appropriate guidance or regulatory actions by integrating the results of risk assessment with engineering data and with social, economic, and political concerns to reach a decision. In this Methodology, the choice of a default fish consumption rate which is protective of 90 percent of the general population is a risk management decision. The choice of an acceptable cancer risk by a State or Tribe is a risk management decision.

Many of the components in the 2000 Human Health Methodology are an amalgam of science, science policy, and/or risk management. For example, most of the default values chosen by EPA are based on examination of scientific data and application of either science policy or risk management. This includes the default assumption of 2 liters a day of drinking water; the assumption of 70 kilograms for an adult body weight; the use of default percent lipid and particulate organic carbon/dissolved organic carbon (POC/DOC) for developing national BAFs; the default fish consumption rates for the general population and sport and subsistence anglers; and the choice of a default cancer risk level. Some decisions are more grounded in science and science policy (such as the choice of default BAFs) and others are more obviously risk management decisions (such as the determination of default fish consumption rates and cancer risk levels). Throughout the 2000 Human Health Methodology, EPA has identified the kind of decision necessary to develop defaults and what the basis for the decision was. More details on the concepts of science analysis, science policy, risk management, and how they are introduced into risk assessments are included in *Risk Assessment in the Federal Government: Managing the Process* (NRC, 1983).

2.3 SETTING CRITERIA TO PROTECT AGAINST MULTIPLE EXPOSURES FROM MULTIPLE CHEMICALS (CUMULATIVE RISK)

EPA is very much aware of the complex issues and implications of cumulative risk and has endeavored to begin developing an overall approach at the Agency-wide level. Assuming that multiple exposures to multiple chemicals are additive is scientifically sound if they exhibit the same toxic endpoints and modes of action. There are numerous publications relevant to cumulative risk that can assist States and Tribes in understanding the complex issues associated with cumulative risk. These include the following:

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- Hertzberg, R.C., G. Rice, and L.K. Teuschler. 1999. Methods for health risk assessment of combustion mixtures. In: *Hazardous Waste Incineration: Evaluating the Human*

Health and Environmental Risks. S. Roberts, C. Teaf and J. Bean, (eds). CRC Press LLC, Boca Raton, FL. Pp. 105-148.

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- USEPA. 1989b. *Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs) and 1989 Update*. Risk Assessment Forum. Washington, DC. EPA/625/3-89/016. March.

The Agency's program offices are also engaged in on-going discussions of the great complexities, methodological challenges, data adequacy needs and other information gaps, as well as the science policy and risk management decisions that will need to be made, as they pursue developing a sound strategy and, eventually, specific guidance for addressing cumulative

risks. As a matter of internal policy, EPA is committed to refining the Methodology as advances in relevant aspects of the science improve, as part of the water quality criteria program.

2.4 CANCER RISK RANGE

For deriving 304(a) criteria or promulgating water quality criteria for States and Tribes under Section 303(c) based on the 2000 Human Health Methodology, EPA intends to use the 10^{-6} risk level, which the Agency believes reflects an appropriate risk for the general population. EPA's program office guidance and regulatory actions have evolved in recent years to target a 10^{-6} risk level as an appropriate risk for the general population. EPA has recently reviewed the policies and regulatory language of other Agency mandates (e.g., the Clean Air Act Amendments of 1990, the Food Quality Protection Act) and believes the target of a 10^{-6} risk level is consistent with Agency-wide practice.

EPA believes that both 10^{-6} and 10^{-5} may be acceptable for the general population and that highly exposed populations should not exceed a 10^{-4} risk level. States or Tribes that have adopted standards based on criteria at the 10^{-5} risk level can continue to do so, if the highly exposed groups would at least be protected at the 10^{-4} risk level. However, EPA is not automatically assuming that 10^{-5} will protect "the highest consumers" at the 10^{-4} risk level. Nor is EPA advocating that States and Tribes automatically set criteria based on assumptions for highly exposed population groups at the 10^{-4} risk level. The Agency is simply endeavoring to add that a specific determination should be made to ensure that highly exposed groups do not exceed a 10^{-4} risk level. EPA understands that fish consumption rates vary considerably, especially among subsistence populations, and it is such great variation among these population groups that may make either 10^{-6} or 10^{-5} protective of those groups at a 10^{-4} risk level. Therefore, depending on the consumption patterns in a given State or Tribal jurisdiction, a 10^{-6} or 10^{-5} risk level could be appropriate. In cases where fish consumption among highly exposed population groups is of a magnitude that a 10^{-4} risk level would be exceeded, a more protective risk level should be chosen. Such determinations should be made by the State or Tribal authorities and are subject to EPA's review and approval or disapproval under Section 303(c) of the CWA.

Adoption of a 10^{-6} or 10^{-5} risk level, both of which States and authorized Tribes have chosen in adopting water quality standards to date, represents a generally acceptable risk management decision, and EPA intends to continue providing this flexibility to States and Tribes. EPA believes that such State or Tribal decisions are consistent with Section 303(c) if the State or authorized Tribe has identified the most highly exposed subpopulation, has demonstrated that the chosen risk level is adequately protective of the most highly exposed subpopulation, and has completed all necessary public participation. States and authorized Tribes also have flexibility in how they demonstrate this protectiveness and obtain such information. A State or authorized Tribe may use existing information as well as collect new information in making this determination. In addition, if a State or authorized Tribe does not believe that the 10^{-6} risk level adequately protects the exposed subpopulations, water quality criteria based on a more stringent risk level may be adopted. This discretion includes combining the 10^{-6} risk level with fish consumption rates for highly exposed population groups.

It is important to understand that criteria for carcinogens are based on chosen risk levels that inherently reflect, in part, the exposure parameters used to derive those values. Therefore, changing the exposure parameters also changes the risk. Specifically, the incremental cancer risk levels are *relative*, meaning that any given criterion associated with a particular cancer risk level is also associated with specific exposure parameter assumptions (e.g., intake rates, body weights). When these exposure parameter values change, so does the relative risk. For a criterion derived on the basis of a cancer risk level of 10^{-6}, individuals consuming up to 10 times the assumed fish intake rate would not exceed a 10^{-5} risk level. Similarly, individuals consuming up to 100 times the assumed rate would not exceed a 10^{-4} risk level. Thus, for a criterion based on EPA's default fish intake rate (17.5 gm/day) and a risk level of 10^{-6}, those consuming a pound per day (i.e., 454 grams/day) would potentially experience between a 10^{-5} and a 10^{-4} risk level (closer to a 10^{-5} risk level). (Note: Fish consumers of up to 1,750 gm/day would not exceed the 10^{-4} risk level.) If a criterion were based on high-end intake rates and the relative risk of 10^{-6}, then an average fish consumer would be protected at a cancer risk level of approximately 10^{-8}. The point is that the risks for different population groups are not the same.

2.5 MICROBIOLOGICAL AMBIENT WATER QUALITY CRITERIA

Guidance for deriving microbiological AWQC is not a part of this Methodology. In 1986, EPA published *Ambient Water Quality Criteria for Bacteria - 1986* (USEPA, 1986a), which updated and revised bacteriological criteria previously published in 1976 in *Quality Criteria for Water* (USEPA, 1976). The inclusion of guidance for deriving microbiological AWQC was considered in the 1992 national workshop that initiated the effort to revise the 1980 Methodology and was recommended by the SAB in 1993. Since that time, however, efforts separate from these Methodology revisions have addressed microbiological AWQC concerns. The purpose of this section is to briefly describe EPA's current recommendations and activities.

EPA's *Ambient Water Quality Criteria for Bacteria - 1986* recommends the use of *Escherichia coli* and enterococci rather than fecal coliforms (USEPA, 1986a). EPA's criteria recommendations are:

- Fresh water: *E. coli* not to exceed 126/100 ml or enterococci not to exceed 33/100 ml; and

- Marine water: enterococci not to exceed 35/100 ml.

These criteria should be calculated as the geometric mean based on five equally spaced samples taken over a 30-day period.

In addition, EPA recommends that States adopt a single sample maximum, based on the expected frequency of use. No sample taken should exceed this value. EPA specifies appropriate single sample maximum values in the 1986 criteria document.

<u>Current Activities and Plans for Future Work</u>

EPA has identified development of microbial water quality criteria as part of its strategy to control waterborne microbial disease, by controlling pathogens in waterbodies and by protecting designated uses, such as recreation and public water supplies. The program fosters an integrated approach to protect both ground-water and surface water sources. EPA plans to conduct additional monitoring for *Cryptosporidium parvum* and *E. coli*, and determine action plans in accordance with the results of this monitoring.

EPA recommends no change at this time in the stringency of its bacterial criteria for recreational waters; existing criteria and methodologies from 1986 will still apply. The recommended methods for *E. coli* and enterococci have been improved. As outlined in the *Action Plan for Beaches and Recreational Waters* (Beach Action Plan, see below), the Agency plans to conduct national studies on improving indicators together with epidemiology studies for new criteria development (USEPA, 1999b). The Agency is also planning to establish improved temporal and spatial monitoring protocols.

In the Beach Action Plan, EPA identifies a multi-year strategy for monitoring recreational water quality and communicating public health risks associated with potentially pathogen-contaminated recreational rivers, lakes, and ocean beaches. It articulates the Agency's rationale and goals in addressing specific problems and integrates all associated program, policy, and research needs and directions. The Beach Action Plan also provides information on timing, products and lead organization for each activity. These include activities and products in the areas of program development, risk communication, water quality indicator research, modeling and monitoring research, and exposure and health effects research.

Recently, EPA approved new 24-hour *E. coli* and enterococcus tests for recreational waters that may be used as an alternative to the 48-hour test (USEPA, 1997). EPA anticipates proposing these methods for inclusion in the 40 CRF 136 in the Fall of 2000. EPA has also published a video with accompanying manual on the original and newer methods for enterococci and *E. coli* (USEPA, 2000).

As part of the Beach Action Plan, EPA made the following recommendations for further Agency study:

- Future criteria development should consider the risk of diseases other than gastroenteritis. EPA intends to consider and evaluate such water-related exposure routes as inhalation and dermal absorption when addressing microbial health effects. The nature and significance of other than the classical waterborne pathogens are to some degree tied to the particular type of waste sources.

- A new set of indicator organisms may need to be developed for tropical water if it is proven that the current fecal indicators can maintain viable cell populations in the soil and water for significant periods of time in uniform tropical conditions. Some potential alternative indicators to be fully explored are coliphage, other bacteriophage, and *Clostridium perfringens*.

- Because animal sources of pathogens of concern for human infection such as *Giardia lamblia*, *Cryptosporidium parvum*, and *Escherichia coli* 0157:H7 may be waterborne or washed into water and thus become a potential source for infection, they should not be ignored in risk assessment. A likely approach would be phylogenetic differentiation; that is, indicators that are specific to, or can discriminate among, animal sources.

- EPA intends to develop additional data on secondary infection routes and infection rates from prospective epidemiology studies and outbreaks from various types of exposure (e.g., shellfish consumption, drinking water, recreational exposure).

- EPA needs to improve sampling strategies for recreational water monitoring including consideration of rainfall and pollution events to trigger sampling.

2.6 RISK CHARACTERIZATION CONSIDERATIONS

On March 21, 1995, EPA's Administrator issued the *EPA Risk Characterization Policy and Guidance* (USEPA, 1995). This policy and guidance is intended to ensure that characterization information from each stage of a risk assessment is used in forming conclusions about risk and that this information is communicated from risk assessors to risk managers, and from EPA to the public. The policy also provides the basis for greater clarity, transparency, reasonableness, and consistency in risk assessments across EPA programs. The fundamental principles which form the basis for a risk characterization are as follows:

- Risk assessments should be transparent, in that the conclusions drawn from the science are identified separately from policy judgments, and the use of default values or methods and the use of assumptions in the risk assessment are clearly articulated.

- Risk characterizations should include a summary of the key issues and conclusions of each of the other components of the risk assessments, as well as describe the likelihood of harm. The summary should include a description of the overall strengths and limitations (including uncertainties) of the assessment and conclusions.

- Risk characterizations should be consistent in general format, but recognize the unique characteristics of each specific situation.

- Risk characterizations should include, at least in a qualitative sense, a discussion of how a specific risk and its context compares with similar risks. This may be accomplished by comparisons with other pollutants or situations on which the Agency has decided to act, or other situations with which the public may be familiar. The discussion should highlight the limitations of such comparisons.

- Risk characterization is a key component of risk communication, which is an interactive process involving exchange of information and expert opinion among individuals, groups, and institutions.

Additional guiding principles include:

- The risk characterization integrates the information from the hazard identification, dose-response, and exposure assessments, using a combination of qualitative information, quantitative information, and information regarding uncertainties.

- The risk characterization includes a discussion of uncertainty and variability in the risk assessment.

- Well-balanced risk characterizations present conclusions and information regarding the strengths and limitations of the assessment for other risk assessors, EPA decision-makers, and the public.

In developing the methodology presented here, EPA has closely followed the risk characterization guiding principles listed above. As States and Tribes adopt criteria using the 2000 Human Health Methodology, they are strongly encouraged to follow EPA's risk characterization guidance. There are a number of areas within the Methodology and criteria development process where risk characterization principles apply:

- Integration of cancer and noncancer assessments with exposure assessments, including bioaccumulation potential determinations, in essence, weighing the strengths and weaknesses of the risk assessment as a whole when developing a criterion.

- Selecting a fish consumption rate, either locally derived or the national default value, within the context of a target population (e.g., sensitive subpopulations) as compared to the general population.

- Presenting cancer and/or noncancer risk assessment options.

- Describing the uncertainty and variability in the hazard identification, the dose-response, and the exposure assessment.

2.7 DISCUSSION OF UNCERTAINTY

2.7.1 Observed Range of Toxicity Versus Range of Environmental Exposure

When characterizing a risk assessment, an important distinction to make is between the observed range of adverse effects (from an epidemiology or animal study) and the environmentally observed range of exposure (or anticipated human exposure) to the contaminant. In many cases, EPA intends to apply default factors to account for uncertainties or incomplete knowledge in developing RfDs or cancer risk assessments using nonlinear low-dose extrapolation to provide a margin of protection. In reality, the actual effect level and the environmental exposure levels may be separated by several orders of magnitude. The difference between the dose causing some observed response and the anticipated human exposure should be described by risk assessors and managers, especially when comparing criteria to environmental levels of a contaminant.

2.7.2 Continuum of Preferred Data/Use of Defaults

In both toxicological and exposure assessments, EPA has defined a continuum of preferred data for toxicological assessments ranging from a highest preference for chronic human data (e.g., studies that examine a long-term exposure of humans to a chemical, usually from occupational and/or residential exposure) and actual field data for many of the exposure parameter values (e.g., locally derived fish consumption rates, waterbody-specific bioaccumulation rates), to default values which are at the lower end of the preference continuum. EPA has supplied default values for all of the risk assessment parameters in the 2000 Human Health Methodology; however, it is important to note that when default values are used, the uncertainty in the final risk assessment may be higher, and the final resulting criterion may not be as applicable to local conditions, than is a risk assessment derived from human/field data. Using defaults assumes generalized conditions and may not capture the actual variability in the population (e.g., sensitive subpopulations/high-end consumers). If defaults are chosen as the basis for criteria, these inherent uncertainties should be communicated to the risk manager and the public. While this continuum is an expression of preference on the part of EPA, it does not imply in any way that any of the choices are unacceptable or scientifically indefensible.

2.7.3 Significant Figures

The number of significant figures in a numeric value is the number of certain digits plus one estimated digit. Digits should not be confused with decimal places. For example, 15.1, 0.0151, and 0.0150 all have 3 significant figures. Decimal places may have been used to maintain the correct number of significant figures, but in themselves they do not indicate significant figures (Brinker, 1984). Since the number of significant figures must include only one estimated digit, the sources of input parameters (e.g., fish consumption and water consumption rates) should be checked to determine the number of significant figures associated with data they provide. However, the original measured values may not be available to determine the number of significant figures in the input parameters. In these situations, EPA recommends utilizing the data as presented.

When developing criteria, EPA recommends rounding the number of significant figures at the end of the criterion calculation to the same number of significant figures in the least precise parameter. This is a generally accepted practice which can be found described in greater detail in APHA (1992) and Brinker (1984). The general rule is that for multiplication or division, the resulting value should not possess any more significant figures than is associated with the factor in the calculation with the least precision. When numbers are added or subtracted, the number that has the fewest decimal places, not necessarily the fewest significant figures, puts the limit on the number of places that justifiably may be carried in the sum or difference. Rounding off a number is the process of dropping one or more digits so that the value contains only those digits that are significant or necessary in subsequent computations (Brinker, 1984). The following rounding procedures are recommended: (1) if the digit 6, 7, 8, or 9 is dropped, increase the preceding digit by one unit; (2) if the digit 0, 1, 2, 3, or 4 is dropped, do not alter the preceding digit; and (3) if the digit 5 is dropped, round off the preceding digit to the nearest even number (e.g., 2.25 becomes 2.2 and 2.35 becomes 2.4) (APHA, 1992; Brinker, 1984).

EPA recommends that calculations of water quality criteria be performed without rounding of intermediate step values. The resulting criterion may be rounded to a manageable number of decimal places. However, in no case should the number of digits presented exceed the number of significant figures implied in the data and calculations performed on them. The term "intermediate step values" refers to values of the parameters in Equations 1-1 through 1-3. The final step is considered the resulting AWQC. Although AWQC are, in turn, used for purposes of establishing water quality-based effluent limits (WQBELs) in National Pollutant Discharge Elimination System (NPDES) permits, calculating total maximum daily loads (TMDLs), and applicable or relevant and appropriate requirements (ARARs) for Superfund, they are considered the final step of this Methodology and, for the purpose of this discussion, where the rounding should occur.

The determination of appropriate significant figures inevitably involves some judgment given that some of the equation parameters are adopted default exposure values. Specifically, the default drinking water intake rate of 2 L/day is a value adopted to represent a majority of the population over the course of a lifetime. Although supported by drinking water consumption survey data, this value was adopted as a policy decision and, as such, does not have to be considered in determining the parameter with the least precision. That is, the resulting AWQC need not always be reduced to one significant digit. Similarly, the 70-kg adult body weight has been adopted Agency-wide and represents a default policy decision.

The following example with a simplified AWQC equation illustrates the rule described above. The example is for hexachlorobutadiene (HCBD), which EPA used to demonstrate the 1998 draft Methodology revisions (USEPA, 1998b). The parameters that were calculated (i.e., not policy adopted values) include values with significant figures of two (the POD and RSC), three (the UF), and four (the FI and BAF). Based on the 2000 Human Health Methodology, the final criterion should be rounded to two significant figures. The bold numbers in parentheses indicate the number of significant figures and those with asterisks also indicate Agency adopted policy values.

$$AWQC = \frac{POD}{UF} \cdot RSC \cdot \left(\frac{BW}{DI + (FI \cdot BAF)} \right) \qquad \text{(Equation 2-1)}$$

Example [Refer to draft HCBD document for details on the POD/UF, RSC and BAF data (EPA 822-R-98-004). Also note that the fish intake rate in this example is the revised value.]:

$$AWQC = \left(\frac{0.054\mathbf{(2)}}{300\mathbf{(3)}} - 1.2 \times 10^{-4}\mathbf{(2)} \right) \times \left(\frac{70\mathbf{(2^*)}}{2\mathbf{(1^*)} + (0.01750\mathbf{(4)} \times 3{,}180\mathbf{(4)})} \right)$$

AWQC = 7.3×10^{-5} mg/L (0.073 µg/L, rounded from 7.285×10^{-2} µg/L)
* represents Agency adopted policy value

A number of the values used in the equation may result in intermediate step values that have more than four figures past the decimal place and may be carried throughout the calculation. However, carrying more than four figures past the decimal place (equivalent to the most precise parameter) is unnecessary as it has no effect on the resulting criterion value.

2.8 OTHER CONSIDERATIONS

2.8.1 Minimum Data Considerations

For many of the preceding technical areas, considerations have been presented for data quality in developing toxicological and exposure assessments. For greater detail and discussion of minimum data recommendations, the reader is referred to the specific sections in the Methodology on cancer and noncancer risk assessments (and especially to the referenced EPA risk assessment guidelines documents), exposure assessment, and bioaccumulation assessment, in addition to the TSD volumes for each.

2.8.2 Site-Specific Criterion Calculation

The 2000 Human Health Methodology allows for site-specific modifications by States and Tribes to reflect local environmental conditions and human exposure patterns. "Local" may refer to any appropriate geographic area where common aquatic environmental or exposure patterns exist. Thus "local" may signify Statewide, regional, a river reach, or an entire river.

Such site-specific criteria may be developed as long as the site-specific data, either toxicological or exposure-related, is justifiable. For example, when using a site-specific fish consumption rate, a State should use a value that represents at least the central tendency of the population surveyed (either sport or subsistence, or both). If a site-specific fish consumption rate for sport anglers or subsistence anglers is lower than an EPA default value, it may be used in calculating AWQC. However, to justify such a level (either higher or lower than EPA defaults), the State should assemble appropriate survey data to arrive at a defensible site-specific fish consumption rate.

Such data must also be submitted to EPA for its review when approving or disapproving State or Tribal water quality standards under Section 303(c). The same conditions apply to site-specific calculations of BAF, percent fish lipid, or the RSC. In the case of deviations from toxicological values (i.e., IRIS values: verified noncancer and cancer assessments), EPA strongly recommends that the data upon which the deviation is based be presented to and approved by the Agency before a criterion is developed.

Additional guidance on site-specific modifications to the 2000 Human Health Methodology is provided in each of the three TSD volumes.

2.8.3 Organoleptic Criteria

Organoleptic criteria define concentrations of chemicals or materials which impart undesirable taste and/or odor to water. Organoleptic effects, while significant from an aesthetic standpoint, are not a significant health concern. In developing and utilizing such criteria, two factors must be appreciated: (1) the limitations of most organoleptic data; and (2) the human health significance of organoleptic properties. In the past, EPA has developed organoleptic criteria if organoleptic data were available for a specific contaminant. The 1980 AWQC National Guidelines made a clear distinction that organoleptic criteria and toxicity-based criteria are derived from completely different endpoints, and that organoleptic criteria have no demonstrated relationship to potential adverse human health effects because there is no toxicological basis. EPA acknowledges that if organoleptic effects (i.e., objectionable taste and odor) cause people to reject the water and its designated uses, then the public is effectively deprived of the natural resource. It is also possible that intense organoleptic characteristics could result in depressed fluid intake which, in turn, might lead to an indirect human health effect via decreased fluid consumption. Although EPA has developed organoleptic criteria in the past and may potentially do so in the future, this will not be a significant part of the water quality criteria program. EPA encourages the development of organoleptic criteria when States and Tribes believe they are needed. However, EPA cautions States and Tribes that the quality of organoleptic data is often significantly less than that of toxicologic data used in establishing health-based criteria. Therefore, a comprehensive evaluation of available organoleptic data should be made, and the selection of the most appropriate database for the criterion should be based on sound scientific judgment.

In 1980, EPA provided recommended criteria summary language when both types of data are available. The following format was used and is repeated here:

> *For comparison purposes, two approaches were used to derive criterion levels for ____. Based on available toxicity data, for the protection of public health the derived level is ____. Using available organoleptic data, for controlling undesirable taste and odor quality of ambient water the estimated level is ____. It should be recognized that organoleptic data as a basis for establishing a water quality criteria have no demonstrated relationship to potential adverse human health effects.*

Similarly, the 1980 Methodology recommended that in those instances where a level to limit toxicity cannot be derived, the following statement should be provided:

> *Sufficient data are not available for ____ to derive a level which would protect against the potential toxicity of this compound.*

2.8.4 Criteria for Chemical Classes

The 2000 Human Health Methodology also allows for the development of a criterion for classes of chemicals, as long as a justification is provided through the analysis of mechanistic data, toxicokinetic data, structure-activity relationship data, and limited acute and chronic toxicity data. When potency differences between members of a class is great (such as in the case

of chlorinated dioxins and furans), toxicity equivalency factors (TEFs) may be more appropriately developed than one class criterion.

A chemical class is defined as any group of chemical compounds which are similar in chemical structure and biological activity, and which frequently occur together in the environment usually because they are generated by the same commercial process. In criterion development, isomers should be regarded as part of a chemical class rather than as a single compound. A class criterion, therefore, is an estimate of risk/safety which applies to more than one member of a class. It involves the use of available data on one or more chemicals of a class to derive criteria for other compounds of the same class in the event that there are insufficient data available to derive compound-specific criteria. The health-based criterion may apply to the water concentration of each member of the class, or may apply to the sum of the water concentrations of the compounds within the class. Because relatively minor structural changes within the class of compounds can have pronounced effects on their biological activities, reliance on class criteria should be minimized depending on the data available.

The following guidance should also be followed when considering the development of a class criterion.

- A detailed review of the chemical and physical properties of the chemicals within the group should be made. A close relationship within the class with respect to chemical activity would suggest a similar potential to reach common biological sites within tissues. Likewise, similar lipid solubilities would suggest the possibility of comparable absorption and distribution.

- Qualitative and quantitative toxicological data for chemicals within the group should be examined. Adequate toxicological data on a number of compounds within a group provides a more reasonable basis for extrapolation to other chemicals of the same class than minimal data on one chemical or a few chemicals within the group.

- Similarities in the nature of the toxicological response to chemicals in the class provides additional support for the prediction that the response to other members of the class may be similar. In contrast, where the biological response has been shown to differ markedly on a qualitative and quantitative basis for chemicals within a class, the extrapolation of a criterion to other members is not appropriate.

- Additional support for the validity of extrapolation of a criterion to other members of a class could be provided by evidence of similar metabolic and toxicokinetic data for some members of the class.

Additional guidance is described in the *Technical Support Document on Health Risk Assessment of Chemical Mixtures* (USEPA, 1990).

2.9.5 Criteria for Essential Elements

Developing criteria for essential elements, particularly metals, must be a balancing act between toxicity and the requirement for good health. The AWQC must consider essentiality and cannot be established at levels that would result in deficiency of the element in the human population. The difference between the recommended daily allowance (RDA) and the daily doses causing a specified risk level for carcinogens or the RfDs for noncarcinogens defines the spread of daily doses within which the criterion may be derived. Because errors are inherent in defining both essential and adverse-effect levels, the criterion is derived from a dose level near the center of such dose ranges.

The process for developing criteria for essential elements should be similar to that used for any other chemical with minor modifications. The RfD represents concern for one end of the exposure spectrum (toxicity), whereas the RDA represents the other end (minimum essentiality). While the RDA and RfD values might occasionally appear to be similar in magnitude to one another, it does not imply incompatibility of the two methodological approaches, nor does it imply inaccuracy or error in either calculation.

2.9 REFERENCES

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3. RISK ASSESSMENT

This section describes the methods used to estimate ambient water quality criteria (AWQC) for the protection of human health for carcinogenic chemicals (Section 3.1) and for noncarcinogenic chemicals (Section 3.2).

3.1 CANCER EFFECTS

3.1.1 Background on EPA Cancer Risk Assessment Guidelines

The current EPA *Guidelines for Carcinogen Risk Assessment* were published in 1986 (USEPA, 1986a, hereafter the "1986 cancer guidelines"). The 1986 cancer guidelines categorize chemicals into alpha-numerical Groups: A, known human carcinogen (sufficient evidence from epidemiological studies or other human studies); B, probable human carcinogen (sufficient evidence in animals and limited or inadequate evidence in humans); C, possible human carcinogen (limited evidence of carcinogenicity in animals in the absence of human data); D, not classifiable (inadequate or no animal evidence of carcinogenicity); and E, evidence of noncarcinogenicity for humans (no evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiological and animal studies). Within Group B there are two subgroups, Groups B1 and B2. Group B1 is reserved for agents for which there is limited evidence of carcinogenicity from epidemiological studies. Group B2 is generally for agents for which there is sufficient evidence from animal studies and for which there is inadequate evidence or no data from epidemiological studies (USEPA, 1986). The system was similar to that used by the International Agency for Research on Cancer (IARC).

The 1986 cancer guidelines include guidance on what constitutes sufficient, limited, or inadequate evidence. In epidemiological studies, sufficient evidence indicates a causal relationship between the agent and human cancer; limited evidence indicates that a causal relationship is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded; inadequate evidence indicates either lack of pertinent data, or a causal interpretation is not credible. In general, although a single study may be indicative of a cause-effect relationship, confidence in inferring a causal association is increased when several independent studies are concordant in showing the association. In animal studies, sufficient evidence includes an increased incidence of malignant tumors or combined malignant and benign tumors:

- In multiple species or strains;
- In multiple experiments (e.g., with different routes of administration or using different dose levels);
- To an unusual degree in a single experiment with regard to high incidence, unusual site or type of tumor, or early age at onset;
- Additional data on dose-response, short-term tests, or structural activity relationships.

In the 1986 cancer guidelines, hazard identification and the weight-of-evidence process focus on tumor findings. The weight-of-evidence approach for making judgments about cancer hazard analyzes human and animal tumor data separately, then combines them to make the overall conclusion about potential human carcinogenicity. The next step of the hazard analysis is an evaluation of supporting evidence (e.g., mutagenicity, cell transformation) to determine whether the overall weight-of-evidence conclusion should be modified.

For cancer risk quantification, the 1986 cancer guidelines recommend the use of linearized multistage model (LMS) as the only default approach. The 1986 cancer guidelines also mention that a low-dose extrapolation model other than the LMS might be considered more appropriate based on biological grounds. However, no guidance is given in choosing other approaches. The 1986 cancer guidelines recommended the use of body weight raised to the 2/3 power ($BW^{2/3}$) as a dose scaling factor between species.

3.1.2 EPA's Proposed Guidelines for Carcinogen Risk Assessment and the Subsequent July, 1999 Draft Revised Cancer Guidelines

In 1996, EPA published *Proposed Guidelines for Carcinogen Risk Assessment* (USEPA, 1996a, hereafter the "1996 proposed cancer guidelines"). After the publication of the 1996 proposed cancer guidelines and a February, 1997 and January, 1999 Science Advisory Board (SAB) review, a revision was made in July, 1999 *Guidelines for Carcinogen Risk Assessment - Review Draft* (hereafter the "1999 draft revised cancer guidelines"; USEPA, 1999a), and an SAB meeting was convened to review this revised document. When final guidelines are published, they will replace the 1986 cancer guidelines. These revisions are designed to ensure that the Agency's cancer risk assessment methods reflect the most current scientific information and advances in risk assessment methodology.

In the meanwhile, the 1986 guidelines are used and extended with principles discussed in the 1999 draft revised cancer guidelines. These principles arise from scientific discoveries concerning cancer made in the last 15 years and from EPA policy of recent years supporting full characterization of hazard and risk both for the general population and potentially sensitive groups such as children. These principles are incorporated in recent and ongoing assessments such as the reassessment of dioxin, consistent with the 1986 guidelines. Until final guidelines are published, information is presented to describe risk under both the 1986 guidelines and 1999 draft revisions.

The 1999 draft revised cancer guidelines call for the full use of all relevant information to convey the circumstances or conditions under which a particular hazard is expressed (e.g., route, duration, pattern, or magnitude of exposure). They emphasize understanding the mode of action (MOA) whereby the agent induces tumors. The MOA underlies the hazard assessment and provides the rationale for dose-response assessments.

The key principles in the 1999 draft revised cancer guidelines include:

a) Hazard assessment is based on the analysis of all biological information rather than just tumor findings.

b) An agent's MOA in causing tumors is emphasized to reduce the uncertainty in describing the likelihood of harm and in determining the dose-response approach(es).

c) The 1999 draft revised cancer guidelines emphasize the conditions under which the hazard may be expressed (e.g., route, pattern, duration and magnitude of exposure). Further, the guidelines call for a *hazard characterization* to integrate the data analysis of all relevant studies into a weight-of-evidence conclusion of hazard and to develop a working conclusion regarding the agent's mode of action in leading to tumor development.

d) A weight-of-evidence narrative with accompanying descriptors (listed in Section 3.1.3.1 below) would replace the current alphanumeric classification system. The narrative summarizes the key evidence for carcinogenicity, describes the agent's MOA, characterizes the conditions of hazard expression, including route of exposure, describes any disproportionate effects on subgroups of the human population (e.g., children), and recommends appropriate dose-response approach(es). Significant strengths, weaknesses, and uncertainties of contributing evidence are also highlighted.

e) Biologically based extrapolation models are the preferred approach for quantifying risk. These models integrate data and conclusions about events in the carcinogenic process throughout the dose-response range from high to low doses. It is anticipated, however, that the necessary data for the parameters used in such models will not be available for most chemicals. The 1999 draft revised cancer guidelines allow for alternative quantitative methods, including several default approaches.

f) Dose-response assessment is a two-step process. In the first step, response data are modeled in the observable range of data and a determination is made of the point of departure (POD) from the observed range to extrapolate to low doses. The second step is extrapolation from the POD to estimate dose-response at lower doses. In addition to modeling tumor data, the 1999 draft revised cancer guidelines call for the use and modeling of other kinds of responses if they are considered to be more informed measures of carcinogenic risk. Nominally, these responses reflect key events in the carcinogenic process integral to the MOA of the agent.

g) Three default approaches are provided–linear, nonlinear, or both when adequate data are unavailable to generate a biologically based model. As the first step for all approaches, curve fitting in the observed range is used to determine a POD. A standard POD is the effective dose corresponding to the lower 95 percent limit on

a dose associated with 10 percent extra risk (LED_{10}).[3] *Linear*: The linear default is a straight line extrapolation from the response at LED_{10} to the origin (zero dose, zero extra risk). *Nonlinear*: The nonlinear default begins with the identified POD and provides a margin of exposure (MOE) analysis rather than estimating the probability of effects at low doses. The MOE analysis is used to determine the appropriate margin between the POD and the exposure level of interest, in this Methodology, the AWQC. The key objective of the MOE analysis is to describe for the risk manager how rapidly responses may decline with dose. Other factors are also considered in the MOE analysis (i.e., nature of the response, slope of the dose-response curve, human sensitivity compared with experimental animals, nature and extent of human variability in sensitivity and human exposure). *Linear and nonlinear*: Section 3.1.3.4E describes the situations when both linear and nonlinear defaults are used.

h) The approach used to calculate an oral human equivalent dose when assessments are based on animal bioassays has been refined and includes a change in the default assumption for interspecies dose scaling. The 1999 draft revised cancer guidelines use body weight raised to the 3/4 power.

EPA health risk assessment practices for both cancer and noncancer endpoints are beginning to come together with recent proposals to emphasize MOA understanding in risk assessment and to model response data in the observable range to derive PODs for data sets and benchmark doses (BMDs) for individual studies. The modeling of observed response data to identify PODs in a standard way will help to harmonize cancer and noncancer dose-response approaches and permit comparisons of cancer and noncancer risk estimates.

3.1.3 Methodology for Deriving AWQC[4] by the 1999 Draft Revised Cancer Guidelines

Following the publication of the *Draft Water Quality Criteria Methodology: Human Health* (USEPA, 1998a) and the accompanying TSD (USEPA, 1998b), EPA received comments from the public. EPA also held an external peer review of the draft Methodology. Both the peer reviewers and the public recommended that EPA incorporate the new approaches into the AWQC Methodology.

Until new guidelines are published, the 1986 cancer guidelines will be used along with principles of the 1999 draft revised cancer guidelines. The 1986 guidelines are the basis for IRIS risk numbers which were used to derive the current AWQC. Each new assessment applying the principles of the 1999 draft revised cancer guidelines will be subject to peer review before being used as the basis of AWQC.

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[3] Use of the LED_{10} as the point of departure is recommended with this Methodology, as it is with the 1999 draft revised cancer guidelines.

[4] Additional information regarding the revised method for assessing carcinogens may be found in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000). Technical Support Document, Volume 1: Risk Assessment* (USEPA, 2000).

The remainder of Section 3 illustrates the methodology for deriving numerical AWQC for carcinogens applying the 1999 draft revised cancer guidelines (USEPA, 1999a). This discussion of the revised methodology for carcinogens focuses primarily on the quantitative aspects of deriving numerical AWQC values. It is important to note that the cancer risk assessment process outlined in the 1999 draft revised cancer guidelines is not limited to the quantitative aspects. A numerical AWQC value derived for a carcinogen is to be based on appropriate hazard characterization and accompanied by risk characterization information.

This section contains a discussion of the weight-of-evidence narrative, that describes all information relevant to a cancer risk evaluation, followed by a discussion of the quantitative aspects of deriving numerical AWQC values for carcinogens. It is assumed that data from an appropriately conducted animal bioassay or human epidemiological study provide the underlying basis for deriving the AWQC value. The discussion focuses on the following: (1) the weight-of-evidence narrative; (2) general considerations and framework for analysis of the MOA; (3) dose estimation; (4) characterizing dose-response relationships in the range of observation and at low, environmentally relevant doses; (5) calculating the AWQC value; (6) risk characterization; and (7) use of Toxicity Equivalent Factors (TEF) and Relative Potency Estimates. The first three topics encompass the quantitative aspects of deriving AWQC for carcinogens.

3.1.3.1 Weight-of-Evidence Narrative[5]

The 1999 draft revised cancer guidelines include a weight-of-evidence narrative that is based on an overall judgment of biological and chemical/physical considerations. Hazard assessment information accompanying an AWQC value for a carcinogen in the form of a weight-of-evidence narrative is described in the footnote. Of particular importance is that the weight-of-evidence narrative explicitly provides adequate support based on human studies, animal bioassays, and other key evidence for the conclusion whether the substance is or is likely to be carcinogenic to humans from exposures through drinking water and/or fish ingestion. The Agency emphasizes the importance of providing an explicit discussion of the MOA for the substance in the weight-of-evidence narrative if data are available, including a discussion that relates the MOA to the quantitative procedures used in the derivation of the AWQC.

3.1.3.2 Mode of Action - General Considerations and Framework for Analysis

[5] The weight-of-evidence narrative is intended for the risk manager, and thus explains in nontechnical language the key data and conclusions, as well as the conditions for hazard expression. Conclusions about potential human carcinogenicity are presented by route of exposure. Contained within this narrative are simple likelihood descriptors that essentially distinguish whether there is enough evidence to make a projection about human hazard (i.e., Carcinogenic to humans; Likely to be carcinogenic to humans; Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential; Data are inadequate for an assessment of human carcinogenic potential; and Not likely to be carcinogenic to humans). Because one encounters a variety of data sets on agents, these descriptors are not meant to stand alone; rather, the context of the weight-of-evidence narrative is intended to provide a transparent explanation of the biological evidence and how the conclusions were derived. Moreover, these descriptors should not be viewed as classification categories (like the alphameric system), which often obscure key scientific differences among chemicals. The new weight-of-evidence narrative also presents conclusions about how the agent induces tumors and the relevance of the mode of action to humans, and recommends a dose-response approach based on the MOA understanding (USEPA, 1996a, 1999a).

An MOA is composed of key events and processes starting with the interaction of an agent with a cell, through operational and anatomical changes, resulting in cancer formation. "Mode" of action is contrasted with "mechanism" of action, which implies a more detailed, molecular description of events than is meant by MOA.

Mode of action analysis is based on physical, chemical, and biological information that helps to explain key events[6] in an agent's influence on development of tumors. Inputs to MOA analysis include tumor data in humans, animals, and among structural analogues as well as the other key data.

There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression. All pertinent studies are reviewed in analyzing an MOA, and an overall weighing of evidence is performed, laying out the strengths, weaknesses, and uncertainties of the case as well as potential alternative positions and rationales. Identifying data gaps and research needs is also part of the assessment.

Mode of action conclusions are used to address the question of human relevance of animal tumor responses, to address differences in anticipated response among humans such as between children and adults or men and women, and as the basis of decisions about the anticipated shape of the dose-response relationship.

In reaching conclusions, the question of "general acceptance" of an MOA will be tested as part of the independent peer review that EPA obtains for its assessment and conclusions.

Framework for Evaluating a Postulated Carcinogenic Mode(s) of Action

The framework is intended to be an analytic tool for judging whether available data support a mode of carcinogenic action postulated for an agent and includes nine elements:

1. Summary description of postulated MOA
2. Identification of key events
3. Strength, consistency, specificity of association
4. Dose-response relationship
5. Temporal relationship
6. Biological plausibility and coherence
7. Other modes of action
8. Conclusion
9. Human relevance, including subpopulations

3.1.3.3 Dose Estimation

[6]A "key event" is an empirically observable, precursor step that is itself a necessary element of the mode of action, or is a marker for such an element.

A. Determining the Human Equivalent Dose by the Oral Route

An important objective in the dose-response assessment is to use a measure of internal or delivered dose at the target site where possible. This is particularly important in those cases where the carcinogenic response information is being extrapolated to humans from animal studies. Generally, by the oral exposure route, the measure of a dose provided in the underlying human studies or animal bioassays is the applied dose, typically given in terms of unit mass per unit body weight per unit time, (e.g., mg/kg-day). When animal bioassay data are used, it is necessary to make adjustments to the applied dose values to account for differences in toxicokinetics between animals and humans that affect the relationship between applied dose and delivered dose at the target organ.

In the estimation of a human equivalent dose, the 1999 draft revised cancer guidelines recommend that when adequate data are available, the doses used in animal studies can be adjusted to equivalent human doses using toxicokinetic information on the particular agent. However, in most cases, there are insufficient data available to compare dose between species. In these cases, the estimate of a human equivalent dose is based on science policy default assumptions. To derive an equivalent human oral dose from animal data, the default procedure in the 1999 draft revised cancer guidelines is to scale daily applied oral doses experienced for a lifetime in proportion to body weight raised to the 3/4 power ($BW^{3/4}$). The adjustment factor is used because metabolic rates, as well as most rates of physiological processes that determine the disposition of dose, scale this way. Thus, the rationale for this factor rests on the empirical observation that rates of physiological processes consistently tend to maintain proportionality with body weight raised to 3/4 power (USEPA, 1992a, 1999a).

The use of $BW^{3/4}$ is a departure from the scaling factor of $BW^{2/3}$ that was based on surface area adjustment and was included in the 1980 AWQC National Guidelines as well as the 1986 cancer guidelines.

B. Dose-Response Analysis

If data on the agent are sufficient to support the parameters of a biologically based model and the purpose of the assessment is such as to justify investing resources supporting its use, this is the preferred approach for both the observed tumor and related response data and for extrapolation below the range of observed data in either animal or human studies.

3.1.3.4 Characterizing Dose-Response Relationships in the Range of Observation and at Low Environmentally Relevant Doses

The first quantitative component in the derivation of AWQC for carcinogens is the dose-response assessment in the range of observation. For most agents, in the absence of adequate data to generate a biologically based model, dose-response relationships in the observed range can be addressed through curve-fitting procedures for response data. It should be noted that the 1999 draft revised cancer guidelines call for modeling of not only tumor data in the observable range, but also other responses thought to be important events preceding tumor development (e.g., DNA adducts, cellular proliferation, receptor binding, hormonal changes). The modeling of

these data is intended to better inform the dose-response assessment by providing insights into the relationships of exposure (or dose) below the observable range for tumor response. These non-tumor response data can only play a role in the dose-response assessment if the agent's carcinogenic mode of action is reasonably understood, as well as the role of that precursor event.

The 1999 draft revised cancer guidelines recommend calculating the lower 95 percent confidence limit on a dose associated with an estimated 10 percent increased tumor or relevant non-tumor response (LED_{10}) for quantitative modeling of dose-response relationships in the observed range. The estimate of the LED_{10} is used as the POD for low-dose extrapolations discussed below. This standard point of departure (LED_{10}) is adopted as a matter of science policy to remain as consistent and comparable from case to case as possible. It is also a convenient comparison point for noncancer endpoints. The rationale supporting use of the LED_{10} is that a 10 percent response is at or just below the limit of sensitivity for discerning a statistically significant tumor response in most long-term rodent studies and is within the observed range for other toxicity studies. Use of lower limit takes experimental variability and sample size into account. The ED_{10} (central estimate) is also presented as a reference for comparison uses, especially for use in relative hazard/potency ranking among agents for priority setting.

For some data sets, a choice of the POD other than the LED_{10} may be appropriate. The objective is to determine the lowest reliable part of the dose-response curve for the beginning of the second step of the dose-response assessment—determine the extrapolation range. Therefore, if the observed response is below the LED_{10}, then a lower point may be a better choice (e.g., LED_5). Human studies more often support a lower POD than animal studies because of greater sample size.

The POD may be a NOAEL when a margin of exposure analysis is the nonlinear dose-response approach. The kinds of data available and the circumstances of the assessment both contribute to deciding to use a NOAEL or LOAEL which is not as rigorous or as ideal as curve fitting, but can be appropriate. If several data sets for key events and tumor response are available for an agent, and they are a mixture of continuous and incidence data, the most practicable way to assess them together is often through a NOAEL/LOAEL approach.

When an LED value estimated from animal data is used as the POD, it is adjusted to the human equivalent dose using an interspecies dose adjustment or a toxicokinetic analysis as described in Section 3.1.3.3.

Analysis of human studies in the observed range is designed on a case-by-case basis depending on the type of study and how dose and response are measured in the study.

A. Extrapolation to Low, Environmentally Relevant Doses

In most cases, the derivation of an AWQC will require an evaluation of carcinogenic risk at environmental exposure levels substantially lower than those used in the underlying study. Various approaches are used to extrapolate risk outside the range of observed experimental data. In the 1999 draft revised cancer guidelines, the choice of extrapolation method is largely

dependent on the mode of action. It should be noted that the term "mode of action" (MOA) is deliberately chosen in the 1999 draft revised cancer guidelines in lieu of the term "mechanism" to indicate using knowledge that is sufficient to draw a reasonable working conclusion without having to know the processes in detail as the term mechanism might imply. The 1999 draft revised cancer guidelines favor the choice of a biologically based model, if the parameters of such models can be calculated from data sources independent of tumor data. It is anticipated that the necessary data for such parameters will not be available for most chemicals. Thus, the 1999 draft revised cancer guidelines allow for several default extrapolation approaches (low-dose linear, nonlinear, or both).

B. Biologically Based Modeling Approaches

If a biologically based approach has been used to characterize the dose-response relationships in the observed range, and the confidence in the model is high, it may be used to extrapolate the dose-response relationship to environmentally relevant doses. For the purposes of deriving AWQC, the environmentally relevant dose would be the risk-specific dose (RSD) associated with incremental lifetime cancer risks in the 10^{-6} to 10^{-4} range for carcinogens for which a linear extrapolation approach is applied.[7] The use of the RSD and the POD/UF to compute the AWQC is presented in Section 3.1.3.5, below. Although biologically-based approaches are appropriate both for characterizing observed dose-response relationships and extrapolating to environmentally relevant doses, it is not expected that adequate data will be available to support the use of such approaches for most substances. In the absence of such data, the default linear approach, the nonlinear (MOE) approach, or both linear and nonlinear approaches will be used.

[7] For discussion of the cancer risk range, see Section 2.4.

C. Default Linear Extrapolation Approach

The default linear approach replaces the LMS approach that has served as the default for EPA cancer risk assessments. Any of the following conclusions leads to selection of a linear dose-response assessment approach:

- There is an absence of sufficient tumor MOA information.
- The chemical has direct DNA mutagenic reactivity or other indications of DNA effects that are consistent with linearity.
- Human exposure or body burden is high and near doses associated with key events in the carcinogenic process (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin).
- Mode of action analysis does not support direct DNA effects, but the dose-response relationship is expected to be linear (e.g., certain receptor-mediated effects).

The procedures for implementing the default linear approach begin with the estimation of a POD as described above. The point of departure, LED_{10}, reflects the interspecies conversion to the human equivalent dose and the other adjustments for less-than-lifetime experimental duration. In most cases, the extrapolation for estimating response rates at low, environmentally relevant exposures is accomplished by drawing a straight line between the POD and the origin (i.e., zero dose, zero extra risk). This is mathematically represented as:

$$y = mx + b \qquad \text{(Equation 3-1)}$$
$$b = 0$$

where:

y	=	Response or incidence
m	=	Slope of the line (cancer potency factor) = $\Delta y/\Delta x$
x	=	Dose
b	=	Slope intercept

The slope of the line, "m" (the estimated cancer potency factor at low doses), is computed as:

$$m = \frac{0.10}{LED_{10}} \qquad \text{(Equation 3-2)}$$

The RSD is then calculated for a specific incremental targeted lifetime cancer risk (in the range of 10^{-6} to 10^{-4}) as:

$$RSD = \frac{\text{Target Incremental Cancer Risk}}{m} \qquad \text{(Equation 3-3)}$$

where:

RSD	=	Risk-specific dose (mg/kg-day)
Target Incremental Cancer Risk[8]	=	Value in the range of 10^{-6} to10^{-4}
m	=	Cancer potency factor $(mg/kg\text{-}day)^{-1}$

The use of the RSD to compute the AWQC is described in Section 3.1.3.5 below.

D. Default Nonlinear Approach

As discussed in the 1999 draft revised cancer guidelines, any of the following conclusions leads to a selection of a nonlinear (MOE) approach to dose-response assessment:

- A tumor MOA supporting nonlinearity applies (e.g., some cytotoxic and hormonal agents such as disruptors of hormonal homeostasis), and the chemical does not demonstrate mutagenic effects consistent with linearity.

- An MOA supporting nonlinearity has been demonstrated, and the chemical has some indication of mutagenic activity, but it is judged not to play a significant role in tumor causation.

Thus, a default assumption of nonlinearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of nonlinearity. The MOA may lead to a dose-response relationship that is nonlinear, with response falling much more quickly than linearly with dose, or being most influenced by individual differences in sensitivity. Alternatively, the MOA may theoretically have a threshold (e.g., the carcinogenicity may be a secondary effect of toxicity or of an induced physiological change that is itself a threshold phenomenon).

The nonlinear approach may be used, for instance, in the case of a bladder tumor inducer, where the chemical is not mutagenic and causes only stone formation in male rat bladders at high doses. This dynamic leads to tumor formation only at the high doses. Stone and subsequent tumor formation are not expected to occur at doses lower than those that induce the physiological changes that lead to stone formation. (More detail on this chemical is provided in the cancer section of the Risk Assessment TSD; USEPA, 2000). EPA does not generally try to distinguish between modes of action that might imply a “true threshold” from others with a

[8]In 1980, the target lifetime cancer risk range was set at 10-7 to 10-5. However, both the expert panel for the AWQC workshop (USEPA, 1993) and the peer review workshop experts (USEPA,1999c) recommended that EPA change the risk range to 10-6 to 10-4, to be consistent with SDWA program decisions. See Section 2.4 for more details.

nonlinear dose-response relationship, because there is usually not sufficient information to distinguish between those possibilities empirically.

The nonlinear MOE approach in the 1986 proposed cancer guidelines compares an observed response rate such as the LED_{10}, NOAEL, or LOAEL with actual or nominal environmental exposures of interest by computing the ratio between the two. In the context of deriving AWQC, the environmentally relevant exposures are nominal targets rather than actual exposures.

If the evidence for an agent indicates nonlinearity (e.g., when carcinogenicity is secondary to another toxicity for which there is a threshold), the MOE analysis for the toxicity is similar to what is done for a noncancer endpoint, and an RfD or RfC for that toxicity may also be estimated and considered in the cancer assessment. However, a threshold of carcinogenic response is not necessarily assumed. It should be noted that for cancer assessment, the MOE analysis begins from a POD that is adjusted for toxicokinetic differences between species to give a human equivalent dose.

To support the use of the MOE approach, risk assessment information provides evaluation of the current understanding of the phenomena that may be occurring as dose (exposure) decreases substantially below the observed data. This gives information about the risk reduction that is expected to accompany a lowering of exposure. The various factors that influence the selection of the UF in an MOE approach are also discussed below.

There are two main steps in the MOE approach. The first step is the selection of a POD. The POD may be the LED_{10} for tumor incidence or a precursor, or in some cases, it may also be appropriate to use a NOAEL or LOAEL value. When animal data are used, the POD is a human equivalent dose or concentration arrived at by interspecies dose adjustment (as discussed in Section 3.1.3.3) or toxicokinetic analysis.

The second step in using MOE analysis to establish AWQC is the selection of an appropriate margin or UF to apply to the POD. This is supported by analyses in the MOE discussion in the risk assessment. The following issues should be considered when establishing the overall UF for the derivation of AWQC using the MOE approach (others may be found appropriate in specific cases):

- The nature of the response used for the dose-response assessment, for instance, whether it is a precursor effect or a tumor response. The latter may support a greater MOE.

- The slope of the observed dose-response relationship at the POD and its uncertainties and implications for risk reduction associated with exposure reduction. (A steeper slope implies a greater reduction in risk as exposure decreases. This may support a smaller MOE).

- Human sensitivity compared with that of experimental animals.

- Nature and extent of human variability and sensitivity.

- Human exposure. The MOE evaluation also takes into account the magnitude, frequency, and duration of exposure. If the population exposed in a particular scenario is wholly or largely composed of a subpopulation of special concern (e.g., children) for whom evidence indicates a special sensitivity to the agent's MOA, an adequate MOE would be larger than for general population exposure.

E. Both Linear and Nonlinear Approaches

Any of the following conclusions leads to selection of both a linear and nonlinear approach to dose-response assessment. Relative support for each dose-response method and advice on the use of that information needs to be documented for the AWQC. In some cases, evidence for one MOA is stronger than for the other, allowing emphasis to be placed on that dose-response approach. In other cases, both modes of action are equally possible, and both dose-response approaches should be emphasized.

- Modes of action for a single tumor type support both linear and nonlinear dose response in different parts of the dose-response curve (e.g., 4,4' methylene chloride).

- A tumor mode of action supports different approaches at high and low doses; e.g., at high dose, nonlinearity, but, at low dose, linearity (e.g., formaldehyde).

- The agent is not DNA-reactive and all plausible modes of action are consistent with nonlinearity, but not fully established.

- Modes of action for different tumor types support differing approaches, e.g., nonlinear for one tumor type and linear for another due to lack of MOA information (e.g., trichloroethylene).

3.1.3.5 <u>AWQC Calculation</u>

A. Linear Approach

The following equation is used for the calculation of the AWQC for carcinogens where an RSD is obtained from the linear approach:

$$AWQC = RSD \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \cdot BAF_i)} \right) \qquad \text{(Equation 3-4)}$$

AWQC	=	Ambient water quality criterion (mg/L)
RSD	=	Risk-specific dose (mg/kg-day)
BW	=	Human body weight (kg)
DI	=	Drinking water intake (L/day)

FI_i	=	Fish intake at trophic level I (I = 2, 3, and 4) (kg/day)
BAF_i	=	Bioaccumulation factor for trophic level I (I = 2, 3, and 4), lipid normalized (L/kg)

B. Nonlinear Approach

In those cases where the nonlinear, MOE approach is used, a similar equation is used to calculate the AWQC [9]

$$AWQC = \frac{POD}{UF} \cdot RSC \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \cdot BAF_i)} \right) \qquad \text{(Equation 3-5)}$$

where variables are defined as for Equation 3-4 and:

POD	=	Point of departure (mg/kg-day)
UF	=	Uncertainty factor (unitless)
RSC	=	Relative source contribution (percentage or subtraction)

Differences between the AWQC values obtained using the linear and nonlinear approaches should be noted. First, the AWQC value obtained using the default linear approach corresponds to a specific estimated incremental lifetime cancer risk level in the range of 10^{-4} to 10^{-6}. In contrast, the AWQC obtained using the nonlinear approach does not describe a specific cancer risk. The AWQC calculations shown above are appropriate for waterbodies that are used as sources of drinking water.

The actual AWQC chosen for the protection of human health is based on a review of all relevant information, including cancer and noncancer data. The AWQC may, or may not, utilize the value obtained from the cancer analysis in the final AWQC value. The endpoint selected for the AWQC will be based on consideration of the weight of evidence and a complete analysis of all toxicity endpoints.

3.1.3.6 Risk Characterization

Risk assessment is an integrative process that is documented in a risk characterization summary. Risk characterization is the final step of the risk assessment process in which all preceding analyses (i.e., hazard, dose-response, and exposure assessments) are tied together to convey the overall conclusions about potential human risk. This component of the risk assessment process characterizes the data in nontechnical terms, explaining the extent and weight of evidence, major points of interpretation and rationale, and strengths and weaknesses of

[9] Although appearing in this equation as a factor to be multiplied, the RSC can also be an amount subtracted.

the evidence, and discussing alternative approaches, conclusions, uncertainties, and variability that deserve serious consideration.

Risk characterization information accompanies the numerical AWQC value and addresses the major strengths and weaknesses of the assessment arising from the availability of data and the current limits of understanding the process of cancer causation. Key issues relating to the confidence in the hazard assessment and the dose-response analysis (including the low-dose extrapolation procedure used) are discussed. Whenever more than one interpretation of the weight of evidence for carcinogenicity or the dose-response characterization can be supported, and when choosing among them is difficult, the alternative views are provided along with the rationale for the interpretation chosen in the derivation of the AWQC value. Where possible, quantitative uncertainty analyses of the data are provided; at a minimum, a qualitative discussion of the important uncertainties is presented.

3.1.3.7 Use of Toxicity Equivalence Factors and Relative Potency Estimates

The 1999 draft revised cancer guidelines state:

> *A toxicity equivalence factor (TEF) procedure is one used to derive quantitative dose-response estimates for agents that are members of a category or class of agents. TEFs are based on shared characteristics that can be used to order the class members by carcinogenic potency when cancer bioassay data are inadequate for this purpose. The ordering is by reference to the characteristics and potency of a well-studied member or members of the class. Other class members are indexed to the reference agent(s) by one or more shared characteristics to generate their TEFs.*

In addition, the 1999 draft revised cancer guidelines state that TEFs are generated and used for the limited purpose of assessment of agents or mixtures of agents in environmental media when better data are not available. When better data become available for an agent, the TEF should be replaced or revised. To date, adequate data to support use of TEFs have been found only for dibenzofurans (dioxins) and coplanar polychlorinated biphenyls (PCBs) (USEPA, 1989, 1999b).

The uncertainties associated with TEFs must be described when this approach is used. This is a default approach to be used when tumor data are not available for individual components in a mixture. Relative potency factors (RPFs) can be similarly derived and used for agents with carcinogenicity or other supporting data. The RPF is conceptually similar to TEFs, but does not have the same level of data to support it and thus has a less rigorous definition compared with the TEF. TEFs and RPFs are used only when there is no better alternative. When they are used, assumptions and uncertainties associated with them are discussed. As of today, there are only three classes of compounds for which relative potency approaches have been examined by EPA: dibenzofurans (dioxins), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). There are limitations to the use of TEF and RFP approaches, and caution should be exercised when using them. More guidance can be found in the draft document for conducting health risk assessment of chemical mixtures, published by the EPA Risk Assessment Forum (USEPA,1999b).

3.1.4 References for Cancer Section

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3.2 NONCANCER EFFECTS

3.2.1 1980 AWQC National Guidelines for Noncancer Effects

In the 1980 AWQC National Guidelines, the Agency evaluated noncancer human health effects from exposure to chemical contaminants using Acceptable Daily Intake (ADI) levels. ADIs were calculated by dividing NOAELs by safety factors (SFs) to obtain estimates of doses of chemicals that would not be expected to cause adverse effects over a lifetime of exposure. In accordance with the National Research Council report of 1977 (NRC, 1977), EPA used SFs of 10, 100, or 1,000, depending on the quality and quantity of the overall database. In general, a factor of 10 was suggested when good-quality data identifying a NOAEL from human studies were available. A factor of 100 was suggested if no human data were available, but the database contained valid chronic animal data. For chemicals with no human data and scant animal data, a factor of 1,000 was recommended. Intermediate SFs could also be used for databases that fell between these categories.

AWQC were calculated using the ADI levels together with standard exposure assumptions about the rates of human ingestion of water and fish, and also accounting for intake from other sources (see Equation 1-1 in the Introduction). Surface water concentrations at or below the calculated criteria concentrations would be expected to result in human exposure levels at or below the ADI. Inherent in these calculations is the assumption that, generally, adverse effects from noncarcinogens exhibit a threshold.

3.2.2 Noncancer Risk Assessment Developments Since 1980

Since 1980, the risk assessment of noncarcinogenic chemicals has changed. To remove the value judgments implied by the words “acceptable” and “safety,” the ADI and SF terms have been replaced with the terms RfD and UF/modifying factor (MF), respectively.

For the risk assessment of general systemic toxicity, the Agency currently uses the guidelines contained in the IRIS background document entitled *Reference Dose (RfD): Description and Use in Health Risk Assessments* (hereafter the “IRIS background document”. That document defines an RfD as “an estimate (with uncertainty spanning approximately an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects over a lifetime” (USEPA,

1993a). The most common approach for deriving the RfD does not involve dose-response modeling. Instead, an RfD for a given chemical is usually derived by first identifying the NOAEL for the most sensitive known toxicity endpoint, that is, the toxic effect that occurs at the lowest dose. This effect is called the critical effect. Factors such as the study protocol, the species of experimental animal, the nature of the toxicity endpoint assessed and its relevance to human effects, the route of exposure, and exposure duration are critically evaluated in order to select the most appropriate NOAEL from among all available studies in the chemical's database. If no appropriate NOAEL can be identified from any study, then the LOAEL for the critical effect endpoint is used and an uncertainly factor for LOAEL-to-NOAEL extrapolation is applied. Using this approach, the RfD is equal to the NOAEL (or LOAEL) divided by the product of UFs and, occasionally, an MF:

$$\text{RfD (mg/kg/day)} = \frac{\text{NOAEL (or LOAEL)}}{\text{UF} \cdot \text{MF}} \qquad \text{(Equation 3-6)}$$

The definitions and guidance for use of the UFs and the MFs are provided in the IRIS background document and are repeated in Table 3-1.

The IRIS background document on the RfD (USEPA, 1993a) provides guidance for critically assessing noncarcinogenic effects of chemicals and for deriving the RfD. Another reference on this topic is Dourson (1994). Furthermore, the Agency has also published separate guidelines for assessing specific toxic endpoints, such as developmental toxicity (USEPA, 1991a), reproductive toxicity (USEPA, 1996a), and neurotoxicity risk assessment (USEPA, 1995). These endpoint-specific guidelines will be used for their respective areas in the hazard assessment step and will complement the overall toxicological assessment. It should be noted, however, that an RfD, derived using the most sensitive known endpoint, is considered protective against all noncarcinogenic effects.

TABLE 3-1. UNCERTAINTY FACTORS AND THE MODIFYING FACTOR

Uncertainty Factor	Definition
UF_H	Use a 1, 3, or 10-fold factor when extrapolating from valid data in studies using long-term exposure to average healthy humans. This factor is intended to account for the variation in sensitivity (intraspecies variation) among the members of the human population.
UF_A	Use an additional factor of 1, 3, or 10 when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty involved in extrapolating from animal data to humans (interspecies variation).
UF_S	Use an additional factor of 1, 3, or 10 when extrapolating from less-than-chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty involved in extrapolating from less-than-chronic NOAELs to chronic NOAELs.
UF_L	Use an additional factor of 1, 3, or 10 when deriving an RfD from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty involved in extrapolating from LOAELs to NOAELs.
UF_D	Use an additional 3- or 10-fold factor when deriving an RfD from an "incomplete" database. This factor is meant to account for the inability of any single type of study to consider all toxic endpoints. The intermediate factor of 3 (approximately ½ $\log_{10}$ unit, i.e., the square root of 10) is often used when there is a single data gap exclusive of chronic data. It is often designated as UF_D.

Modifying Factor

Use professional judgment to determine the MF, which is an additional uncertainty factor that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and database not explicitly treated above (e.g., the number of species tested). The default value for the MF is 1.

Note: With each UF or MF assignment, it is recognized that professional scientific judgment must be used. The total product of the uncertainty factors and modifying factor should not exceed 3,000.

Similar to the procedure used in the 1980 AWQC National Guidelines, the revised method of deriving AWQC for noncarcinogens uses the RfD together with various assumptions concerning intake of the contaminant from both water and non-water sources of exposure. The objective of an AWQC for noncarcinogens is to ensure that human exposure to a substance related to its presence in surface water, combined with exposure from other sources, does not exceed the RfD. The algorithm for deriving AWQC for noncarcinogens using the RfD is presented as Equation 1-1 in the Introduction.

3.2.3 Issues and Recommendations Concerning the Derivation of AWQC for Noncarcinogens

During a review of the 1980 AWQC National Guidelines (USEPA, 1993b), the Agency identified several issues that must be resolved in order to develop a final revised methodology for deriving AWQC based on noncancer effects. These issues, as discussed below, mainly concern the derivation of the RfD as the basis for such an AWQC. Foremost among these issues is whether the Agency should revise the present method or adopt entirely new procedures that use quantitative dose-response modeling for the derivation of the RfD. Other issues include the following:

- Presenting the RfD as a single point value or as a range to reflect the inherent imprecision of the RfD;
- Selecting specific guidance documents for derivation of noncancer health effect levels;
- Considering severity of effect in the development of the RfD;
- Using less-than-90-day studies as the basis for RfDs;
- Integrating reproductive/developmental, immunotoxicity, and neurotoxicity data into the RfD calculation;
- Applying toxicokinetic data in risk assessments; and
- Considering the possibility that some noncarcinogenic effects do not exhibit a threshold.

3.2.3.1 Using the Current NOAEL/UF-Based RfD Approach or Adopting More Quantitative Approaches for Noncancer Risk Assessment

The current NOAEL/UF-based RfD methodology, or its predecessor ADI/SF methodology, have been used since 1980. This approach assumes that there is a threshold exposure below which adverse noncancer health effects are not expected to occur. Exposures above this threshold are believed to pose some risk to exposed individuals; however, the current approach does not address the nature and magnitude of the risk above the threshold level (i.e., the shape of the dose-response curve above the threshold). The NOAEL/UF-based RfD approach is intended primarily to ensure that the RfD value derived from the available data falls below the population effects threshold. However, the NOAEL/UF-based RfD procedure has

limitations. In particular, this method requires that one of the actual experimental doses used by the researchers in the critical study be selected as the NOAEL or LOAEL value. The determination that a dose is a NOAEL or LOAEL will depend on the biological endpoints used and the statistical significance of the data. Statistical significance will depend on the number and spacing of dose groups and the numbers of animals used in each dose group. Studies using a small number of animals can limit the ability to distinguish statistically significant differences among measurable responses seen in dose groups and control groups. Furthermore, the determination of the NOAEL or LOAEL also depends on the dose spacing of the study. Doses are often widely spaced, typically differing by factors of three to ten. A study can identify a NOAEL and a LOAEL from among the doses studied, but the "true" effects threshold cannot be determined from those results. The study size and dose spacing limitations also limit the ability to characterize the nature of the expected response to exposures between the observed NOAEL and LOAEL values.

The limitations of the NOAEL/UF approach have prompted development of alternative approaches that incorporate more quantitative dose-response information. The traditional NOAEL approach for noncancer risk assessment has often been a source of controversy and has been criticized in several ways. For example, experiments involving fewer animals tend to produce higher NOAELs and, as a consequence, may produce higher RfDs. Larger sample sizes, on the other hand, should provide greater experimental sensitivity and lower NOAELs. The focus of the NOAEL approach is only on the dose that is the NOAEL, and the NOAEL must be one of the experimental doses. It also ignores the shape of the dose-response curve. Thus, the slope of the dose-response plays little role in determining acceptable exposures for human beings. Therefore, in addition to the NOAEL/UF-based RfD approach described above, EPA will accept other approaches that incorporate more quantitative dose-response information in appropriate situations for the evaluation of noncancer effects and the derivation of RfDs. However, the Agency wishes to emphasize that it still believes the NOAEL/UF RfD methodology is valid and can continue to be used to develop RfDs.

Two alternative approaches that may have relevance in assisting in the derivation of the RfD for a chemical are the BMD and the categorical regression approaches. These alternative approaches may overcome some of the inherent limitations in the NOAEL/UF approach. For example, the BMD analyses for developmental effects show that NOAELs from studies correlate well with a 5 percent response level (Allen et al., 1994). The BMD and the categorical regression approaches usually have greater data requirements than the RfD approach. Thus, it is unlikely that any one approach will apply to every circumstance; in some cases, different approaches may be needed to accommodate the varying databases for the range of chemicals for which water quality criteria must be developed. Acceptable approaches will satisfy the following criteria: (1) meet the appropriate risk assessment goal; (2) adequately describe the toxicity database and its quality; (3) characterize the endpoints properly; (4) provide a measure of the quality of the "fit" of the model when a model is used for dose-response analysis; and (5) describe the key assumptions and uncertainties.

A. The Benchmark Dose

The BMD is defined as the dose estimated to produce a predetermined level of change in response (the Benchmark Response level, or BMR) relative to control. The BMDL is defined as the statistical lower confidence limit on the BMD. In the derivation of an RfD, the BMDL is used as the dose to which uncertainty factors are applied instead of the NOAEL. The BMD approach first models a dose-response curve for the critical effect(s) using available experimental data. Several mathematical algorithms can be used to model the dose-response curve, such as polynomial or Weibull functions. To define a BMD from the modeled curve for quantal data, the assessor first selects the BMR. The choice of the BMR is critical. For quantal endpoints, a particular level of response is chosen (e.g., 1 percent, 5 percent, or 10 percent). For continuous endpoints, the BMR is the degree of change from controls and is based on what is considered a biologically significant change. The BMD is derived from the BMR dose by applying the desired confidence limit calculation. The RfD is obtained by dividing the BMD by one or more uncertainty factors, similar to the NOAEL approach. Because the BMD is used like the NOAEL to obtain the RfD, the BMR should be selected at or near the low end of the range of increased risks that can be detected in a study of typical size. Generally, this falls in the range between the ED_{01} and the ED_{10}.

The Agency will accept use of a BMD approach to derive RfDs for those agents for which there is an adequate database. There are a number of technical decisions associated with the application of the BMD technique. These include the following:

- The definition of an adverse response;
- Selection of response data to model;
- The form of the data used (continuous versus quantal);
- The choice of the measures of increased risk (extra risk versus additional risk);
- The choice of mathematical model (including use of nonstandard models for unusual data sets);
- The selection of the BMR;
- Methods for calculating the confidence interval;
- Selection of the appropriate BMD as the basis for the RfD (when multiple endpoints are modeled from a single study, when multiple models are applied to a single response, and when multiple BMDs are calculated from different studies); and
- The use of uncertainty factors with the BMD approach.

These topics are discussed in detail in Crump et al. (1995) and in the Risk Assessment TSD Volume (USEPA, 2000). The use of the BMD approach has been discussed in general terms by several authors (Gaylor, 1983; Crump, 1984; Dourson et al., 1985; Kimmel and Gaylor, 1988; Brown and Erdreich, 1989; Kimmel, 1990). The International Life Sciences Institute

(ILSI) also held a major workshop on the BMD in September 1993; the workshop proceedings are summarized in ILSI (1993) and in Barnes et al. (1995). For further information on these technical issues, the reader is referred to the publications referenced above.

The BMD approach addresses several of the quantitative or statistical criticisms of the NOAEL approach. These are discussed at greater length in Crump et al. (1995) and are summarized here. First, the BMD approach uses all the dose-response information in the selected study rather than just a single data point, such as the NOAEL or LOAEL. By using response data from all of the dose groups to model a dose-response curve, the BMD approach allows for consideration of the steepness of the slope of the curve when estimating the ED_{10}. The use of the full data set also makes the BMD approach less sensitive to small changes in data than the NOAEL approach, which relies on the statistical comparison of individual dose groups. The BMD approach also allows consistency in the consideration of the level of effect (e.g., a 10 percent response rate) across endpoints.

The BMD approach accounts more appropriately for the size of each dose group than the NOAEL approach. Laboratory tests with fewer animals per dose group tend to yield higher NOAELs, and thus higher RfDs, because statistically significant differences in response rates are harder to detect. Therefore, in the NOAEL approach, dose groups with fewer animals lead to a higher (less conservative) RfD. In contrast, with the BMD approach, smaller dose groups will tend to have the effect of extending the confidence interval around the ED_{10}; therefore, the lower confidence limit on the ED_{10} (the BMD) will be lower. With the BMD approach, greater uncertainty (smaller test groups) leads to a lower (more conservative) RfD.

There are some issues to be resolved before the BMD approach is used routinely. These were identified in a 1996 Peer Consultation Workshop (USEPA, 1996b). Methods for routine use of the BMD are currently under development by EPA. Several RfCs and RfDs based on the BMD approach are included in EPA's IRIS database. These include reference values for methylmercury based on delayed postnatal development in humans; carbon disulfide based on neurotoxicity; 1,1,1,2-tetrafluoroethane based on testicular effects in rats; and antimony trioxide based on chronic pulmonary interstitial inflammation in female rats.

Various mathematical approaches have been proposed for modeling developmental toxicity data (e.g., Crump, 1984; Kimmel and Gaylor, 1988; Rai and Van Ryzin, 1985; Faustman et al., 1989), which could be used to calculate a BMD. Similar methods can be used to model other types of toxicity data, such as neurotoxicity data (Gaylor and Slikker, 1990, 1992; Glowa and MacPhail, 1995). The choice of the mathematical model may not be critical, as long as estimation is within the observed dose range. Since the model fits a mathematical equation to the observed data, the assumptions in a particular model regarding the existence or absence of a threshold for the effect may not be pertinent (USEPA, 1997). Thus, any model that suitably fits the empirical data is likely to provide a reasonable estimate of a BMD. However, research has shown that flexible models that are nonsymmetric (e.g., the Weibull) are superior to symmetric models (e.g., the probit) in estimating the BMD because the data points at the higher doses have less influence on the shape of the curve than at low doses. In addition, models should incorporate fundamental biological factors where such factors are known (e.g., intralitter correlation for developmental toxicity data) in order to account for as much variability in the

data as possible. The Agency is currently using the BMD approach in risk assessments where the data support its use. Draft guidelines for application of the BMD approach also are being developed by the Agency.

Use of BMD methods involves fitting mathematical models to dose-response data obtained primarily from toxicology studies. When considering available models to use for a BMD analysis, it is important to select the model that fits the data the best and is the most biologically appropriate. EPA has developed software following several years of research and development, expert peer review, public comment, subsequent revision, and quality assurance testing. The software (BMDS, Version 1.2) can be downloaded from http://www.epa.gov/ncea/bmds.htm. BMDS facilitates these operations by providing simple data-management tools, a comprehensive help manual, an online help system, and an easy-to-use interface to run multiple models on the same dose-response data.

As part of this software package, EPA has included sixteen (16) different models that are appropriate for the analysis of dichotomous (quantal) data (Gamma, Logistic, Log-Logistic, Multistage, Probit, Log-Probit, Quantal-Linear, Quantal-Quadratic, Weibull), continuous data (Linear, Polynomial, Power, Hill), and nested developmental toxicology data (NLogistic, NCTR, Rai & Van Ryzin). Results from all models include a reiteration of the model formula and model run options chosen by the user, goodness-of-fit information, the BMD, and the estimate of the lower-bound confidence limit on the benchmark dose (BMDL). Model results are presented in textual and graphical output files which can be printed or saved and incorporated into other documents.

B. Categorical Regression

Categorical regression is an emerging technique that may have relevance for the derivation of RfDs or for estimating risk above the RfD (Dourson et al., 1997; Guth et al., 1997). The categorical regression approach, like the BMD approach, can be used to estimate a dose that corresponds to a given probability of adverse effects. This dose would then be divided by UFs to establish an RfD. However, unlike the BMD approach, the Categorical regression approach can incorporate information on different health endpoints in a single dose-response analysis. For those health effects for which studies exist, responses to the substance in question are grouped into severity categories; for example (1) no effect, (2) no adverse effect, (3) mild-to-moderate adverse effect, and (4) frank effect. These categories correspond to the dose categories currently used in setting the RfD, namely, the no-observed-effect level (NOEL), NOAEL, LOAEL, and frank-effect level (FEL), respectively. Logistic transformation or other applicable mathematical operations are used to model the probability of experiencing effects in a certain category as a function of dose (Harrell, 1986; Hertzberg, 1989). The "acceptability" of the fit of the model to the data can be judged using several statistical measures, including the χ^2 statistic, correlation coefficients, and the statistical significance of its model parameter estimates.

The resulting mathematical equation can be used to find a dose (or the lower confidence bound on the dose) at which the probability of experiencing adverse effects does not exceed a selected level, e.g., 10 percent. This dose (like the NOAEL or BMD) would then be divided by

relevant UFs to calculate an RfD. For more detail on how to employ the categorical regression approach, see the discussion in the Risk Assessment TSD (USEPA, 2000).

As with the BMD approach, the categorical regression approach has the advantage of using more of the available dose-response data to account for response variability as well as accounting for uncertainty due to sample size through the use of confidence intervals. Additional advantages of categorical regression include the combining of data sets prior to modeling, thus allowing the calculation of the slope of a dose-response curve for multiple adverse effects rather than only one effect at a time. Another advantage is the ability to estimate risks for different levels of severity from exposures above the RfD.

On the other hand, as with BMD, opinions differ over the amount and adequacy of data necessary to implement the method. The categorical regression approach also requires judgments regarding combining data sets, judging goodness-of-fit, and assigning severity to a particular effect. Furthermore, this approach is still in the developmental stage. It is not recommended for routine use, but may be used when data are available and justify the extensive analyses required.

C. Summary

Whether a NOAEL/UF-based methodology, a BMD, a categorical regression model, or other approach is used to develop the RfD, the dose-response-evaluation step of a risk assessment process should include additional discussion about the nature of the toxicity data and its applicability to human exposure and toxicity. The discussion should present the range of doses that are effective in producing toxicity for a given agent; the route, timing, and duration of exposure; species specificity of effects; and any toxicokinetic or other considerations relevant to extrapolation from the toxicity data to human-health-based AWQC. This information should always accompany the characterization of the adequacy of the data.

3.2.3.2 Presenting the RfD as a Single Point or as a Range for Deriving AWQC

Although the RfD has traditionally been presented and used as a single point, its definition contains the phrase ". . . an estimate (with uncertainty spanning perhaps an order of magnitude) . . ." (USEPA, 1993a). Underlying this concept is the reasoning that the selection of the critical effect and the total uncertainty factor used in the derivation of the RfD is based on the "best" scientific judgment, and that competent scientists examining the same database could derive RfDs which varied within an order of magnitude.

In one instance, IRIS presented the RfD as a point value within an accompanying range. EPA derived a single number as the RfD for arsenic (0.3 μg/kg-day), but added that "strong scientific arguments can be made for various values within a factor of 2 or 3 of the currently recommended RfD value, i.e., 0.1 to 0.8 μg/kg/day" (USEPA, 1993c). EPA noted that regulatory managers should be aware of the flexibility afforded them through this action.

There are situations in which the risk manager can select an alternative value to use in place of the RfD in the AWQC calculations. The domain from which this alternative value can

be selected is restricted to a defined range around the point estimate. As explained further below, the Agency is recommending that sometimes the use of a value other than the calculated RfD point estimate is appropriate in characterizing risk. The selection of an alternative value within an appropriate range must be determined for each individual situation, since several factors affect the selection of the alternative value. Observing similar effects in several animal species, including humans, can increase confidence in the selection of the critical effect and thereby narrow the range of uncertainty. There are other factors that can affect the precision. These include the slope of the dose-response curve, seriousness of the observed effect, dose spacing, and possibly the route for the experimental doses. Dose spacing and the number of animals in the study groups used in the experiment can also affect the confidence in the RfD.

To derive the AWQC, the calculated point estimate of the RfD is the default. Based on consideration of the available data, the use of another number within the range defined by the product of the UF(s) (and MF, if used) could be justified in some specific situations. This means that there are risk considerations which indicate that some value in the range other than the point estimate may be more appropriate, based on human health or environmental fate considerations. For example, the bioavailability of the contaminant in fish tissues is one factor to consider. If bioavailability from fish tissues is much lower than that from water and the RfD was derived from a study in which the contaminant exposure was from drinking water, the alternative to the calculated RfD could be selected from the high end of the range and justified using the quantitative difference in bioavailability.

Most inorganic contaminants, particularly divalent cations, have bioavailability values of 20 percent or less from a food matrix, but are much more available (about 80 percent or higher) from drinking water. Accordingly, the external dose necessary to produce a toxic internal dose would likely be higher for a study where the exposure occurred through the diet rather than the drinking water. As a result, the RfD from a dietary study would likely be higher than that for the drinking water study if equivalent external doses had been used. Conversely, in cases where the NOAEL that was the basis for the RfD came from a dietary study, the alternative value could be slightly lower than the calculated RfD.

Because the uncertainty around the dose-response relationship increases as extrapolation below the observed data increases, the use of an alternative point within the range may be more appropriate in characterizing the risk than the use of the calculated RfD, especially in situations when the uncertainty is high. Therefore, as a matter of policy, the 2000 Human Health Methodology permits the selection of a single point within a range about the calculated RfD to be used as the basis of the AWQC if an adequate justification of the alternative point is provided. More complete discussion of this option, including limitations on the span of the range, is provided in the Risk Assessment TSD (USEPA, 2000).

3.2.3.3 Guidelines to be Adopted for Derivation of Noncancer Health Effects Values

The Agency currently is using the IRIS background document as the general basis for the risk assessment of noncarcinogenic effects of chemicals (USEPA, 1993a). EPA recommends continued use of this document for this purpose. However, it should be noted that the process for evaluating chemicals for inclusion in IRIS is undergoing revision (USEPA, 1996c). The

revised assessments for many chemicals are now available on IRIS and can be consulted as examples of the RfD development process and required supporting documentation.

3.2.3.4 Treatment of Uncertainty Factors/Severity of Effects During the RfD Derivation and Verification Process

During the RfD derivation and toxicology review process, EPA considers the uncertainty in extrapolating between animal species and within individuals of a species, as well as specific uncertainties associated with the completeness of the database. The Agency's RfD Work Group has always considered the severity of the observed effects induced by the chemical under review when choosing the value of the UF with a LOAEL. For example, during the derivation and verification of the RfD for zinc (USEPA, 1992), an uncertainty factor less than the standard factor of 10 (UF of 3) was assigned to the relatively mild decrease in erythrocyte superoxide dismutase activity in human subjects. EPA recommends that the severity of the critical effect be assessed when deriving an RfD and that risk managers be made aware of the severity of the effect and the weight placed on this attribute of the effect when the RfD was derived.

3.2.3.5 Use of Less-Than-90-Day Studies to Derive RfDs

Generally, less-than-90-day experimental studies are not used to derive an RfD. This is based on the rationale that studies lasting for less than 90 days may be too short to detect various toxic effects. However, EPA, has in certain circumstances, derived an RfD based on a less-than-90-day study. For example, the RfD for nonradioactive effects of uranium is based on a 30-day rabbit study (USEPA, 1989). The short-term exposure period was used, because it was adequate for determining doses that cause chronic toxicity. In other cases, it may be appropriate to use a less-than-90-day study because the critical effect is expressed in less than 90 days. For example, the RfD for nitrate was derived and verified using studies that were less than 3-months in duration (USEPA, 1991b). For nitrate, the critical effect of methemoglobinemia in infants occurs in less than 90 days. When it can be demonstrated from other data in the toxicological database that the critical adverse effect is expressed within the study period and that a longer exposure duration would not exacerbate the observed effect or cause the appearance of some other adverse effect, the Agency may choose to use less-than-90-day studies as the basis of the RfD. Such values would have to be used with care because of the uncertainty in determining if other effects might be expressed if exposure was of greater duration than 90 days.

3.2.3.6 Use of Reproductive/Developmental, Immunotoxicity, and Neurotoxicity Data as the Basis for Deriving RfDs

All relevant toxicity data have some bearing on the RfD derivation and verification and are considered by EPA. The "critical" effect is the adverse effect most relevant to humans or, in the absence of an effect known to be relevant to humans, the adverse effect that occurs at the lowest dose in animal studies. If the critical effect is neurotoxicity, EPA will use that endpoint as the basis for the derivation and verification of an RfD, as it did for the RfD for acrylamide. Moreover, the Agency is continually revising its procedures for noncancer risk assessment. For example, EPA has released guidelines for deriving developmental RfDs (RfD_{DT}, USEPA, 1991a), for using reproductive toxicity (USEPA, 1996a), and neurotoxicity (USEPA, 1995) data

in risk assessments. The Agency is currently working on guidelines for using immunotoxicity data to derive RfDs. In addition, the Agency is proceeding with the process of generating acceptable emergency health levels for hazardous substances in acute exposure situations based on established guidelines (NRC, 1993).

3.2.3.7 Applicability of Toxicokinetic Data in Risk Assessment

All pertinent toxicity data should be used in the risk assessment process, including toxicokinetic and mechanistic data. The Agency has used toxicokinetic data in deriving the RfD for cadmium and other compounds and currently is using toxicokinetic data to better characterize human inhalation exposures from animal inhalation experiments during derivation/verification of RfCs. In analogy to the RfD, the RfC is considered to be an estimate of a concentration in the air that is not anticipated to cause adverse noncancer effects over a lifetime of inhalation exposure (USEPA, 1994; Jarabek, 1995a). For RfCs, different dosimetry adjustments are made to account for the differences between laboratory animals and humans in gas uptake and disposition or in particle clearance and retention. This procedure results in calculation of a "human equivalent concentration." Based on the use of these procedures, an interspecies UF of 3 (i.e., approximately $10^{0.5}$), instead of the standard factor of 10, is used in the RfC derivation (Jarabek, 1995b).

Toxicokinetics and toxicodynamics of a chemical each contribute to a chemical's observed toxicity, and specifically, to observed differences among species in sensitivity. Toxicokinetics describes the disposition (i.e., deposition, absorption, distribution, metabolism, and elimination of chemicals in the body) and can be approximated using toxicokinetic models. Toxicodynamics describes the toxic interaction of the agent with the target cell. In the absence of specific data on their relative contributions to the toxic effects observed in species, each is considered to account for approximately one-half of the difference in observed effects for humans compared with laboratory animals. The implication of this assumption is that an interspecies uncertainty factor of 3 rather than 10 could be used for deriving an RfD when valid toxicokinetic data and models can be applied to obtain an oral "human equivalent applied dose" (Jarabek, 1995b). If specific data exist on the relative contribution of either element to observed effects, that proportion will be used. The role exposure duration may play, and whether or not the chemical or its damage may accumulate over time in a particular scenario, also requires careful consideration (Jarabek, 1995c).

3.2.3.8 Consideration of Linearity (or Lack of a Threshold) for Noncarcinogenic Chemicals

It is quite possible that there are chemicals with noncarcinogenic endpoints that have no threshold for effects. For example, in the case of lead, it has not been possible to identify a threshold for effects on neurological development. Other examples could include genotoxic teratogens and germline mutagens. Genotoxic teratogens act by causing mutational events during organogenesis, histogenesis, or other stages of development. Germline mutagens interact with germ cells to produce mutations which may be transmitted to the zygote and expressed during one or more stages of development. However, there are few chemicals which currently have sufficient mechanistic information about these possible modes of action. It should be recognized that although an MOA consistent with linearity is possible (especially for agents

known to be mutagenic), this has yet to be reasonably demonstrated for most toxic endpoints other than cancer.

EPA has recognized the potential for nonthreshold noncarcinogenic endpoints and discussed this issue in the *Guidelines for Developmental Toxicity Risk Assessment* (USEPA, 1991a) and in the 1986 *Guidelines for Mutagenicity Risk Assessment* (USEPA, 1986). An awareness of the potential for such teratogenic/mutagenic effects should be established in order to deal with such data. However, without adequate data to support a genetic or mutational basis for developmental or reproductive effects, the default becomes a UF or MOA approach, which are procedures utilized for noncarcinogens assumed to have a threshold. Therefore, genotoxic teratogens and germline mutagens should be considered an exception while the traditional uncertainty factor approach is the general rule for calculating criteria or values for chemicals demonstrating developmental/reproductive effects. For the exceptional cases, since there is no well-established mechanism for calculating criteria protective of human health from the effects of these agents, criteria will be established on a case-by-case basis. Other types of nonthreshold noncarcinogens must also be handled on a case-by-case basis.

3.2.3.9 Minimum Data Guidance

For details on minimum data guidance for RfD development, see the Risk Assessment TSD (USEPA, 2000).

3.2.4 References for Noncancer Effects

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4. EXPOSURE

The derivation of AWQC for the protection of human health requires information about both the toxicological endpoints of concern for water pollutants and the pathways of human exposure to those pollutants. The two primary pathways of human exposure to pollutants present in a particular ambient waterbody that have been considered in deriving AWQC are direct ingestion of drinking water obtained from that waterbody and the consumption of fish/shellfish obtained from that waterbody. The water pathway also includes other exposures from household uses (e.g., showering). The derivation of an AWQC involves the calculation of the maximum water concentration for a pollutant (i.e., the water quality criteria level) that ensures drinking water and/or fish ingestion exposures will not result in human intake of that pollutant in amounts that exceed a specified level based upon the toxicological endpoint of concern.

The equation for noncancer effects is presented again here, in simplified form, to emphasize the exposure-related parameters (in bold). [Note: the RSC parameter also applies to nonlinear low-dose extrapolation for cancer effects and the other exposure parameters apply to all three of the equations (see Section 1.6).]

$$AWQC = RfD \bullet \boldsymbol{RSC} \bullet \frac{(\boldsymbol{BW})}{\left[\boldsymbol{DI} + (\boldsymbol{FI} \bullet BAF)\right]} \qquad \text{(Equation 4-1)}$$

where:

AWQC	=	Ambient Water Quality Criterion (mg/L)
RfD	=	Reference dose for noncancer effects (mg/kg-day)
RSC	=	Relative source contribution factor to account for non-water sources of exposure
BW	=	Human body weight (kg)
DI	=	Drinking water intake (L/day)
FI	=	Fish intake (kg/day)
BAF	=	Bioaccumulation factor (L/kg)

The following subsections discuss exposure issues relevant to the 2000 Human Health Methodology: exposure policy issues; consideration of non-water sources of exposure (the Relative Source Contribution approach); and the factors used in AWQC computation. In relevant sections, science policy and risk management decisions made by EPA are discussed.

4.1 EXPOSURE POLICY ISSUES

This section discusses broad policy issues related to exposure concerning the major objectives that the Agency believes should be met in setting AWQC.

An Exposure Assessment TSD provides greater detail on numerous topics discussed in this guidance: suggested sources of contaminant concentration and exposure intake information; suggestions of survey methods for obtaining and analyzing exposure data necessary for deriving AWQC; summaries of studies on fish consumption among sport fishers and subsistence fishers; more detailed presentation of parameter values (e.g., fish consumption rates, body weights); and additional guidance on the application of the RSC approach.

4.1.1 Sources of Exposure Associated With Ambient Water

4.1.1.1 Appropriateness of Including the Drinking Water Pathway in AWQC

EPA intends to continue including the drinking water exposure pathway in the derivation of its national default human health criteria (AWQC), as has been done since the 1980 AWQC National Guidelines were first published.

EPA recommends inclusion of the drinking water exposure pathway where drinking water is a designated use for the following reasons: (1) Drinking water is a designated use for surface waters under the CWA and, therefore, criteria are needed to assure that this designated use can be protected and maintained. (2) Although rare, there are some public water supplies that provide drinking water from surface water sources without treatment. (3) Even among the majority of water supplies that do treat surface waters, existing treatments may not necessarily be effective for reducing levels of particular contaminants. (4) In consideration of the Agency's goals of pollution prevention, ambient waters should not be contaminated to a level where the burden of achieving health objectives is shifted away from those responsible for pollutant discharges and placed on downstream users to bear the costs of upgraded or supplemental water treatment.

This policy decision has been supported by the States, most of the public stakeholders, and by external peer reviewers. As with the other exposure parameters, States and authorized Tribes have the flexibility to use alternative intake rates if they believe that drinking water consumption is substantively different than EPA's recommended default assumptions of 2 L/day for adults and 1 L/day for children. EPA recommends that States and authorized Tribes use an intake rate that would be protective of a majority of consumers and will consider whether an alternative assumption is adequately protective of a State's or Tribe's population based on the information or rationale provided at the time EPA reviews State and Tribal water quality standards submissions.

4.1.1.2 Setting Separate AWQC for Drinking Water and Fish Consumption

In conjunction with the issue of the appropriateness of including the drinking water pathway explicitly in the derivation of AWQC for the protection of human health, EPA intends to continue its practice of setting a single AWQC for both drinking water and fish/shellfish consumption, and a separate AWQC based on ingestion of fish/shellfish alone. This latter criterion applies in those cases where the designated uses of a waterbody include supporting fishable uses under Section 101(a) of the CWA and, thus, fish or shellfish for human consumption, but not as a drinking water supply source (e.g., non-potable estuarine waters).

EPA does not believe that national water quality criteria for protection of drinking water uses only are particularly useful for two reasons. First, State and Tribal standards for human health are set to protect Section 101(a) uses (e.g., "fishable, swimmable uses") under the CWA. Second, most waters have multiple designated uses. Additionally, the water quality standards program protects aquatic life. The 2000 Human Health Methodology revisions do not change EPA's policy to apply aquatic life criteria to protect aquatic species where they are more sensitive (i.e., when human health criteria would not be protective enough) or where human health via fish or water ingestion is not an issue.

4.1.1.3 Incidental Ingestion from Ambient Surface Waters

The 2000 Human Health Methodology does not routinely include criteria to address incidental ingestion of water from recreational uses. EPA has considered whether there are cases where water quality criteria for the protection of human health based only on fish ingestion (or only criteria for the protection of aquatic life) may not adequately protect recreational users from health effects resulting from incidental water ingestion.

EPA reviewed information that provided estimates of incidental water ingestion rates averaged over time. EPA generally believes that the averaged amount is negligible and will not have any impact on the chemical criteria values representative of both drinking water and fish ingestion. A lack of impact on the criteria values would likely also be true for chemical criteria based on fish consumption only, unless the chemical exhibits no bioaccumulation potential. However, EPA also believes that incidental/accidental water ingestion could be important for the development of microbial contaminant water quality criteria, and for either chemical or microbial criteria for States where recreational uses such as swimming and boating are substantially higher than the national average. EPA also notes that some States have indicated they already have established incidental ingestion rates for use in developing criteria. Therefore, although EPA will not use this intake parameter when deriving its national 304(a) chemical criteria, limited guidance is provided in the Exposure Assessment TSD volume in order to assist States and authorized Tribes that face situations where this intake parameter could be of significance.

4.2 CONSIDERATION OF NON-WATER SOURCES OF EXPOSURE WHEN SETTING AWQC

4.2.1 Policy Background

The 2000 Human Health Methodology uses different approaches for addressing non-water exposure pathways in setting AWQC for the protection of human health depending upon the toxicological endpoint of concern. With those substances for which the appropriate toxic endpoint is carcinogenicity based on a linear low-dose extrapolation, only the two water sources (i.e., drinking water and fish ingestion) are considered in the derivation of the AWQC. Non-water sources are not considered explicitly. In the case of carcinogens based on linear low-dose extrapolation, the AWQC is being determined with respect to the *incremental* lifetime risk posed by a substance's presence in water, and is not being set with regard to an individual's total risk from all sources of exposure. Thus, the AWQC represents the water concentration that would be

expected to increase an individual's lifetime risk of carcinogenicity from exposure to the particular pollutant by no more than one chance in one million, regardless of the additional lifetime cancer risk due to exposure, if any, to that particular substance from other sources.

Furthermore, health-based criteria values for one medium based on linear low-dose extrapolation typically vary from values for other media in terms of the concentration value, and often the associated risk level. Therefore, the RSC concept could not even theoretically apply unless all risk assessments for a particular carcinogen based on linear low-dose extrapolation resulted in the same concentration value and same risk level; that is, an apportionment would need to be based on a single risk value and level.

In the case of substances for which the AWQC is set on the basis of a carcinogen based on a nonlinear low-dose extrapolation or for a noncancer endpoint where a threshold is assumed to exist, non-water exposures are considered when deriving the AWQC using the RSC approach. The rationale for this approach is that for pollutants exhibiting threshold effects, the objective of the AWQC is to ensure that an individual's total exposure does not exceed that threshold level.

There has been some discussion of whether it is, in fact, necessary in most cases to explicitly account for other sources of exposure when computing the AWQC for pollutants exhibiting threshold effects. It has been argued that because of the conservative assumptions generally incorporated in the calculation of RfDs (or POD/UF values) used as the basis for the AWQC derivation, total exposures slightly exceeding the RfD are unlikely to produce adverse effects.

EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion or multiple criteria, when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD or the POD/UF. The policy of considering multiple sources of exposure when deriving health-based criteria has become common in EPA's program office risk characterizations and criteria and standard-setting actions. Numerous EPA workgroups have evaluated the appropriateness of factoring in such exposures, and the Agency concludes that it is important for adequately protecting human health. Consequently, EPA risk management policy has evolved significantly over the last six years. Various EPA program initiatives and policy documents regarding aggregate exposure and cumulative risk have been developed, including the consideration of inhalation and dermal exposures. Additionally, accounting for other exposures has been included in recent mandates (e.g., the Food Quality Protection Act) and, thus, is becoming a requirement for the Agency. The Exposure Decision Tree approach has been shared with other EPA offices, and efforts to coordinate policies on aggregate exposure, where appropriate, have begun. EPA intends to continue developing policy guidance on the RSC issue and guidance to address the concern that human health may not be adequately protected if criteria allow for higher levels of exposure that, combined, may exceed the RfD or POD/UF. EPA also intends to refine the 2000 Human Health Methodology in the future to incorporate additional guidance on inhalation and dermal exposures. As stated previously, EPA is required to derive national water quality criteria under Section 304(a) of the CWA and does not intend to derive site-specific criteria. However, States and authorized Tribes have the flexibility to make alternative exposure and RSC estimates based on local data, and EPA strongly encourages this.

Uncertainty factors used in the derivation of the RfD (or POD/UF) to account for intra- and interspecies variability and the incompleteness of the toxicity data set(s)/animal studies are specifically relevant to the chemical's internal toxicological action, irrespective of the sources of exposure that humans may be experiencing. The Agency's policy is to consider and account for other sources of exposure in order to set protective health criteria. EPA believes that multiple route exposures may be particularly important when uncertainty factors associated with the RfD are small. Although EPA is well aware that RfDs are not all equivalent in their derivation, EPA does not believe that uncertainty in the toxicological data should result in less stringent criteria by ignoring exposure sources. However, the RSC policy approach does allow less stringent assumptions when multiple sources of exposure are not anticipated.

The AWQC are designed to be protective criteria, generally applicable to the waters of the United States. While EPA cannot quantitatively predict the actual human health risk associated with combined exposures above the RfD or POD/UF, a combination of health criteria for multiple media exceeding the RfD or POD/UF may not be sufficiently protective. Therefore, EPA's policy is to routinely account for all sources and routes of non-occupational exposure when setting AWQC for noncarcinogens and for carcinogens based on nonlinear low-dose extrapolations. EPA believes that maintaining total exposure below the RfD (or POD/UF) is a reasonable health goal and that there are circumstances where health-based criteria for a chemical should not exceed the RfD (or POD/UF), either alone (if only one criterion is relevant, along with other intake sources considered as background exposures) or in combination. EPA believes its RSC policy ensures this goal.

Also, given the inability to reasonably predict future changes in exposure patterns, the uncertainties in the exposure estimates due to typical data inadequacy, possible unknown sources of exposure, and the potential for some populations to experience greater exposures than indicated by the available data, EPA believes that utilizing the entire RfD (or POD/UF) does not ensure adequate protection.

4.2.2 The Exposure Decision Tree Approach

As indicated in Section 1, EPA has, in the past, used a "subtraction" method to account for multiple sources of exposure to pollutants. In the subtraction method, other sources of exposure (i.e., those other than the drinking water and fish exposures) are subtracted from the RfD (or POD/UF). However, EPA also previously used a "percentage" method for the same purpose. In this approach, the percentage of total exposure typically accounted for by the exposure source for which the criterion is being determined, referred to as the relative source contribution (RSC), is applied to the RfD to determine the maximum amount of the RfD "apportioned" to that source. With both procedures, a "ceiling" level of 80 percent of the RfD and a "floor level" of 20 percent of the RfD are applied.

The subtraction method is considered acceptable when only one criterion is relevant for a particular chemical. The percentage method is recommended in the context of the above goals when multiple media criteria are at issue. The percentage method does not simply depend on the amount of a contaminant in the prospective criterion source only. It is intended to reflect health considerations, the relative portions of other sources, and the likelihood for ever-changing levels

in each of those multiple sources (due to ever-changing sources of emissions and discharges). Rather than simply defaulting in every instance, the Agency attempts to compare multiple source exposures with one another to estimate their relative contribution to the total–given that understanding the degree to which their concentrations vary, or making any distributional analysis, is often not possible. The criteria levels, when multiple criteria are at issue, are based on the actual levels, with an assumption that there may be enough relative variability such that an apportionment (relating that percentage to the RfD) is a reasonable way of accounting for the uncertainty regarding that variability.

The specific RSC approach recommended by EPA, which we will use for the derivation of AWQC for noncarcinogens and carcinogens assessed using nonlinear low-dose extrapolation, is called the Exposure Decision Tree and is described below. To account for exposures from other media when setting an AWQC (i.e., non-drinking water/non-fish ingestion exposures, and inhalation or dermal exposures), the Exposure Decision Tree for determining proposed RfD or POD/UF apportionments represents a method of comprehensively assessing a chemical for water quality criteria development. This method considers the adequacy of available exposure data, levels of exposure, relevant sources/media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the same chemical). The Decision Tree addresses most of the disadvantages associated with the exclusive use of either the percentage or subtraction approaches, because they are not arbitrarily chosen prior to determining the following: specific population(s) of concern, whether these populations are relevant to multiple-source exposures for the chemical in question (i.e., whether the population is actually or potentially experiencing exposure from multiple sources), and whether levels of exposure, regulatory agendas, or other circumstances make apportionment of the RfD or POD/UF desirable. Both subtraction and percentage methods are potentially utilized under different circumstances with the Exposure Decision Tree approach, and the Decision Tree is recommended with the idea that there is enough flexibility to use other procedures if information on the contaminant in question suggests it is not appropriate to follow the Decision Tree. EPA recognizes that there may be other valid approaches in addition to the Exposure Decision Tree.

The Exposure Decision Tree approach allows flexibility in the RfD (or POD/UF) apportionment among sources of exposure. When adequate data are available, they are used to make protective exposure estimates for the population(s) of concern. When other sources or routes of exposure are anticipated but data are not adequate, there is an even greater need to make sure that public health protection is achieved. For these circumstances, a series of qualitative alternatives is used (with the less adequate data or default assumptions) that allow for the inadequacies of the data while protecting human health. Specifically, the Decision Tree makes use of chemical information when actual monitoring data are inadequate. It considers information on the chemical/physical properties, uses of the chemical, and environmental fate and transformation, as well as the likelihood of occurrence in various media. Review of such information, when available, and determination of a reasonable exposure characterization for the chemical will result in a water quality criterion that more accurately reflects exposures than automatically using a default value. Although the 20 percent default will still generally be used when information is not adequate, the need for using it should be reduced. There may also be some situations where EPA would consider the use of an 80 percent default (see Section 4.2.3).

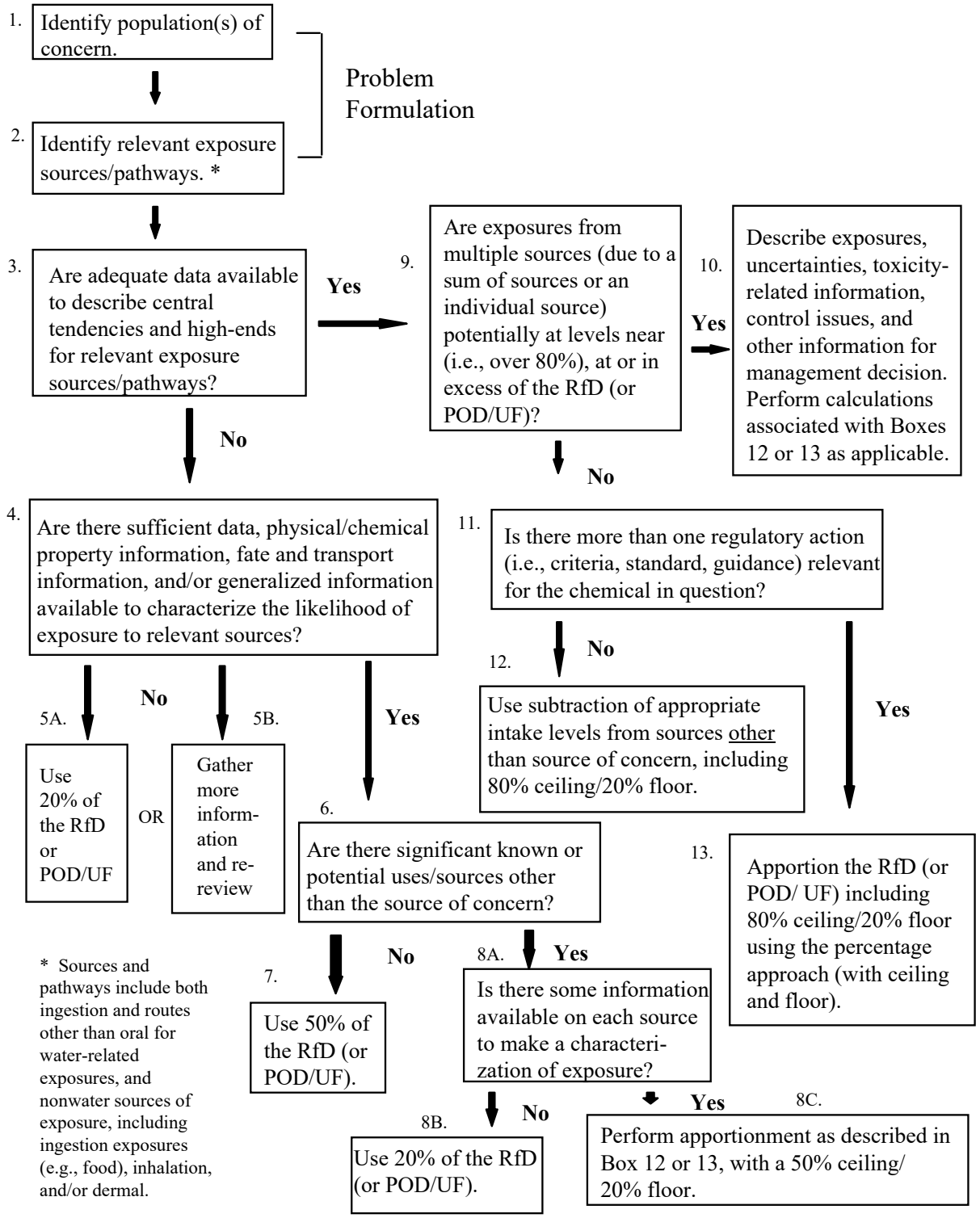
The Decision Tree also allows for use of either the subtraction or percentage method to account for other exposures, depending on whether one or more health-based criterion is relevant for the chemical in question. The subtraction method is considered acceptable when only one criterion is relevant for a particular chemical. In these cases, other sources of exposure can be considered "background" and can be subtracted from the RfD (or POD/UF).

EPA cautions States and Tribes when using the subtraction method in these circumstances. The subtraction method results in a criterion allowing the maximum possible chemical concentration in water after subtracting other sources. As such, it removes any cushion between pre-criteria levels (i.e., actual "current" levels) and the RfD, thereby setting criteria at the highest levels short of exceeding the RfD. It is somewhat counter to the goals of the CWA for maintaining and restoring the nation's waters. It is also directly counter to Agency policies, explicitly stated in numerous programs, regarding pollution prevention. EPA has advocated that it is good health policy to set criteria such that exposures are kept low when current levels are already low. The subtraction method generally results in criteria levels of a contaminant in a particular medium at significantly higher levels than the percentage method and, in this respect, is contradictory to such goals. In fact, many chemicals have pre-criteria levels in environmental media substantially lower (compared to the RfD) than the resulting criteria allow.

When more than one criterion is relevant to a particular chemical, apportioning the RfD (or POD/UF) via the percentage method is considered appropriate to ensure that the combination of criteria and, thus, the potential for resulting exposures do not exceed the RfD (or POD/UF). The Exposure Decision Tree (with numbered boxes) is shown in Figure 4-1. The explanation in the text on the following pages must be read in tandem with the Decision Tree figure; the text in each box of the figure only nominally identifies the process and conditions for determining the outcome for that step of the Decision Tree. The underlying objective is to maintain total exposure below the RfD (or POD/UF) while generally avoiding an extremely low limit in a single medium that represents just a nominal fraction of the total exposure. To meet this objective, all proposed numeric limits lie between 80 percent and 20 percent of the RfD (or POD/UF). Again, EPA will use the Exposure Decision Tree approach when deriving its AWQC but also recognizes that departures from the approach may be appropriate in certain cases. EPA understands that there may be situations where the Decision Tree procedure is not practicable or

Figure 4-1

Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment



may be simply irrelevant after considering the properties, uses, and sources of the chemical in question. EPA endorses such flexibility by States and authorized Tribes when developing alternative water quality criteria in order to choose other procedures that are more appropriate for setting health-based criteria and, perhaps, apportioning the RfD or POD/UF, as long as reasons are given as to why it is not appropriate to follow the Exposure Decision Tree approach and as long as the steps taken to evaluate the potential sources and levels of exposure are clearly described. Often, however, the common situation of multiple exposure sources for a chemical is likely to merit a Decision Tree evaluation for the purpose of developing human health water quality criteria for a given chemical.

It is clear that this will be an interactive process; input by exposure assessors will be provided to, and received from, risk managers throughout the process, given that there may be significant implications regarding control issues (i.e., cost/feasibility), environmental justice issues, etc. In cases where the Decision Tree is not chosen, communication and concurrence about the decision rationale and the alternative water quality criteria are of great importance.

Descriptions of the boxes within the Decision Tree are separated by the following process headings to facilitate an understanding of the major considerations involved. The decision to perform, or not to perform, an apportionment could actually be made at several points during the Decision Tree process. Working through the process is most helpful for identifying possible exposure sources and the potential for exposure, determining the relevancy of the Decision Tree to developing an AWQC for a particular chemical and, possibly, determining the appropriateness of using an alternative approach to account for overall exposure. "Relevancy" here means determining whether more than one criterion, standard, or other guidance is being planned or is in existence for the chemical in question. Additional guidance for States and Tribes that wish to use the Exposure Decision Tree is provided in the Exposure Assessment TSD.

4.2.2.1 Problem Formulation

Initial Decision Tree discussion centers around the first two boxes: identification of population(s) of concern (Box 1) and identification of relevant exposure sources and pathways (Box 2). The term "problem formulation" refers to evaluating the population(s) and sources of exposure in a manner that allows determination of the potential for the population of concern to experience exposures from multiple sources for the chemical in question. Also, the data for the chemical in question must be representative of each source/medium of exposure and be relevant to the identified population(s). Evaluation includes determining whether the levels, multiple criteria or regulatory standards, or other circumstances make apportionment of the RfD or POD/UF reasonable. The initial problem formulation also determines the exposure parameters chosen, the intake assumptions chosen for each route, and any environmental justice or other social issues that aid in determining the population of concern. The term "data," as used here and discussed throughout this section, refers to ambient sampling data (whether from Federal, regional, State, or area-specific studies) and not internal human exposure measurements.

4.2.2.2 Data Adequacy

In Box 3, it is necessary that adequate data exist for the relevant sources/pathways of exposure if one is to avoid using default procedures. The adequacy of data is a professional judgment for each individual chemical of concern, but EPA recommends that the minimum acceptable data for Box 3 are exposure distributions that can be used to determine, with an acceptable 95 percent confidence interval, the central tendency and high-end exposure levels for each source. In fact, distributional data may exist for some or most of the sources of exposure.

There are numerous factors to consider in order to determine whether a dataset is adequate. These include: (1) sample size (i.e., the number of data points); (2) whether the data set is a random sample representative of the target population (if not, estimates drawn from it may be biased no matter how large the sample); (3) the magnitude of the error that can be tolerated in the estimate (estimator precision); (4) the sample size needed to achieve a given precision for a given parameter (e.g., a larger sample is needed to precisely estimate an upper percentile than a mean or median value); (5) an acceptable analytical method detection limit; and (6) the functional form and variability of the underlying distribution, which determines the estimator precision (e.g., whether the distribution is normal or lognormal and whether the standard deviation is 1 or 10). Lack of information may prevent assessment of each of these factors; monitoring study reports often fail to include background information or sufficient summary statistics (and rarely the raw data) to completely characterize data adequacy. Thus, a case-by-case determination of data adequacy may be necessary.

That being stated, there are some guidelines, as presented below, that lead to a rough rule-of-thumb on what constitutes an "adequate" sample size for exposure assessment. Again, first and foremost, the representativeness of the data for the population evaluated and the analytical quality of the data must be acceptable. If so, the primary objective then becomes estimating an upper percentile (e.g., say the 90th) and a central tendency value of some exposure distribution based on a random sample from the distribution. Assuming that the distribution of exposures is unknown, a nonparametric estimate of the 90th percentile is required. The required estimate, based on a random sample of *n* observations from a target population, is obtained by ranking the data from smallest to largest and selecting the observation whose rank is 1 greater than the largest integer in the product of 0.9 times *n*. For example, in a data set of 25 points, the nonparametric estimate of the 90th percentile is the 23rd largest observation.

In addition to this point estimate, it is useful to have an upper confidence bound on the 90th percentile. To find the rank of the order statistic that gives an upper 95 percent confidence limit on the 90th percentile, the smallest value of *r* that satisfies the following formula is determined:

$$0.95 \approx \sum_{i=0}^{r-1} \binom{n}{i} 0.9^{i}\, 0.1^{n-i} \qquad \text{(Equation 4-2)}$$

where:

r	=	the rank order of the observation
n	=	the number of observations
I	=	integer from 0 to r - 1

For relatively small data sets, the above formula will lead to selecting the largest observation as the upper confidence limit on the 90th percentile. However, the problem with using the maximum is that, in many environmental datasets, the largest observation is an outlier and would provide an unrealistic upper bound on the 90th percentile. It would, therefore, be preferable if the sample size *n* were large enough so that the formula yielded the second largest observation as the confidence limit (see for example Gibbons, 1971).

This motivates establishing the following criterion for setting an "adequate" sample size: pick the smallest *n* such that the nonparametric upper 95 percent confidence limit on the 90th percentile is the second largest value. Application of the above formula with *r* set to *n*-1 yields *n* = 45 for this minimum sample size.

For the upper 95 percent confidence limit to be a useful indicator of a high-end exposure, it must not be overly conservative (too large relative to the 90th percentile). It is, therefore, of interest to estimate the expected magnitude of the ratio of the upper 95 percent confidence limit to the 90th percentile. This quantity generally cannot be computed, since it is a function of the unknown distribution. However, to get a rough idea of its value, consider the particular case of a normal distribution. If the coefficient of variation (i.e., the standard deviation divided by the mean) is between 0.5 and 2.0, the expected value of the ratio in samples of 45 will be approximately 1.17 to 1.31; i.e., the upper 95 percent confidence limit will be only about 17 to 31 percent greater than the 90th percentile on the average.

It should be noted that the nonparametric estimate of the 95 percent upper confidence limit based on the second largest value can be obtained even if the data set has only two detects (it is assumed that the two detects are greater than the detection limit associated with all non-detects). This is an argument for using nonparametric rather than parametric estimation, since use of parametric methods would require more detected values. On the other hand, if non-detects were not a problem and the underlying distribution were known, a parametric estimate of the 90th percentile would generally be more precise.

As stated above, adequacy also depends on whether the samples are relevant to and representative of the population at risk. Data may, therefore, be adequate for some decisions and inadequate for others; this determination requires some professional judgment.

If the answer to Box 3 is no, based on the above determination of adequacy, then the decision tree moves to Box 4. As suggested by the separate boxes, the available data that will be reviewed as part of Box 4 do not meet the requirements necessary for Box 3. In Box 4, any limited data that are available (in addition to information about the chemical/physical properties, uses, and environmental fate and transformation, as well as any other information that would characterize the likelihood of exposure from various media for the chemical) are evaluated to

make a qualitative determination of the relation of one exposure source to another. Although this information should always be reviewed at the outset, it is recommended that this information also be used to estimate the health-based water quality criteria. The estimate should be rather conservative (as indicated in the Decision Tree), given that it is either not based on actual monitoring data or is based on data that has been considered to be inadequate for a more accurate quantitative estimate. Therefore, greater uncertainties exist and accounting for variability is not really possible. Whether the available data are adequate and sufficiently representative will likely vary from chemical to chemical and may depend on the population of concern. If there are some data and/or other information to make a characterization of exposure, a determination can be made as to whether there are significant known or potential uses for the chemical/sources of exposure other than the source of concern (i.e., in this case, the drinking water and fish intakes relevant to developing an AWQC) that would allow one to anticipate/quantify those exposures (Box 6). If there are not, then it is recommended that 50 percent of the RfD or POD/UF can be safely apportioned to the source of concern (Box 7). While this leaves half of the RfD or POD/UF unapportioned, it is recommended as the maximum apportionment due to the lack of data needed to more accurately quantify actual or potential exposures. If the answer to the question in Box 6 is yes (there is multiple source information available for the exposures of concern), and some information is available on each source of exposure (Box 8A), apply the procedure in either Box 12 or Box 13 (depending on whether one or more criterion is relevant to the chemical), using a 50 percent ceiling (Box 8C)–again due to the lack of adequate data. If the answer to the question in Box 8A is no (there is no available information to characterize exposure), then the 20 percent default of the RfD or POD/UF is used (Box 8B).

If the answer to the question in Box 4 is no; that is, there are not sufficient data/information to characterize exposure, EPA intends to generally use the "default" assumption of 20 percent of the RfD or POD/UF (Box 5A) when deriving or revising the AWQC. It may be better to gather more data or information and re-review when this information becomes available (Box 5B). EPA has done this on occasion when resources permit the acquisition of additional data to enable better estimates of exposure instead of the default. If this is not possible, then the assumption of 20 percent of the RfD or POD/UF (Box 5A) should be used. Box 5A is likely to be used infrequently with the Exposure Decision Tree approach, given that the information described in Box 4 should be available in most cases. However, EPA intends to use 20 percent of the RfD (or POD/UF), which has also been used in past water program regulations, as the default value.

4.2.2.3 Regulatory Actions

If there are adequate data available to describe the central tendencies and high ends from each exposure source/pathway, then the levels of exposure relative to the RfD or POD/UF are compared (Box 9). If the levels of exposure for the chemical in question are not near (currently defined as greater than 80 percent), at, or in excess of the RfD or POD/UF, then a subsequent determination is made (Box 11) as to whether there is more than one health-based criterion or regulatory action relevant for the given chemical (i.e., more than one medium-specific criterion,

standard or other guidance being planned, performed or in existence for the chemical). The subtraction method is considered acceptable when only one criterion (standard, etc.) is relevant for a particular chemical. In these cases, other sources of exposure can be considered "background" and can be subtracted from the RfD (or POD/UF). When more than one criterion is relevant to a particular chemical, apportioning the RfD (or POD/UF) via the percentage method is considered appropriate to ensure that the combination of health criteria, and thus the potential for resulting exposures, do not exceed the RfD (or POD/UF).

As indicated in Section 2, for EPA's national 304(a) criteria, the RSC intake estimates of non-water exposures (e.g., non-fish dietary exposures) will be based on arithmetic mean values when data are available. The assumed body weight used in calculating the national criteria will also be based on average values. The drinking water and fish intake values are 90th percentile estimates. EPA believes that these assumptions will be protective of a majority of the population and recommends them for State and Tribal use. However, States and authorized Tribes have the flexibility to choose alternative intake rate and exposure estimate assumptions to protect specific population groups that they have chosen.

4.2.2.4 Apportionment Decisions

If the answer to the question in Box 11 is no (there is not more than one relevant medium-specific criterion/regulatory action), then the recommended method for setting a health-based water quality criterion is to utilize a subtraction calculation (Box 12). Specifically, appropriate intake values for each exposure source other than the source of concern are subtracted out. EPA will rely on average values commonly used in the Agency for food ingestion and inhalation rates, combined with mean contaminant concentration values, for calculating RSC estimates to subtract. Alternatively, contaminant concentrations could be selected based on the variability associated with those concentrations for each source. This implies that a case-by-case determination of the variability and the resulting intake chosen would be made, as each chemical evaluated can be expected to have different variations in concentration associated with each source of intake. However, EPA anticipates that the available data for most contaminants will not allow this for determination (based on past experience). Guidance addressing this possibility is addressed in the Exposure Assessment TSD. EPA does not recommend that high-end intakes be subtracted for every exposure source, since the combination may not be representative of any actually exposed population or individual. The subtraction method would also include an 80 percent ceiling and a 20 percent floor.

If the answer to the question in Box 11 is yes (there is more than one medium-specific criterion/regulation relevant), then the recommended method for setting health-based water quality criteria is to apportion the RfD or POD/UF among those sources for which health-based criteria are being set (Box 13). This is done via a percentage approach (with a ceiling and floor). This simply refers to the percentage of overall exposure contributed by an individual exposure source. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50 percent. The health-based criteria would, in turn, be set at 50 percent of the RfD or POD/UF. This method also utilizes an appropriate combination of intake values for each

exposure source based on values commonly used in the Agency for food ingestion and inhalation rates, combined with mean contaminant concentration values.

Finally, if the levels of exposure for the chemical in question are near (currently defined as greater than 80 percent), at, or in excess of the RfD or POD/UF (i.e., the answer in Box 9 is yes), then the estimates of exposures and related uncertainties, recommended apportionment (either box 12 or 13), toxicity-related information, control issues, and other information are to be presented to managers for a decision (Box 10). The high levels referred to in Box 9 may be due to one source contributing that high level (while other sources contribute relatively little) or due to more than one source contributing levels that, in combination, approach or exceed the RfD or POD/UF. Management input may be necessary due to the control issues (i.e., cost and feasibility concerns), especially when multiple criteria are at issue. In practice, risk managers are routinely a part of decisions regarding regulatory actions and will be involved with any recommended outcome of the Exposure Decision Tree or, for that matter, any alternative to the Exposure Decision Tree. However, because exposures approach or exceed the RfD or POD/UF and because the feasibility of controlling different sources of exposure are complicated issues, risk managers will especially need to be directly involved in final decisions in these circumstances.

It is emphasized here that the procedures in these circumstances are not different than the procedures when exposures are not at or above the RfD (or POD/UF). Therefore, in these cases, estimates should be performed as with Boxes 11, 12, and 13. The recommendation should be made based on health-based considerations only, just as when the chemical in question was not a Box 10 situation. If the chemical is relevant to one health criterion or regulatory action only, the other sources of exposure could be subtracted from the RfD or POD/UF to determine if there is any leftover amount for setting the criterion. If the chemical is a multiple media criteria issue, then an apportionment should be made, even though it is possible that all sources would need to be reduced. Regardless of the outcome of Box 9, all apportionments made (via the methods of Boxes 12 or 13) should include a presentation of the uncertainty in the estimate and in the RfD or POD/UF for a more complete characterization.

The process for a Box 10 situation (versus a situation that is not) differs in that the presentations for Boxes 12 and 13 are based on apportionments (following the review of available information and a determination of appropriate exposure parameters) that must address additional control issues and may result in more selective reductions. With Box 10, one or several criteria possibilities ("scenarios") could be presented for comparison along with implications of the effects of various control options. It is appropriate to present information in this manner to risk managers given the complexity of these additional control issues.

4.2.3 Additional Points of Clarification on the Exposure Decision Tree Approach for Setting AWQC

As with Box 9, if a determination is made in Box 8A (i.e., information is available to characterize exposure) that exposures are near, at, or above the RfD (or POD/UF) based on the available information, the apportionments made need to be presented to risk managers for decision. If information is lacking on some of the multiple exposure sources, then EPA would use a default of 20 percent of the RfD or POD/UF (Box 8B).

Results of both Boxes 12 and 13 rely on the 80 percent ceiling and 20 percent floor. The 80 percent ceiling was implemented to ensure that the health-based goal will be low enough to provide adequate protection for individuals whose total exposure to a contaminant is, due to any of the exposure sources, higher than currently indicated by the available data. This also increases the margin of safety to account for possible unknown sources of exposure. The 20 percent floor has been traditionally rationalized to prevent a situation where small fractional exposures are being controlled. That is, below that point, it is more appropriate to reduce other sources of exposure, rather than promulgating standards for *de minimus* reductions in overall exposure.

If it can be demonstrated that other sources and routes of exposure are not anticipated for the pollutant in question (based on information about its known/anticipated uses and chemical/physical properties), then EPA would use the 80 percent ceiling. EPA qualifies this policy with the understanding that as its policy on cumulative risk assessment continues to develop, the 80 percent RSC may prove to be underprotective.

In the cases of pollutants for which substantial data sets describing exposures across all anticipated pathways of exposure exist, and probabilistic analyses have been conducted based on those data, consideration will be given to the results of those assessments as part of the Exposure Decision Tree approach for setting AWQC.

For many chemicals, the rate of absorption from ingestion can differ substantially from absorption by inhalation. There is also available information for some chemicals that demonstrates appreciable differences in gastrointestinal absorption depending on whether the chemical is ingested from water, soil, or food. For some contaminants, the absorption of the contaminant from food can differ appreciably for plant compared with animal food products. Regardless of the apportionment approach used, EPA recommends using existing data on differences in bioavailability between water, air, soils, and different foods when estimating total exposure for use in apportioning the RfD or POD/UF. The Agency has developed such exposure estimates for cadmium (USEPA, 1994). In the absence of data, EPA will assume equal rates of absorption from different routes and sources of exposure.

4.2.4 Quantification of Exposure

When selecting contaminant concentration values in environmental media and exposure intake values for the RSC analysis, it is important to realize that each value selected (including those recommended as default assumptions in the AWQC equation) may be associated with a distribution of values for that parameter. Determining how various subgroups fall within the distributions of overall exposure and how the combination of exposure variables defines what population is being protected is a complicated and, perhaps, unmanageable task, depending on the amount of information available on each exposure factor included. Many times, the default assumptions used in EPA risk assessments are derived from the evaluation of numerous studies and are considered to generally represent a particular population group or a national average. Therefore, describing with certainty the exact percentile of a particular population that is protected with a resulting criteria is often not possible.

By and large, the AWQC are derived to protect the majority of the general population from chronic adverse health effects. However, as stated above in Section 4.1.1.1, States and authorized Tribes are encouraged to consider protecting population groups that they determine are at greater risk and, thus, would be better protected using alternative exposure assumptions. The ultimate choice of the contaminant concentrations used in the RSC estimate and the exposure intake rates requires the use of professional judgment. This is discussed in greater detail in the Exposure Assessment TSD.

4.2.5 Inclusion of Inhalation and Dermal Exposures

EPA intends to develop policy guidelines to apply to this Methodology for explicitly incorporating inhalation and dermal exposures. When estimating overall exposure to pollutants for AWQC development, EPA believes that the sources of inhalation and dermal exposures considered should include, on a case-by-case basis, both non-oral exposures from water and other inhalation and dermal sources (e.g., ambient or indoor air, soil). When the policy guidelines are completed, this Methodology will be refined to include that guidance.

A number of drinking water contaminants are volatile and thus diffuse from water into the air where they may be inhaled. In addition, drinking water is used for bathing and, thus, there is at least the possibility that some contaminants in water may be dermally absorbed. Volatilization may increase exposure via inhalation and decrease exposure via ingestion and dermal absorption. The net effect of volatilization and dermal absorption upon total exposure to volatile drinking water contaminants is unclear in some cases and varies from chemical to chemical. Dermal exposures are also important to consider for certain population groups, such as children and other groups with high soil contact.

With regard to additional non-water related exposures, it is clear that the type and magnitude of toxicity produced via inhalation, ingestion, and dermal contact may differ; that is, the route of exposure can affect absorption of a chemical and can otherwise modify its toxicity. For example, an inhaled chemical such as hydrogen fluoride may produce localized effects on the lung that are not observed (or only observed at much higher doses) when the chemical is administered orally. Also, the active form of a chemical (and principal toxicity) can be the parent compound and/or one or more metabolites. With this Methodology, EPA recommends that differences in absorption and toxicity by different routes of exposure be determined and accounted for in dose estimates and applied to the exposure assessment. EPA acknowledges that the issue of whether the doses received from inhalation and ingestion exposures are cumulative (i.e., toward the same threshold of toxicity) is complicated. Such a determination involves evaluating the chemical's physical characteristics, speciation, and reactivity. A chemical may also exhibit different metabolism by inhalation versus oral exposure and may not typically be metabolized by all tissues. In addition, a metabolite may be much more or much less toxic than the parent compound. Certainly with a systemic effect, if the chemical absorbed via different routes enters the bloodstream, then there is some likelihood that it will contact the same target organ. Attention also needs to be given to the fact that both the RfD and RfC are derived based on the administered level. Toxicologists generally believe that the effective concentration of the active form of a chemical(s) at the site(s) of action determines the toxicity. If specific differences between routes of exposure are not known, it may be reasonable to assume that the

internal concentration at the site from any route contributes as much to the same effect as any other route. A default of assuming equal absorption has often been used. However, for many of the chemicals that the Agency has reviewed, there is a substantial amount of information already known to determine differences in rates of absorption. For example, absorption is, in part, a function of blood solubility (i.e., Henry's Constant) and better estimations than the default can be made.

The RSC analyses that accompany the 2000 Human Health Methodology accommodate inclusion of inhalation exposures. Even if different target organs are involved between different routes of exposure, a conservative policy may be appropriate to keep all exposures below a certain level. A possible alternative is to set allowable levels (via an equation) such that the total of ingestion exposures over the ingestion RfD added to the total of inhalation exposures over the inhalation RfC is not greater than 1 (Note: the RfD is typically presented in mg/kg-day and the RfC is in mg/m^3). Again, EPA intends to develop guidance for this Methodology to explicitly incorporate inhalation and dermal exposures, and will refine the Methodology when that guidance is completed.

4.3 EXPOSURE FACTORS USED IN THE AWQC COMPUTATION

This section presents values for the specific exposure factors that EPA will use in the derivation of AWQC. These include human body weight, drinking water consumption rates, and fish ingestion rates.

When choosing exposure factor values to include in the derivation of a criterion for a given pollutant, EPA recommends considering values that are relevant to population(s) that is (are) most susceptible to that pollutant. In addition, highly exposed populations should be considered when setting criteria. In general, exposure factor values specific to adults and relevant to lifetime exposures are the most appropriate values to consider when determining criteria to protect against effects from long-term exposure which, by and large, the human health criteria are derived to protect. However, infants and children may have higher rates of water and food consumption per unit body weight compared with adults and also may be more susceptible to some pollutants than adults (USEPA, 1997a). There may be instances where acute or subchronic developmental toxicity makes children the population group of concern. In addition, exposure of pregnant women to certain toxic chemicals may cause developmental effects in the fetus (USEPA, 1997b). Exposures resulting in developmental effects may be of concern for some contaminants and should be considered along with information applicable to long-term health effects when setting AWQC. (See Section 3.2 for further discussion of this issue.) Short-term exposure may include multiple intermittent or continuous exposures occurring over a week or so. Exposure factor values relevant for considering chronic toxicity, as well as exposure factor values relevant for short-term exposure developmental concerns, that could result in adverse health effects are discussed in the sections below. In appropriate situations, EPA may consider developing criteria for developmental health effects based on exposure factor values specific to children or to women of childbearing age. EPA encourages States and Tribes to do the same when health risks are associated with short-term exposures.

EPA believes that the recommended exposure factor default intakes for adults in chronic exposure situations are adequately protective of the population over a lifetime. In providing additional exposure intake values for highly exposed subpopulations (e.g., sport anglers, subsistence fishers), EPA is providing flexibility for States and authorized Tribes to establish criteria specifically targeted to provide additional protection using adjusted values for exposure parameters for body weight, drinking water intake, and fish consumption. The exposure factor values provided for women of childbearing age and children would only be used in the circumstances indicated above.

Each of the following sections recommends exposure parameter values for use in developing AWQC. These are based on both science policy decisions that consider the best available data, as well as risk management judgments regarding the overall protection afforded by the choice in the derivation of AWQC. These will be used by EPA to derive new, or revise existing, 304(a) national criteria.

4.3.1 Human Body Weight Values for Dose Calculations

The source of data for default human body weights used in deriving the AWQC is the third *National Health and Nutrition Examination Survey* (NHANES III). NHANES III represents a very large interview and examination endeavor of the National Center for Health Statistics (NCHS) and included participation from the Centers for Disease Control (CDC). The NHANES III was conducted on a nationwide probability sample of over 30,000 persons from the civilian, non-institutionalized population of the United States. The survey began in October 1988 and was completed in October 1994 (WESTAT, 2000; McDowell, 2000). Body weight data were taken from the NHANES III Examination Data File. Sampling weights were applied to all persons examined in the Mobile Examination Centers (MECs) or at home, as was recommended by the NHANES data analysts (WESTAT, 2000).

The NHANES III survey has numerous strengths and very few weaknesses. Its primary strengths are the national representativeness, large sample size, and precise estimates due to this large sample size. Another strength is its high response rate; the examination rate was 73 percent overall, 89 percent for children under 1 year old, and approximately 85 percent for children 1 to 5 years old (McDowell, 2000). Interview response rates were even higher, but the body weight data come from the NHANES examinations; that is, all body weights were carefully measured by survey staff, rather than the use of self-reported body weights. The only significant potential weakness of the NHANES data is the fact that the data are now between 6 and 12 years old. Given that there were upward trends in body weight from NHANES II to NHANES III, and that NCHS has indicated the prevalence of overweight people increased in all age groups, the data could underestimate current body weights if that trend has continued (WESTAT, 2000).

The NHANES III collected standard body measurements of sample subjects, including height and weight, that were made at various times of the day and in different seasons of the year. This technique was used because one’s weight may vary between winter and summer and may fluctuate with recency of food and water intake and other daily activities (McDowell, 2000).

As with the other exposure assumptions, States and authorized Tribes are encouraged to use alternative body weight assumptions for population groups other than the general population and to use local or regional data over default values as more representative of their target population group(s).

4.3.1.1 Rate Protective of Human Health from Chronic Exposure

EPA recommends maintaining the default body weight of 70 kg for calculating AWQC as a representative average value for both male and female adults. As previously indicated, exposure factor values specific to adults are recommended to protect against effects from long-term exposure. The value of 70 kg is based on the following information. In the analysis of the NHANES III database, median and mean values for female adults 18-74 years old are 65.8 and 69.5 kg, respectively (WESTAT, 2000). For males in the same age range, the median and mean values are 79.9 and 82.1 kg, respectively. The mean body weight value for men and women ages 18 to 74 years old from this survey is 75.6 kg (WESTAT, 2000). This mean value is higher than the mean value for adults ages 20-64 years old of 70.5 kg from a study by the National Cancer Institute (NCI) which primarily measured drinking water intake (Ershow and Cantor, 1989). The NCI study is described in the subsection on Drinking Water Intake Rates that follows (Section 4.3.2). The value from the NHANES III database is also higher than the value given in the revised EPA *Exposure Factors Handbook* (USEPA, 1997b), which recommends 71.8 kg for adults, based on the older NHANES II data. The Handbook also acknowledges the commonly used 70 kg value and encourages risk assessors to use values which most accurately reflect the exposed population. However, the point is also made that the 70 kg value is used in the derivation of cancer slope factors and unit risks that appear in IRIS. Consistency is advocated between the dose-response relationship and exposure factors assumed. Therefore, if a value higher than 70 kg is used, the assessor needs to adjust the dose-response relationship as described in the Appendix to Chapter 1, Volume 1 of the Handbook (USEPA, 1997b).

4.3.1.2 Rates Protective of Developmental Human Health Effects

As noted above, pregnant women may represent a more appropriate population for which to assess risks from exposure to chemicals in ambient waters in some cases, because of the potential for developmental effects in fetuses. In these cases, body weights representative of women of childbearing age may be appropriate to adequately protect offspring from such health effects. To determine a mean body weight value appropriate to this population, separate body weight values for women in individual age groups within the range of 15 to 44 years old were analyzed from the NHANES III data (WESTAT, 2000). The resulting median and mean body weight values are 63.2 and 67.3 kg, respectively. Ershow and Cantor (1989) present body weight values specifically for pregnant women included in the survey; median and mean weights are 64.4 and 65.8 kilograms, respectively. Ershow and Cantor (1989), however, do not indicate the ages of these pregnant women. Based on this information for women of childbearing age and pregnant women, EPA recommends use of a body weight value of 67 kg in cases where pregnant women are the specific population of concern and the chemical of concern exhibits reproductive and/or developmental effects (i.e., the critical effect upon which the RfD or POD/UF is based). Using the 67 kg assumption would result in lower (more protective) criteria than criteria based on 70 kg.

As discussed earlier, because infants and children generally have a higher rate of water and food consumption per unit body weight compared with adults, a higher intake rate per unit body weight may be needed when comparing estimated exposure doses with critical doses when RfDs are based on health effects in children. To calculate intake rates relevant to such effects, the body weight of children should be used. As with the default body weight for pregnant women, EPA is not recommending the development of additional AWQC (i.e., similar to drinking water health advisories) that focus on acute or short-term effects, since these are not seen routinely as having a meaningful role in the water quality criteria program. However, there may be circumstances where the consideration of exposures for these groups is warranted. Although the AWQC generally are based on chronic health effects data, they are intended to also be protective with respect to adverse effects that may reasonably be expected to occur as a result of elevated shorter-term exposures. EPA acknowledges this as a potential course of action and is, therefore, recommending these default values which EPA would consider in an appropriate circumstance and for States and authorized Tribes to utilize in such situations.

EPA is recommending an assumption of 30 kg as a default child's body weight to calculate AWQC to provide additional protection for children when the chemical of concern indicates health effects in children are of predominant concern (i.e., test results show children are more susceptible due to less developed immune systems, neurological systems, and/or lower body weights). The value is based on the mean body weight value of 29.9 kg for children ages 1 to14 years old, which combines body weight values for individual age groups within this larger group. The mean value is based on body weight information from NHANES III for individual-year age groups between one and 14 years old (WESTAT, 2000). A mean body weight of 28 kg is obtained using body weight values from Ershow and Cantor (1989) for five age groups within this range of 0-14 years and applying a weighting method for different ages by population percentages from the U.S. Bureau of the Census. The 30 kg assumption is also consistent with the age range for children used with the estimated fish intake rates. Unfortunately, fish intake rates for finer age group divisions are not possible due to the limited sampling base from the fish intake survey; there is limited confidence in calculated values (e.g., the mean) for such fine age groups. Given this limitation, the broad age category of body weight for children is suitable for use with the default fish intake assumption.

Given the hierarchy of preferences regarding the use of fish intake information (see Section 4.3.3), States may have more comprehensive data and prefer to target a more narrow, younger age group. If States choose to specifically evaluate toddlers, EPA recommends using 13 kg as a default body weight assumption for children ages 1 to 3 years old. The median and mean values of body weight for children 1 to 3 years old are 13.2 and 13.1 kg, respectively, based on an analysis of the NHANES III database (WESTAT, 2000). The NHANES III median and mean values for females between 1 and 3 years old are 13.0 and 12.9 kg, respectively, and are 13.4 and 13.4 kg for males, respectively. Median and mean body weight values from the earlier Ershow and Cantor (1989) study for children ages 1 to 3 years old were 13.6 and 14.1 kg, respectively. Finally, if infants are specifically evaluated, EPA recommends a default body weight of 7 kg based on the NHANES III analysis. Median and mean body weights for both male and female infants (combined) 2 months old were 6.3 and 6.3 kg, respectively, and for infants 3 months old were 7.0 and 6.9 kg, respectively. With the broader age category of males and females 2 to 6 months old, median and mean body weights were 7.4 and 7.4 kg, respectively. The NHANES

analysis did not include infants under 2 months of age. Although EPA is not recommending body weight values for newborns, the NCHS National Vital Statistics Report indicates that, for 1997, the median birth weight ranged from 3 to 3.5 kg, according to WESTAT (2000).

Body weight values for individual ages within the larger range of 0-14 years are listed in the Exposure Assessment TSD for those States and authorized Tribes who wish to use body weight values for these individual groups. States and Tribes may wish to consider certain general developmental ages (e.g., infants, pre-adolescents, etc.), or certain specific developmental landmarks (e.g., neurological development in the first four years), depending on the chemical of concern. EPA encourages States and authorized Tribes to choose a body weight intake from the tables presented in the TSD, if they believe a particular age subgroup is more appropriate.

4.3.2 Drinking Water Intake Rates

The basis for the drinking water intake rates (also for the fish intake rates presented in Section 4.3.3) is the 1994-96 Continuing Survey of Food Intake by Individuals *(*CSFII) conducted by the U.S. Department of Agriculture (USDA, 1998). The CSFII survey collects dietary intake information from nationally representative samples of non-institutionalized persons residing in United States households. Households in these national surveys are sampled from the 50 states and the District of Columbia. Each survey collects daily consumption records for approximately 10,000 food codes across nine food groups. These food groups are (1) milk and milk products; (2) meat, poultry, and fish; (3) eggs; (4) dry beans, peas, legumes, nuts, and seeds; (5) grain products; (6) fruit; (7) vegetables; (8) fats, oils, and salad dressings; and (9) sweets, sugars, and beverages. The survey also asks each respondent how many fluid ounces of plain drinking water he or she drank during each of the survey days. In addition, the CSFII collects household information, including the source of plain drinking water, water used to prepare beverages, and water used to prepare foods. Data provide "up-to-date information on food intakes by Americans for use in policy formation, regulation, program planning and evaluation, education, and research." The survey is "the cornerstone of the National Nutritional Monitoring and Related Research Program, a set of related federal activities intended to provide regular information on the nutritional status of the United States population" (USDA, 1998).

The 1994-96 CSFII was conducted according to a stratified, multi-area probability sample organized using estimates of the 1990 United States population. Stratification accounted for geographic location, degree of urbanization, and socioeconomics. Each year of the survey consisted of one sample with oversampling for low-income households.

Survey participants provided two non-consecutive, 24-hour days of dietary data. Both days' dietary recall information was collected by an in-home interviewer. Interviewers provided participants with an instructional booklet and standard measuring cups and spoons to assist them in adequately describing the type and amount of food ingested. If the respondent referred to a cup or bowl in their own home, a 2-cup measuring cup was provided to aid in the calculation of the amount consumed. The sample person could fill their own bowl or cup with water to represent the amount eaten or drunk, and the interviewer could then measure the amount consumed by pouring it into the 2-cup measure. The Day 2 interview occurred three to 10 days

after the Day 1 interview, but not on the same day of the week. The interviews allowed participants "three passes" through the daily intake record to maximize recall (USDA, 1998). Proxy interviews were conducted for children aged six and younger and sampled individuals unable to report due to mental or physical limitations. The average questionnaire administration time for Day 1 intake was 30 minutes, while Day 2 averaged 27 minutes.

Two days of dietary recall data were provided by 15,303 individuals across the three survey years. This constitutes an overall two-day response rate of 75.9 percent. Survey weights were corrected by the USDA for nonresponse.

All three 1994-96 CSFII surveys are multistage, stratified-cluster samples. Sample weights, which project the data from a sampled individual to the population, are based on the probability of an individual being sampled at each stage of the sampling design. The sample weights associated with each individual reporting two days of consumption data were adjusted to correct for nonresponse bias.

The 1994-96 CSFII surveys have advantages and limitations for estimating per capita water (or fish) consumption. The primary advantage of the CSFII surveys is that they were designed and conducted by the USDA to support unbiased estimation of food consumption across the population in the United States and the District of Columbia. Second, the survey is designed to record daily intakes of foods and nutrients and support estimation of food consumption.

One limitation of the 1994-96 CSFII surveys is that individual food consumption data were collected for only two days–a brief period which does not necessarily depict "usual intake." Usual dietary intake is defined as "the long-run average of daily intakes by an individual." Upper percentile estimates may differ for short-term and longer-term data because short-term food consumption data tend to be inherently more variable. It is important to note, however, that variability due to duration of the survey does not result in bias of estimates of overall mean consumption levels. Also, the multistage survey design does not support interval estimates for many of the subpopulations of interest because of sparse representation in the sample. Subpopulations with sparse representation include Native Americans on reservations and certain ethnic groups. While these individuals are participants in the survey, they are not present in sufficient numbers to support consumption estimates.

Despite these limitations, the CSFII is considered one of the best sources of current information on consumption of water and fish-containing foods. The objective of estimating per capita water and fish consumption by the United States population is compatible with the statistical design and scope of the CSFII survey.

4.3.2.1 Rate Protective of Human Health from Chronic Exposure

EPA recommends maintaining the default drinking water intake rate of 2 L/day to protect most consumers from contaminants in drinking water. EPA believes that the 2 L/day assumption is representative of a majority of the population over the course of a lifetime. EPA also notes that there is comparatively little variability in water intake within the population compared with

fish intake (i.e., drinking water intake varies, by and large, by about a three-fold range, whereas fish intake can vary by 100-fold). EPA believes that the 2 L/day assumption continues to represent an appropriate risk management decision. The results of the 1994-96 CSFII analysis indicate that the arithmetic mean, 75th, and 90th percentile values for adults 20 years and older are 1.1, 1.5, and 2.2 L/day, respectively (USEPA, 2000a). The 2 L/day value represents the 86th percentile for adults. These values can also be compared to data from an older National Cancer Institute (NCI) study, which estimated intakes of tapwater in the United States based on the USDA's 1977-78 Nationwide Food Consumption Survey (NFCS). The arithmetic mean, 75th, and 90th percentile values for adults 20 - 64 years old were 1.4, 1.7, and 2.3 L/day, respectively (Ershow and Cantor, 1989). The 2 L/day value represents the 88th percentile for adults from the NCI study.

The 2 L/day assumption was used with the original 1980 AWQC National Guidelines and has also been used in EPA's drinking water program. EPA believes that the newer studies continue to support the use of 2 L/day as a reasonable and protective consumption rate that represents the intake of most water consumers in the general population. However, individuals who work or exercise in hot climates could have water consumption rates significantly above 2 L/day, and EPA believes that States and Tribes should consider regional or occupational variations in water consumption.

4.3.2.2 Rates Protective of Developmental Human Health Effects

Based on the 1994-96 CSFII study data, EPA also recommends 2 L/day for women of childbearing age. The analysis for women of childbearing age (ages 15-44) indicate mean, 75th, and 90th percentile values of 0.9, 1.3, and 2.0 L/day, respectively. These rates compare well with those based on an analysis of tapwater intake by pregnant and lactating women by Ershow et al. (1991), based on the older USDA data, for women ages 15-49. Arithmetic mean, 75th and 90th percentile values were 1.2, 1.5, and 2.2 L/day, respectively, for pregnant women. For lactating women, the arithmetic mean, 75th and 90th percentile values were 1.3, 1.7, and 1.9 L/day, respectively.

As noted above, because infants and children have a higher daily water intake per unit body weight compared with adults, a water consumption rate measured for children is recommended for use when RfDs are based on health effects in children. Use of this water consumption rate should result in adequate protection for infants and children when setting criteria based on health effects for this target population. EPA recommends a drinking water intake of 1 L/day to, again, represent a majority of the population of children that consume drinking water. The results of the 1994-96 CSFII analysis indicate that for children from 1 to 10 years of age, the arithmetic mean, 75th, and 90th percentile values are 0.4, 0.6, and 0.9 L/day, respectively (USEPA, 2000a). The 1 L/day value represents the 93rd percentile for this group. The arithmetic mean, 75th, and 90th percentile values for smaller children, ages 1 to 3 years, are 0.3, 0.5, and 0.7 L/day, respectively. The 1 L/day value represents the 97th percentile of the group ages 1 to 3 years old. For the category of infants under 1 year of age, the arithmetic mean, 75th, and 90th percentile values are 0.3, 0.7, and 0.9 L/day, respectively. These data can similarly be compared to those of the older National Cancer Institute (NCI) study. The arithmetic mean, 75th, and 90th percentile values for children 1 to 10 years old were 0.74, 0.96, and 1.3 L/day,

respectively. The mean, 75th, and 90th percentile values for children 1 to 3 years old in the NCI study were 0.6, 0.8, and 1.2 L/day, respectively. Finally, the mean, 75th, and 90th percentile values for infants less than 6 months old were 0.3, 0.3, and 0.6 L/day, respectively (Ershow and Cantor, 1989).

4.3.2.3 Rates Based on Combining Drinking Water Intake and Body Weight

As an alternative to considering body weight and drinking water intake rates separately, EPA is providing rates based on intake per unit body weight data (in units of ml/kg) in the Exposure Assessment TSD, with additional discussion on their use. These rates are based on self-reported body weights from the CSFII survey respondents for the 1994-96 data. While EPA intends to derive or revise national default criteria on the separate intake values and body weights, in part due to the strong input received from its State stakeholders, the ml/kg-BW/day values are provided in the TSD for States or authorized Tribes that prefer their use. It should be noted that in their 1993 review, EPA's Science Advisory Board (SAB) felt that using drinking water intake rate assumptions on a per unit body weight basis would be more accurate, but did not believe this change would appreciably affect the criteria values (USEPA, 1993).

4.3.3 Fish Intake Rates

The basis for the fish intake rates is the 1994-96 CSFII conducted by the USDA, and described above in Section 4.3.2.

4.3.3.1 Rates Protective of Human Health from Chronic Exposure

EPA recommends a default fish intake rate of 17.5 grams/day to adequately protect the general population of fish consumers, based on the 1994 to 1996 data from the USDA's CSFII Survey. EPA will use this value when deriving or revising its national 304(a) criteria. This value represents the 90th percentile of the 1994-96 CSFII data. This value also represents the uncooked weight estimated from the CSFII data, and represents intake of freshwater and estuarine finfish and shellfish only. For deriving AWQC, EPA has also considered the States' and Tribes' needs to provide adequate protection from adverse health effects to highly exposed populations such as recreational and subsistence fishers, in addition to the general population. Based on available studies that characterize consumers of fish, recreational fishers and subsistence fishers are two distinct groups whose intake rates may be greater than the general population. It is, therefore, EPA's decision to discuss intakes for these two groups, in addition to the general population.

EPA recommends default fish intake rates for recreational and subsistence fishers of 17.5 grams/day and 142.4 grams/day, respectively. These rates are also based on uncooked weights for fresh/estuarine finfish and shellfish only. However, because the level of fish intake in highly exposed populations varies by geographical location, EPA suggests a four preference hierarchy for States and authorized Tribes to follow when deriving consumption rates that encourages use of the best local, State, or regional data available. A thorough discussion of the development of this policy method and relevant data sources is contained in the Exposure Assessment TSD. The hierarchy is also presented here because EPA strongly emphasizes that States and authorized

Tribes should consider developing criteria to protect highly exposed population groups and use local or regional data over the default values as more representative of their target population group(s). The four preference hierarchy is: (1) use of local data; (2) use of data reflecting similar geography/population groups; (3) use of data from national surveys; and (4) use of EPA's default intake rates.

The recommended four preference hierarchy is intended for use in evaluating fish intake from fresh and estuarine species only. Therefore, to protect humans who additionally consume marine species of fish, the marine portion should be considered an *other source of exposure* when calculating an RSC for dietary intake. Refer to the Exposure Assessment TSD for further discussion. States and Tribes need to ensure that when evaluating overall exposure to a contaminant, marine fish intake is not double-counted with the other dietary intake estimate used. Coastal States and authorized Tribes that believe accounting for total fish consumption (i.e., fresh/estuarine and marine species) is more appropriate for protecting the population of concern may do so, provided that the marine intake component is not double-counted with the RSC estimate. Tables of fish consumption intakes based on the CSFII in the TSD provide rates for fresh/estuarine species, marine species, and total (combined) values to facilitate this option for States and Tribes. Throughout this section, the terms "fish intake" or "fish consumption" are used. These terms refer to the consumption of finfish and shellfish, and the CSFII survey includes both. States and Tribes should ensure that when selecting local or regionally-specific studies, both finfish and shellfish are included when the population exposed are consumers of both types.

EPA's first preference is that States and authorized Tribes use the results from fish intake surveys of local watersheds within the State or Tribal jurisdiction to establish fish intake rates that are representative of the defined populations being addressed for the particular waterbody. Again, EPA recommends that data indicative of fresh/estuarine species only be used which is, by and large, most appropriate for developing AWQC. EPA also recommends the use of uncooked weight intake values, which is discussed in greater detail with the fourth preference. States and authorized Tribes may use either high-end values (such as the 90^{th} or 95^{th} percentile values) or average values for an identified population that they plan to protect (e.g., subsistence fishers, sport fishers, or the general population). EPA generally recommends that arithmetic mean values should be the lowest value considered by States or Tribes when choosing intake rates for use in criteria derivation. When considering geometric mean (median) values from fish consumption studies, States and authorized Tribes need to ensure that the distribution is based on survey respondents who reported consuming fish because surveys based on both consumers and nonconsumers can often result in median values of zero. If a State or Tribe chooses values (whether the central tendency or high-end values) from studies that particularly target high-end consumers, these values should be compared to high-end fish intake rates for the general population to make sure that the high-end consumers within the general population would be protected by the chosen intake rates. EPA believes this is a reasonable procedure and is also consistent with the recent Great Lakes Water Quality Initiative (known as the "GLI") (USEPA, 1995). States and authorized Tribes may wish to conduct their own surveys of fish intake, and EPA guidance is available on methods to conduct such studies in *Guidance for Conducting Fish and Wildlife Consumption Surveys* (USEPA, 1998). Results from broader geographic regions in which the State or Tribe is located can also be used, but may not be as applicable as results from

local watersheds. Since such studies would ultimately form the basis of a State or Tribe's AWQC, EPA would review any surveys of fish intake for consistency with the principles of EPA's guidance as part of the Agency's review of water quality standards under Section 303(c).

If surveys conducted in the geographic area of the State or Tribe are not available, EPA's second preference is that States and authorized Tribes consider results from existing fish intake surveys that reflect similar geography and population groups (e.g., from a neighboring State or Tribe or a similar watershed type), and follow the method described above regarding target values to derive a fish intake rate. Again, EPA recommends the use of uncooked weight intake values and the use of fresh/estuarine species data only. Results of existing local and regional surveys are discussed in greater detail in the TSD.

If applicable consumption rates are not available from local, State, or regional surveys, EPA's third preference is that States and authorized Tribes select intake rate assumptions for different population groups from national food consumption surveys. EPA has analyzed one such national survey, the 1994-96 CSFII. As described in Section 4.3.2, this survey, conducted annually by the USDA, collects food consumption information from a probability sample of the population of all 50 states. Respondents to the survey provide two days of dietary recall data. A detailed description of the combined 1994-96 CSFII survey, the statistical methodology, and the results and uncertainties of the EPA analyses are provided in a separate EPA report (USEPA, 2000b). The Exposure Assessment TSD for this Methodology presents selected results from this report including point and interval estimates of combined finfish and shellfish consumption for the mean, 50^{th} (median), 90^{th}, 95^{th}, and 99^{th} percentiles. The estimated fish consumption rates are by fish habitat (i.e., freshwater/estuarine, marine and all habitats) for the following population groups: (1) all individuals; (2) individuals age 18 and over; (3) women ages 15-44; and (4) children age 14 and under. Three kinds of estimated fish consumption rates are provided: (1) per capita rates (i.e., rates based on consumers and nonconsumers of fish from the survey period– refer to the TSD for further discussion); (2) consumers-only rates (i.e., rates based on respondents who reported consuming finfish or shellfish during the two-day reporting period); and (3) per capita consumption by body weight (i.e., per capita rates reported as milligrams of fish per kilogram of body weight per day).

EPA's fourth preference is that States and authorized Tribes use as fish intake assumptions the following default rates, based on the 1994-96 CSFII data, that EPA believes are representative of fish intake for different population groups: 17.5 grams/day for the general adult population and sport fishers, and 142.4 grams/day for subsistence fishers. These are risk management decisions that EPA has made after evaluating numerous fish intake surveys. These values represent the uncooked weight intake of freshwater/estuarine finfish and shellfish. As with the other preferences, EPA requests that States and authorized Tribes routinely consider whether there is a substantial population of sport fishers or subsistence fishers when developing site-specific estimates, rather than automatically basing them on the typical individual. Because the combined 1994-96 CSFII survey is national in scope, EPA will use the results from this survey to estimate fish intake for deriving national criteria. EPA has recognized the data gaps and uncertainties associated with the analysis of the 1994-96 CSFII survey in the process of making its default recommendations. The estimated mean of freshwater and estuarine fish ingestion for adults is 7.50 grams/day, and the median is 0 grams/day. The estimated 90^{th}

percentile is 17.53 grams/day; the estimated 95th percentile is 49.59 grams/day; and the estimated 99th percentile is 142.41 grams/day. The median value of 0 grams/day may reflect the portion of individuals in the population who never eat fish as well as the limited reporting period (2 days) over which intake was measured. By applying as a default 17.5 grams/day for the general adult population, EPA intends to select an intake rate that is protective of a majority of the population (again, the 90th percentile of consumers and nonconsumers according to the 1994-96 CSFII survey data). Trophic level breakouts are: TL2 = 3.8 grams/day; TL3 = 8.0 grams/day; and TL4 = 5.7 grams/day. EPA further considers 17.5 grams/day to be indicative of the average consumption among sport fishers based on averages in the studies reviewed, which are presented in the Exposure Assessment TSD. Similarly, EPA believes that the assumption of 142.4 grams/day is within the range of average consumption estimates for subsistence fishers based on the studies reviewed. Experts at the 1992 National Workshop that initiated the effort to revise this Methodology acknowledged that the national survey high-end values are representative of average rates for highly exposed groups such as subsistence fishermen, specific ethnic groups, or other highly exposed people. EPA is aware that some local and regional studies indicate greater consumption among Native American, Pacific Asian American, and other subsistence consumers, and recommends the use of those studies in appropriate cases, as indicated by the first and second preferences. Again, States and authorized Tribes have the flexibility to choose intake rates higher than an average value for these population groups. If a State or authorized Tribe has not identified a separate well-defined population of high-end consumers and believes that the national data from the 1994-96 CSFII are representative, they may choose these recommended rates.

As indicated above, the default intake values are based on the uncooked weights of the fish analyzed. There has been some question regarding whether to use cooked or uncooked weights of fish intake for deriving the AWQC. Studies show that, typically, with a filet or steak of fish, the weight loss in cooking is about 20 percent; that is, the uncooked weight is approximately 20 percent higher (Jacobs et al., 1998). This obviously means that using uncooked weights results in a slightly higher intake rate and slightly more stringent AWQC. In researching consumption surveys for this proposal, EPA has found that some surveys have reported rates for cooked fish, others have reported uncooked rates, and many more are unclear as to whether cooked or uncooked rates are used. The basis of the CSFII survey was prepared or *as consumed* intakes; that is, the survey respondents estimated the weight of fish that they consumed. This was also true with the GLI (which was specifically based on studies describing consumption rates of cooked fish) and, by and large, cooked fish is what people consume. However, EPA's *Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories* recommends analysis and advisories based on uncooked fish (USEPA, 1997a). EPA considered the potential confusion over the fact that the uncooked weights are used in the fish advisory program. Further, the measures of a contaminant in fish tissue samples that are applicable to compliance monitoring and the permitting program are related to the uncooked weights. The choice of intakes is also complicated by factors such as the effect of the cooking process, the different parts of a fish where a chemical may accumulate, and the method of preparation.

After considering all of the above (in addition to public input received), EPA will derive its national default criteria based on the uncooked weight fish intakes. The Exposure

Assessment TSD provides additional guidance on site-specific modifications. Specifically, an alternate approach is described for calculating AWQC with the *as consumed* weight–which is more directly associated with human exposure and risk–and then adjusting the value by the approximate 20 percent loss to an uncooked equivalent (thereby representing the same relative risk as the *as consumed* value). This approach results in a different AWQC value (than using the uncooked weights) and represents a more direct translation of the *as consumed* risk to the uncooked equivalent. However, EPA understands that it is more scientifically rigorous and may be too intensive of a process for States and Tribes to rely on. The option is presented in the TSD to offer States and authorized Tribes greater flexibility with their water quality standards program.

The default fish intake values also reflect specific designations of species classified in accordance with information regarding the life history of the species or based on landings information form the National Marine Fisheries Service. Most significantly, salmon has been reclassified from a freshwater/estuarine species to a marine species. As marine harvested salmon represents approximately 99 percent of salmon consumption in the 1994-96 CSFII Survey, removal reduces the overall fresh/estuarine fish consumption rate by 13 percent. Although they represent a very small percentage of freshwater/estuarine intake, land-locked and farm-raised salmon consumed by 1994-96 CSFII respondents are still included. The rationale for the default intake species designations is explained in the Exposure Assessment TSD. Once again, EPA emphasizes the flexibility for States and authorized Tribes to use alternative assumptions based on local or regional data to better represent their population groups of concern.

4.3.3.2 Rates Protective of Developmental Human Health Effects

Exposures resulting in health effects in children or developmental effects in fetuses may be of primary concern. As discussed at the beginning of this section on exposure factors used, in a situation where acute or sub-chronic toxicity and exposure are the basis of an RfD (or POD/UF), EPA will consider basing its national default criteria on children or women of childbearing age, depending on the target population at greatest risk. EPA recommends that States and authorized Tribes use exposure factors for children or women of childbearing age in these situations. As stated previously, EPA is not recommending the development of additional AWQC but is acknowledging that basing a criterion on these population groups is a potential course of action and is, therefore, recommending the following default intake rates for such situations.

EPA's preferences for States and authorized Tribes in selecting values for intake rates relevant for children is the same as that discussed above for establishing values for average daily consumption rates for chronic effects; i.e., in decreasing order of preference, results from fish intake surveys of local watersheds, results from existing fish intake surveys that reflect similar geography and population groups, the distribution of intake rates from nationally based surveys (e.g., the CSFII), or lastly, the EPA default rates. When an RfD is based on health effects in children, EPA recommends a default intake rate of 156.3 grams/day for assessing those contaminants that exhibit adverse effects. This represents the 90th percentile consumption rate for actual consumers of freshwater/estuarine finfish and shellfish for children ages 14 and under using the combined 1994 to 1996 results from the CSFII survey. The value was calculated based

on data for only those children who ate fish during the 2-day survey period, and the intake was averaged over the number of days during which fish was actually consumed. EPA believes that by selecting the data for consumers only, the 90th percentile is a reasonable intake rate to approximate consumption of fresh/estuarine finfish and shellfish within a short period of time for use in assessments where adverse effects in children are of primary concern. As discussed previously, EPA will use a default body weight of 30 kg to address potential acute or subchronic effects from fish consumption by children. EPA is also providing these default intake values for States and authorized Tribes that choose to provide additional protection when developing criteria that they believe should be based on health effects in children. This is consistent with the rationale in the recent GLI (USEPA, 1995) and is an approach that EPA believes is reasonable. Distributional information on intake values relevant for assessing exposure when health effects to children are of concern is presented in the Exposure Assessment TSD.

There are also cases in which pregnant women may be the population of most concern, due to the possibility of developmental effects that may result from exposures of the mother to toxicants. In these cases, fish intake rates specific to females of childbearing age are most appropriate when assessing exposures to developmental toxicants. When an RfD is based on developmental toxicity, EPA proposes a default intake rate of 165.5 grams/day for assessing exposures for women of childbearing age from contaminants that cause developmental effects. This is equivalent to the 90th percentile consumption rate for actual consumers of freshwater/ estuarine finfish and shellfish for women ages 15 to 44 using the combined 1994 to1996 results from the CSFII survey. As with the rate for children, this value represents only those women who ate fish during the 2-day survey period. As discussed previously, EPA will use a default body weight of 67 kg for women of childbearing age.

4.3.3.3 Rates Based on Combining Fish Intake and Body Weight

As with the drinking water intake values, EPA is providing values for fish intake based on a per unit body weight basis (in units of mg/kg) in the Exposure Assessment TSD. These rates use the self-reported body weights of the 1994-96 CSFII survey. Again, while EPA intends to derive or revise national default criteria on the separate intake values and body weights, the mg/kg-BW/day values are provided in the TSD for States or authorized Tribes that prefer their use.

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5. BIOACCUMULATION

5.1 INTRODUCTION

Aquatic organisms can accumulate certain chemicals in their bodies when exposed to these chemicals through water, their diet, and other sources. This process is called bioaccumulation. The magnitude of bioaccumulation by aquatic organisms varies widely depending on the chemical but can be extremely high for some highly persistent and hydrophobic chemicals. For such highly bioaccumulative chemicals, concentrations in aquatic organisms may pose unacceptable human health risks from fish and shellfish consumption even when concentrations in water are too low to cause unacceptable health risks from drinking water consumption alone. These chemicals may also biomagnify in aquatic food webs, a process whereby chemical concentrations increase in aquatic organisms of each successive trophic level due to increasing dietary exposures (e.g., increasing concentrations from algae, to zooplankton, to forage fish, to predatory fish).

In order to prevent harmful exposures to waterborne chemicals through the consumption of contaminated fish and shellfish, national 304(a) water quality criteria for the protection of human health must address the process of chemical bioaccumulation in aquatic organisms. For deriving national 304(a) criteria to protect human health, EPA accounts for potential bioaccumulation of chemicals in fish and shellfish through the use of national bioaccumulation factors (BAFs). A national BAF is a ratio (in L/kg) that relates the concentration of a chemical in water to its expected concentration in commonly consumed aquatic organisms in a specified trophic level. An illustration of how national BAFs are used in the derivation of 304(a) criteria for carcinogens using linear low-dose extrapolation is shown in the following equation:

$$AWQC = RSD \bullet \left(\frac{BW}{DI + \sum_{i=2}^{4} (FI_i \bullet BAF_i)} \right) \qquad \text{(Equation 5-1)}$$

where:

RSD	=	Risk specific dose (mg/kg-day)
BW	=	Human body weight (kg)
DI	=	Drinking water intake (L/day)
FI_i	=	Fish intake at trophic level I, where I=2, 3, and 4;
BAF_i	=	National bioaccumulation factor at trophic level I, where I=2, 3, and 4

The purpose of this chapter is to present EPA's recommended methodology for deriving national bioaccumulation factors for setting national 304(a) water quality criteria to protect human health. A detailed scientific basis of the recommended national BAF methodology is provided in the Bioaccumulation TSD. While the methodology detailed in this chapter is

intended to be used by EPA for deriving national BAFs, EPA encourages States and authorized Tribes to derive BAFs that are specific to certain regions or waterbodies, where appropriate. Guidance to States and authorized Tribes for deriving site-specific BAFs is provided in the Biaccumulation TSD.

5.1.1 Important Bioaccumulation and Bioconcentration Concepts

Several attributes of the bioaccumulation process are important to understand when deriving national BAFs for use in setting national 304(a) criteria. First, the term "bioaccumulation" refers to the uptake and retention of a chemical by an aquatic organism from all surrounding media (e.g., water, food, sediment). The term "bioconcentration" refers to the uptake and retention of a chemical by an aquatic organism from water only. For some chemicals (particularly those that are highly persistent and hydrophobic), the magnitude of bioaccumulation by aquatic organisms can be substantially greater than the magnitude of bioconcentration. Thus, an assessment of bioconcentration alone would underestimate the extent of accumulation in aquatic biota for these chemicals. Accordingly, EPA's guidelines presented in this chapter emphasize the measurement of chemical bioaccumulation by aquatic organisms, whereas EPA's 1980 Methodology emphasized the measurement of bioconcentration.

Another noteworthy aspect of bioaccumulation process is the issue of steady-state conditions. Specifically, both bioaccumulation and bioconcentration can be viewed simply as the result of competing rates of chemical uptake and depuration (chemical loss) by an aquatic organism. The rates of chemical uptake and depuration can be affected by various factors including the properties of the chemical, the physiology of the organism in question, water quality and other environmental conditions, ecological characteristics of the waterbody (e.g., food web structure), and the concentration and loadings history of the chemical. When the rates of chemical uptake and depuration are equal, tissue concentrations remain constant over time and the distribution of the chemical between the organism and its source(s) is said to be at steady-state. For constant chemical exposures and other conditions, the steady-state concentration in the organism represents the highest accumulation potential of the chemical in that organism under those conditions. The time required for a chemical to achieve steady state has been shown to vary according to the properties of the chemical and other factors. For example, some highly hydrophobic chemicals can require long periods of time to reach steady state between environmental compartments (e.g., many months), while highly hydrophilic chemicals usually reach steady-state relatively quickly (e.g., hours to days).

Since national 304(a) criteria for the protection of human health are typically designed to protect humans from harmful lifetime or long-term exposures to waterborne contaminants, the assessment of bioaccumulation that equals or approximates steady-state accumulation is one of the principles underlying the derivation of national BAFs. For some chemicals that require relatively long periods of time to reach steady-state in tissues of aquatic organisms, changes in water column concentrations may occur on a much more rapid time scale compared to the corresponding changes in tissue concentrations. Thus, if the system departs substantially from steady-state conditions and water concentrations are not averaged over a sufficient time period, the ratio of the tissue concentration to a water concentration may have little resemblance to the steady-state ratio and have little predictive value of long-term bioaccumulation potential.

Therefore, BAF measurements should be based on water column concentrations which are averaged over a sufficient period of time (e.g., a duration comparable to the time required for the chemical to reach steady-state). In addition, BAF measurements should be based on adequate spatial averaging of both tissue and water column concentrations for use in deriving 304(a) criteria for the protection of human health.

For this reason, a BAF is defined in this Methodology as representing the ratio (in L/kg-tissue) of a concentration of a chemical in tissue to its concentration in the surrounding water in situations where the organism and its food are exposed and the ratio does not change substantially over time (i.e., the ratio which reflects bioaccumulation at or near steady-state). A bioconcentration factor (BCF) is the ratio (in L/kg-tissue) of the concentration of a substance in tissue of an aquatic organism to its concentration in the ambient water, in situations where the organism is exposed through the water only and the ratio does not change substantially over time.

5.1.2 Goal of the National BAF

The goal of EPA's national BAF is to represent the long-term, average bioaccumulation potential of a chemical in edible tissues of aquatic organisms that are commonly consumed by humans throughout the United States. National BAFs are not intended to reflect fluctuations in bioaccumulation over short time periods (e.g., a few days) because 304(a) human health criteria are generally designed to protect humans from long-term exposures to waterborne chemicals. National BAFs are also intended to account for some major chemical, biological, and ecological attributes that can affect bioaccumulation in bodies of water across the United States. For example, separate procedures are provided for deriving national BAFs depending on the type of chemical (i.e., nonionic organic, ionic organic, inorganic and organometallic). In addition, EPA's national BAFs are derived separately for each trophic level to account for potential biomagnification of some chemicals in aquatic food webs and broad physiological differences between trophic levels that may influence bioaccumulation. Because lipid content of aquatic organisms and the amount of organic carbon in the water column have been shown to affect bioaccumulation of nonionic organic chemicals, EPA's national BAFs are adjusted to reflect the lipid content of commonly consumed fish and shellfish and the freely dissolved fraction of the chemical in ambient water for these chemicals.

5.1.3 Changes to the 1980 Methodology

Numerous scientific advances have occurred in the area of bioaccumulation since the publication of the 1980 Methodology for deriving AWQC for the protection of human health (USEPA, 1980). These advances have significantly increased our ability to assess and predict the bioaccumulation of chemicals in aquatic biota. As a result, EPA has revised the bioaccumulation portion of the 1980 Methodology to reflect the current state of the science and to improve accuracy in assessing bioaccumulation for setting 304(a) criteria for the protection of human health. The changes contained in the bioaccumulation portion of the 2000 Human Health Methodology are mostly designed to:

- Improve the ability to incorporate chemical exposure from sediments and aquatic food webs in assessing bioaccumulation potential,

- Expand the ability to account for site-specific factors which affect bioaccumulation, and

- Incorporate new data and assessment tools into the bioaccumulation assessment process.

A summary of the key changes that have been incorporated into the bioaccumulation portion of the 2000 Human Health Methodology and appropriate comparisons to the1980 Methodology are provided below.

5.1.3.1 Overall Approach

The 1980 Methodology for deriving 304(a) criteria for the protection of human health emphasized the assessment of bioconcentration (uptake from water only) through the use of the BCF. Based on the 1980 Methodology, measured BCFs were usually determined from laboratory data unless field data demonstrated consistently higher or lower accumulation compared with laboratory data. In these cases, "field BCFs" (currently termed field-measured BAFs) were recommended for use. For lipophilic chemicals where lab or field-measured data were unavailable, EPA recommended predicting BCFs from the octanol-water partition coefficient and the following equation from Veith et al. (1979): "log BCF = (0.85 log K_{ow}) - 0.70".

The 2000 Human Health Methodology revisions contained in this chapter emphasize the measurement of bioaccumulation (uptake from water, sediment, and diet) through the use of the BAF. Consistent with the 1980 Methodology, measured data are preferred over predictive approaches for determining the BAF (i.e., field-measured BAFs are generally preferred over predicted BAFs). However, the 2000 Human Health Methodology contains additional methods for deriving a national BAF that were not available in 1980. The preference for using the BAF methods also differs depending on the type and properties of the chemical. For example, the BAF derivation procedure differs for each of three broadly defined chemical categories: (1) nonionic organic, (2) ionic organic, and (3) inorganic and organometallic chemicals. Furthermore, within the category of nonionic organic chemicals, different procedures are used to derive the BAF depending on a chemicals' hydrophobicity and extent of chemical metabolism that would be expected to occur in aquatic biota.

5.1.3.2 Lipid Normalization

In the 1980 Methodology, BCFs for lipophilic chemicals were normalized by the lipid fraction in the tissue of fish and shellfish used to determine the BCF. Lipid normalization enabled BCFs to be averaged across tissues and organisms. Once the average lipid-normalized BCF was determined, it was adjusted by the consumption-weighted lipid content of commonly consumed aquatic organisms in the United States to obtain an overall consumption-weighted BCF. A similar procedure has been retained in the 2000 Human Health Methodology, whereby BAFs for nonionic organic chemicals are lipid normalized and adjusted by the consumption-weighted lipid content of commonly consumed organisms to obtain a BAF for criteria

calculations. However, the 2000 Human Health Methodology uses more up-to-date lipid data and consumption data for deriving the consumption-weighted BAFs.

5.1.3.3 Bioavailability

Bioconcentration factors derived according to the 1980 Methodology were based on the total concentration of the chemical in water, for both lipophilic and nonlipophilic chemicals. In the 2000 Human Health Methodology, BAFs for nonionic organic chemicals are derived using the most bioavailable fraction (i.e., the freely dissolved fraction) to account for the influence of particulate and dissolved organic carbon on a chemical's bioavailability. Such BAFs are then adjusted to reflect the expected bioavailability at the sites of interest (i.e., by adjusting for organic carbon concentrations at the sites of interest). Procedures for accounting for the effect of organic carbon on bioaccumulation were published previously by EPA under the Great Lakes Water Quality Initiative (GLWQI or GLI) rulemaking (USEPA, 1995a,b). Bioavailability is also considered in developing BAFs for the other chemical classes defined in the 2000 Human Health Methodology (e.g., ionic organics, inorganics/organometallics) but is done so on a chemical-by-chemical basis.

5.1.3.4 Trophic Level Considerations

In the 1980 Methodology, BCFs were determined and used for criteria derivation without explicit regard to the trophic level of the aquatic organism (e.g., benthic filter feeder, forage fish, predatory fish). Over the past two decades, much information has been assembled which demonstrates that an organism's trophic position in the aquatic food web can have an important effect on the magnitude of bioaccumulation of certain chemicals. In order to account for the variation in bioaccumulation that is due to trophic position of the organism, the 2000 Human Health Methodology recommends that BAFs be determined and applied on a trophic level-specific basis.

5.1.3.5 Site-Specific Adjustments

The 1980 Methodology contained little guidance for making adjustments to the national BCFs to reflect site- or region-specific conditions. The 2000 Human Health Methodology has greatly expanded the guidance to States and authorized Tribes for making adjustments to national BAFs to reflect local conditions. This guidance is contained in the Bioaccumulation TSD. In the Bioaccumulation TSD, guidance and data are provided for adjusting national BAFs to reflect the lipid content in locally consumed aquatic biota and the organic carbon content in the waterbodies of concern. This guidance also allows the use of appropriate bioaccumulation models for deriving site-specific BAFs. EPA also plans to publish detailed guidance on designing and conducting field bioaccumulation studies for measuring BAFs and biota-sediment accumulation factors (BSAFs). In general, EPA encourages States and authorized Tribes to make site-specific modifications to EPA's national BAFs provided such adjustments are scientifically defensible and adequately protect the designated use of the waterbody.

While the aforementioned revisions are new to EPA's Methodology for deriving national 304(a) criteria for the protection of human health, many of these refinements have been

incorporated in prior Agency guidance and regulations. For example, the use of food chain multipliers to account for the biomagnification of nonionic organic chemicals in aquatic food webs when measured data are unavailable was introduced by EPA in three documents*: Technical Support Document for Water Quality-Based Toxics Control* (USEPA, 1991), a draft document entitled *Assessment and Control of Bioconcentratable Contaminants in Surface Waters* (USEPA, 1993), and in the *Great Lakes Water Quality Initiative* (GLI) (USEPA, 1995b). Similarly, procedures for predicting BAFs using BSAFsand incorporating the effect of organic carbon on bioavailability were used to derive water quality criteria under the GLI.

5.1.4 Organization of This Section

The methodology for deriving national BAFs for use in deriving National 304(a) Human Health AWQC is provided in the following sections. Important terms used throughout this chapter are defined in Section 5.2. Section 5.3 provides an overview of the BAF derivation guidelines. Detailed procedures for deriving national BAFs are provided in Section 5.4 for nonionic organic chemicals, in Section 5.5 for ionic organic chemicals, and in Section 5.6 for inorganics and organometallic chemicals. Literature cited is provided in Section 5.7.

5.2 DEFINITIONS

The following terms and definitions are used throughout this chapter.

Bioaccumulation. The net accumulation of a substance by an organism as a result of uptake from all environmental sources.

Bioconcentration. The net accumulation of a substance by an aquatic organism as a result of uptake directly from the ambient water, through gill membranes or other external body surfaces.

Bioaccumulation Factor (BAF). The ratio (in L/kg-tissue) of the concentration of a substance in tissue to its concentration in the ambient water, in situations where both the organism and its food are exposed and the ratio does not change substantially over time. The BAF is calculated as:

$$BAF = \frac{C_t}{C_w} \qquad \text{(Equation 5-2)}$$

where:

C_t = Concentration of the chemical in the specified wet tissue
C_w = Concentration of chemical in water

Bioconcentration Factor (BCF). The ratio (in L/kg-tissue) of the concentration of a substance in tissue of an aquatic organism to its concentration in the ambient water, in situations where the organism is exposed through the water only and the ratio does not change substantially over time. The BCF is calculated as:

$$BCF = \frac{C_t}{C_w} \qquad \text{(Equation 5-3)}$$

where:

C_t = Concentration of the chemical in the specified wet tissue
C_w = Concentration of chemical in water

Baseline BAF (BAF_{ℓ}^{fd}). For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), a BAF (in L/kg-lipid) that is based on the concentration of freely dissolved chemical in the ambient water and the lipid normalized concentration in tissue.

Baseline BCF (BCF_{ℓ}^{fd}). For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), a BCF (in L/kg-lipid) that is based on the concentration of freely dissolved chemical in the ambient water and the lipid normalized concentration in tissue.

Biomagnification. The increase in tissue concentration of a chemical in organisms at successive trophic levels through a series of predator-prey associations, primarily through the mechanism of dietary accumulation.

Biomagnification Factor (BMF). The ratio (unitless) of the tissue concentration of a chemical in a predator at a particular trophic level to the tissue concentration in its prey at the next lower trophic level for a given waterbody and chemical exposure. For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), a BMF can be calculated using lipid-normalized concentrations in the tissue of organisms at two successive trophic levels as:

$$BMF_{(TL,\ n)} = \frac{C_{\ell\ (TL,\ n)}}{C_{\ell\ (TL,\ n-1)}} \qquad \text{(Equation 5-4)}$$

where:

$C_{\ell\ (TL,\ n)}$ = Lipid-normalized concentration in appropriate tissue of predator organism at a given trophic level (TL “n”)

$C_{\ell\ (TL,\ n-1)}$ = Lipid-normalized concentration in appropriate tissue of prey organism at the next lower trophic level from the predator (TL "n-1")

For inorganic, organometallic, and certain ionic organic chemicals where lipid and organic carbon partitioning does not apply, a BMF can be calculated using chemical concentrations in the tissue of organisms at two successive trophic levels as:

$$BMF_{(TL,\ n)} = \frac{C_{t\ (TL,\ n)}}{C_{t\ (TL,\ n-1)}} \qquad \text{(Equation 5-5)}$$

where:

$C_{t\ (TL,\ n)}$ = Concentration in appropriate tissue of predator organism at trophic level "n" (may be either wet weight or dry weight concentration so long as both the predator and prey concentrations are expressed in the same manner)

$C_{t\ (TL,\ n-1)}$ = Concentration in appropriate tissue of prey organism at the next lower trophic level from the predator (may be either wet weight or dry weight concentration so long as both the predator and prey concentrations are expressed in the same manner)

Biota-Sediment Accumulation Factor (BSAF). For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), the ratio of the lipid-normalized concentration of a substance in tissue of an aquatic organism to its organic carbon-normalized concentration in surface sediment (expressed as kg of sediment organic carbon per kg of lipid), in situations where the ratio does not change substantially over time, both the organism and its food are exposed, and the surface sediment is representative of average surface sediment in the vicinity of the organism. The BSAF is defined as:

$$BSAF = \frac{C_{\ell}}{C_{soc}} \qquad \text{(Equation 5-6)}$$

where:

C_{ℓ} = The lipid-normalized concentration of the chemical in tissues of the biota (µg/g lipid)

C_{soc} = The organic carbon-normalized concentration of the chemical in the surface sediment (µg/g sediment organic carbon)

Depuration. The loss of a substance from an organism as a result of any active or passive process.

Food Chain Multiplier (FCM). For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), the ratio of a baseline BAF_{ℓ}^{fd} for an organism of a particular trophic level to the baseline BCF_{ℓ}^{fd} (usually determined for organisms in trophic level one). For inorganic, organometallic, and certain ionic organic chemicals where lipid and organic carbon partitioning does not apply, a FCM is based on total (wet or dry weight) concentrations of the chemical in tissue.

Freely Dissolved Concentration. For nonionic organic chemicals, the concentration of the chemical that is dissolved in ambient water, excluding the portion sorbed onto particulate or dissolved organic carbon. The freely dissolved concentration is considered to represent the most bioavailable form of an organic chemical in water and, thus, is the form that best predicts bioaccumulation. The freely dissolved concentration can be determined as:

$$C_w^{fd} = (C_w^t) \cdot (f_{fd}) \qquad \text{(Equation 5-7)}$$

where:

C_w^{fd}	=	Freely dissolved concentration of the organic chemical in ambient water
C_w^t	=	Total concentration of the organic chemical in ambient water
f_{fd}	=	Fraction of the total chemical in ambient water that is freely dissolved

Hydrophilic. A term that refers to the extent to which a chemical is attracted to partitioning into the water phase. Hydrophilic organic chemicals have a greater tendency to partition into polar phases (e.g., water) compared to chemicals of hydrophobic chemicals.

Hydrophobic. A term that refers to the extent to which a chemical avoids partitioning into the water phase. Highly hydrophobic organic chemicals have a greater tendency to partition into nonpolar phases (e.g., lipid, organic carbon) compared with chemicals of lower hydrophobicity.

Lipid-normalized Concentration (C_{ℓ}). The total concentration of a contaminant in a tissue or whole organism divided by the lipid fraction in that tissue or whole organism. The lipid-normalized concentration can be calculated as:

$$C_{\ell} = \frac{C_t}{f_{\ell}} \qquad \text{(Equation 5-8)}$$

where:

C_t	=	Concentration of the chemical in the wet tissue (either whole organism or specified tissue)

f_ℓ = Fraction lipid content in the organism or specified tissue

Octanol-water Partition Coefficient (K_{ow}). The ratio of the concentration of a substance in the n-octanol phase to its concentration in the aqueous phase in an equilibrated two-phase octanol-water system. For log K_{ow}, the log of the octanol-water partition coefficient is a base 10 logarithm.

Organic Carbon-normalized Concentration (C_{soc}). For sediments, the total concentration of a contaminant in sediment divided by the fraction of organic carbon in sediment. The organic carbon-normalized concentration can be calculated as:

$$C_{soc} = \frac{C_s}{f_{oc}} \qquad \text{(Equation 5-9)}$$

where:

C_s = Concentration of chemical in sediment
f_{oc} = Fraction organic carbon in sediment

Uptake. Acquisition by an organism of a substance from the environment as a result of any active or passive process.

5.3 FRAMEWORK FOR DETERMINING NATIONAL BIOACCUMULATION FACTORS

5.3.1 Four Different Methods

Bioaccumulation factors used to derive national BAFs can be measured or predicted using some or all of the following four methods, depending on the type of chemical and its properties. These methods are:

(1) a measured BAF obtained from a field study (i.e., a field-measured BAF);

(2) a BAF predicted from a field-measured BSAF;

(3) a BAF predicted from a laboratory-measured BCF (with or without adjustment by an FCM); and

(4) a BAF predicted from a chemical's octanol-water partition coefficient (K_{ow}), with or without adjustment using an FCM.

A brief summary of each of the four methods is provided below. Additional details on the use of these four methods is provided in Section 5.4 (for nonionic organics), Section 5.5 (for ionic organics) and Section 5.6 (for inorganics and organometallics).

1. **Field-Measured BAF.** Use of a field-measured BAF, which is the most direct measure of bioaccumulation, is the only method that can be used to derive a national BAF for all types of chemicals (i.e., nonionic organic, ionic organic, and inorganic and organometallic chemicals). A field-measured BAF is determined from a field study using measured chemical concentrations in the aquatic organism and its surrounding water. Because field studies are conducted in natural aquatic ecosystems, a field-measured BAF reflects an organism's exposure to a chemical through all relevant exposure pathways (i.e., water, sediment, and diet). A field-measured BAF also reflects any metabolism of a chemical that might occur in the aquatic organism or its food web. Therefore, field-measured BAFs are appropriate for all chemicals, regardless of the extent of chemical metabolism in biota.

2. **Field-measured BSAF.** For nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies), a BAF can also be predicted from BSAFs. A BSAF is similar to a field-measured BAF in that the concentration of a chemical in biota is measured in the field and reflects an organism's exposure to all relevant exposure routes. A BSAF also reflects any chemical metabolism that might occur in the aquatic organism or its food web. However, unlike a field-measured BAF which references the biota concentration to the water concentration, a BSAF references the biota concentration to the sediment concentration. Use of the BSAF procedure is restricted to organic chemicals which are classified as being moderately to highly hydrophobic.

3. **Lab-measured BCF.** A laboratory-measured BCF can also be used to estimate a BAF for organic and inorganic chemicals. However, unlike a field-measured BAF or a BAF predicted from a field-measured BSAF, a laboratory-measured BCF only reflects the accumulation of chemical through the water exposure route. Laboratory-measured BCFs may therefore under estimate BAFs for chemicals where accumulation from sediment or dietary sources is important. In these cases, laboratory-measured BCFs can be multiplied by a FCM to reflect accumulation from non-aqueous (i.e., food chain) pathways of exposure. Since a laboratory-measured BCF is determined using the measured concentration of a chemical in an aquatic organism and its surrounding water, a laboratory-measured BCF reflects any metabolism of the chemical that occurs in the organism, but not in the food web.

4. **K_{ow}.** A chemical's octanol-water partition coefficient, or K_{ow}, can also be used to predict a BAF for nonionic organic chemicals. This procedure is appropriate only for nonionic organic chemicals (and certain ionic organic chemicals where similar lipid and organic carbon partitioning behavior applies). The K_{ow} has been extensively correlated with the BCF for nonionic organic chemicals that are poorly metabolized by aquatic organisms. Therefore, where substantial metabolism is known to occur in biota, the K_{ow} is not used

to predict the BAF. For nonionic organic chemicals where chemical exposure through the food web is important, use of the K_{ow} alone will under predict the BAF. In such cases, the K_{ow} is adjusted with a FCM similar to the BCF procedure above.

5.3.2 Overview of BAF Derivation Framework

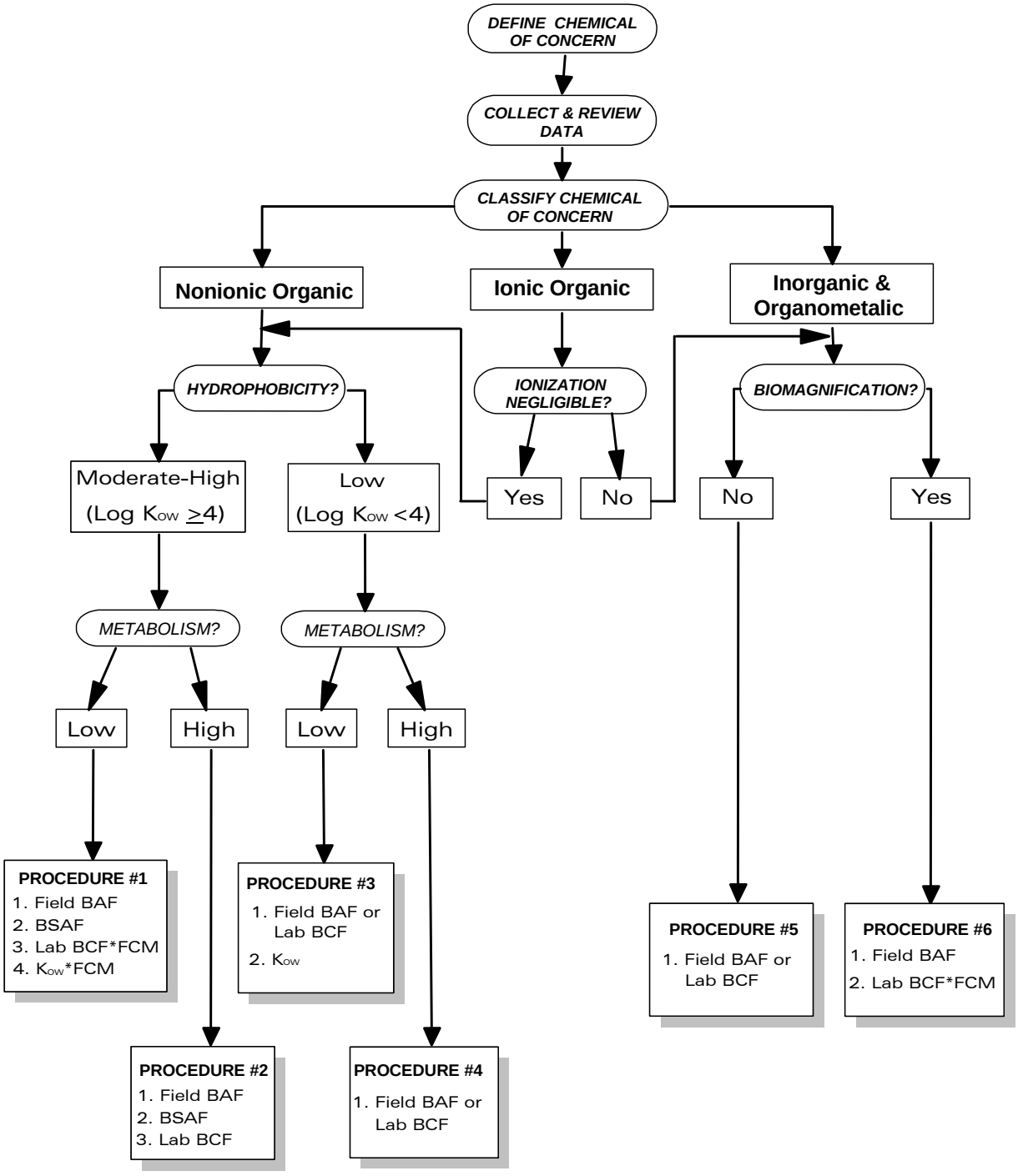
Although up to four methods can be used to derive a BAF as described in the previous section, it is evident that these methods do not apply equally to all types of chemicals. In addition, experience demonstrates that the required data will usually not be available to derive a BAF value using all of the applicable methods. As a result, EPA has developed the following guidelines to direct users in selecting the most appropriate method(s) for deriving a national BAF.

Figure 5-1 shows the overall framework of EPA's national BAF methodology. This framework illustrates the major steps and decisions that will ultimately lead to calculating a national BAF using one of six hierarchical procedures shown at the bottom of Figure 5-1. Each procedure contains a hierarchy of the BAF derivation methods discussed above, the composition of which depends on the chemical type and certain chemical properties (e.g., its degree of hydrophobicity and expected degree of metabolism and biomagnification). The number assigned to each BAF method within a procedure indicates its general order of preference for deriving a national BAF value. The goal of the framework and accompanying guidelines is to enable full use of available data and methods for deriving a national BAF value while appropriately restricting the use of certain methods to reflect their inherent limitations.

The first step in the framework is to define the chemical of concern. As described in Section 5.3.3, the chemical used to derive the national BAF should be consistent with the chemical used to derive the critical health assessment value. The second step is to collect and review all relevant data on bioconcentration and bioaccumulation of the chemical of concern (see Section 5.3.4). Once pertinent data are reviewed, the third step is to classify the chemical of concern into one of three broadly defined chemical categories: (1) nonionic organic chemicals, (2) ionic organic chemicals, and (3) and inorganic and organometallic chemicals. Guidance for classifying chemicals into these three categories is provided in Section 5.3.5.

After a chemical has been classified into one of the three categories, other information is used to select one of six hierarchical procedures to derive the national BAF. The specific procedures for deriving a BAF for each chemical group are discussed in Section 5.4 for nonionic organics, Section 5.5 for ionic organics, and Section 5.6 for inorganics and organometallics.

Figure 5-1. Framework for Deriving a National BAF



Detailed guidance concerning the first three steps of the derivation process (i.e, defining the chemical of concern, collecting and reviewing data, and classifying the chemical of concern) is provided in the following three sections.

5.3.3 Defining the Chemical of Concern

Defining the chemical of concern is the first step in deriving a national BAF. This step involves precisely defining the form(s) of the chemical upon which the national BAF value will be derived. Although this step is usually straightforward for single chemicals, complications can arise when the chemical of concern occurs as a mixture. The following guidelines should be followed for defining the chemical of concern.

1. Information for defining the chemical of concern should be obtained from the health and exposure assessment portions of the criteria derivation effort. The chemical(s) used to derive the national BAF should be consistent with the chemical(s) used to derive the reference dose (RfD), point of departure/uncertainty factor (POD/UF), or cancer potency factor.

2. In most cases, the RfD, POD/UF, or cancer potency factor will be based on a single chemical. In some cases, the RfD, POD/UF, or cancer potency factor will be based on a mixture of compounds, typically within the same chemical class (e.g., toxaphene, chlordane). In these situations, the national BAF should be derived in a manner that is consistent with the mixture used to express the health assessment.

    a. If sufficient data are available to reliably assess the bioaccumulation of each relevant compound contained in the mixture, then the national BAF(s) should be derived using the BAFs for the individual compounds of the mixture and appropriately weighted to reflect the mixture composition used to establish the RfD, POD/UF, or cancer potency factor. An example of this approach is shown in the derivation of BAFs for PCBs in the GLI Rulemaking (USEPA, 1997).

    b. If sufficient data are not available to reliably assess the bioaccumulation of individual compounds of the mixture, then the national BAF(s) should be derived using BAFs for the same or appropriately similar chemical mixture as that used to establish the RfD, POD/UF, or cancer potency value.

5.3.4 Collecting and Reviewing Data

The second step in deriving a national BAF is to collect and review all relevant bioaccumulation data for the chemical of concern. The following guidance should be followed for collecting and reviewing bioaccumulation data for deriving national BAFs.

1. All data on the occurrence and accumulation of the chemical of concern in aquatic animals and plants should be collected and reviewed for adequacy.

2. A comprehensive literature search strategy should be used for gathering bioaccumulation-related data. An example of a comprehensive literature search strategy is provided in the Bioaccumulation TSD.

3. All data that are used should contain sufficient supporting information to indicate that acceptable measurement procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator.

4. Questionable data, whether published or unpublished, should not be used. Guidance for assessing the acceptability of bioaccumulation and bioconcentration studies is found in Sections 5.4, 5.5, and 5.6.

5.3.5 Classifying the Chemical of Concern

The next step in deriving a national BAF consists of classifying the chemical of concern into one of three categories: nonionic organic, ionic organic, and inorganic and organometallic (Figure 5-1). This step helps to determine which of the four methods described in Section 5.3.1 are appropriate for deriving BAFs. The following guidance applies for classifying the chemical of concern.

1. **Nonionic Organic Chemicals.** For the purposes of the 2000 Human Health Methodology, nonionic organic chemicals are those organic compounds that do not ionize substantially in natural bodies of water. These chemicals are also referred to as neutral or nonpolar organics in the scientific literature. Due to their neutrality, nonionic organic chemicals tend to associate with other neutral (or near neutral) compartments in aquatic ecosystems (e.g., lipid, organic carbon). Examples of nonionic organic chemicals which have been widely studied in terms of their bioaccumulation include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans, many chlorinated pesticides, and polynuclear aromatic hydrocarbons (PAHs). Procedures for deriving a national BAF for nonionic organic chemicals are provided in Section 5.4.

2. **Ionic Organic Chemicals.** For the purposes of the 2000 Human Health Methodology, ionic organic chemicals are considered to include those chemicals that contain functional groups with exchangeable protons such as hydroxyl, carboxylic, and sulfonic groups and functional groups that readily accept protons such as amino and aromatic heterocyclic nitrogen (pyridine) groups. Ionic organic chemicals undergo ionization in water, the extent of which depends on pH and the pKa of the chemical. Because the ionized species of these chemicals behave differently from the neutral species, separate guidance is provided for deriving BAFs for ionic organic chemicals. Procedures for deriving national BAFs for ionic organic chemicals are provided in Section 5.5.

3. **Inorganic and Organometallic Chemicals.** The inorganic and organometallic category is considered to include inorganic minerals, other inorganic compounds and elements, metals (e.g., copper, cadmium, chromium, zinc), metalloids (selenium, arsenic) and

organometallic compounds (e.g., methylmercury, tributyltin, tetraalkyllead). Procedures for deriving BAFs for inorganic and organometallic chemicals are provided in Section 5.6.

5.4 NATIONAL BIOACCUMULATION FACTORS FOR NONIONIC ORGANIC CHEMICALS

5.4.1 Overview

This section contains the methodology for deriving national BAFs for nonionic organic chemicals as defined in Section 5.3.5. The four general steps of this methodology are:

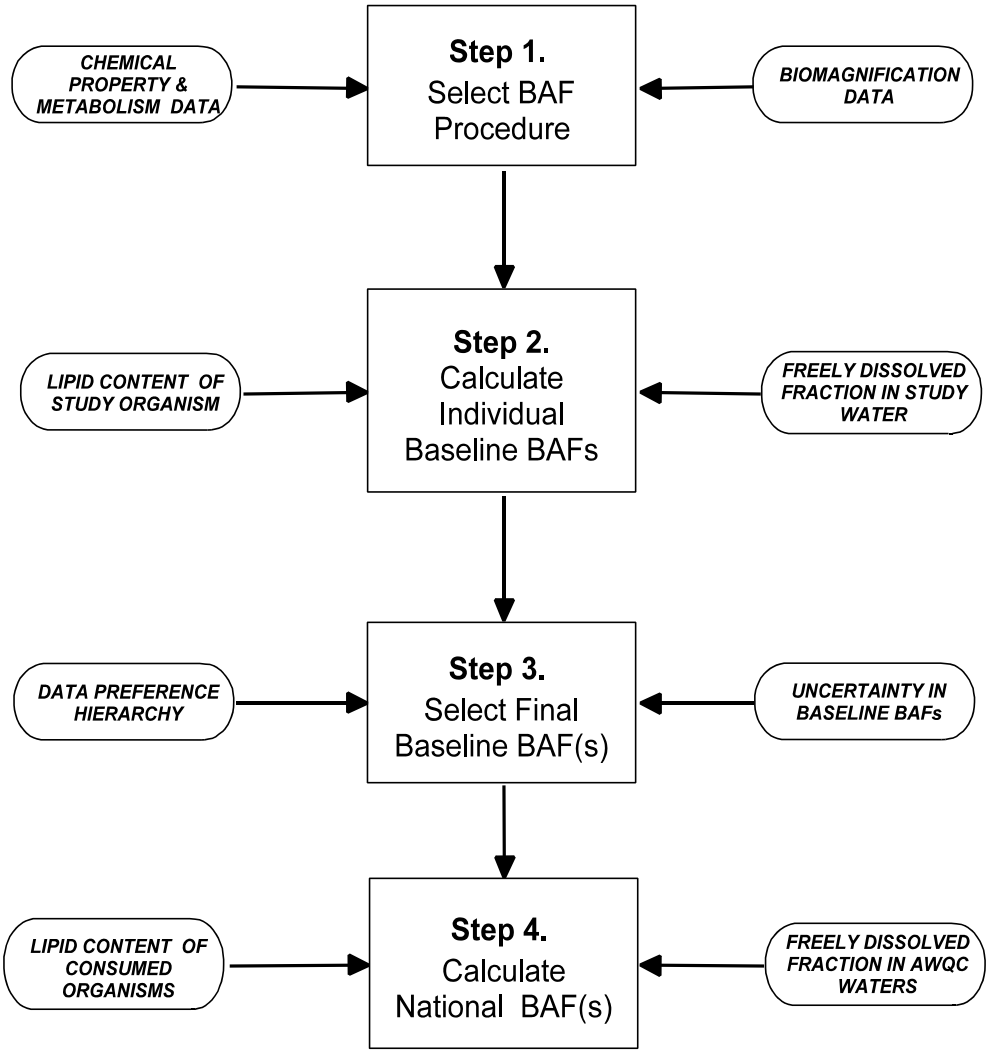
1. Selecting the BAF derivation procedure,
2. Calculating individual baseline BAF_{ℓ}^{fd}s,
3. Selecting the final baseline BAF_{ℓ}^{fd}s, and
4. Calculating the national BAFs from the final baseline BAF_{ℓ}^{fd}s.

A schematic of this four-step process is shown in Figure 5-2.

Step 1 of the methodology (selecting the BAF derivation procedure) determines which of the four BAF procedures summarized in Figure 5-1 will be appropriate for deriving the national BAF. Step 2 involves calculating individual, species-specific BAF_{ℓ}^{fd}s using all of the methods available within the selected BAF derivation procedure. Calculating the individual baseline BAF_{ℓ}^{fd}s involves using data from the field site or laboratory where the original data were collected to account for site-specific factors which affect the bioavailability of the chemical to aquatic organisms (e.g., lipid content of study organisms and freely dissolved concentration in study water). Step 3 of the methodology consists of selecting the final baseline BAF_{ℓ}^{fd}s from the individual baseline BAF_{ℓ}^{fd}s by taking into account the uncertainty in the individual BAFs and the data preference hierarchy selected in Step 1. The final step is to calculate a BAF (or BAFs) that will be used in the derivation of 304(a) criteria (i.e., referred to as the national BAF). This step involves adjusting the final baseline BAF_{ℓ}^{fd}(s) to reflect certain factors that affect bioavailablity of the chemical to aquatic organisms in waters to which the national 304(a) criteria will apply (e.g., the freely dissolved fraction expected in U.S. waters and the lipid content of consumed aquatic organisms). Baseline BAF_{ℓ}^{fd}s are not used directly in the derivation of the 304(a) criteria because they do not reflect the conditions that affect bioavailability in U.S. waters.

Section 5.4.2 below provides detailed guidance for selecting the appropriate BAF derivation procedure (Step 1 of the process). Guidance on calculating individual baseline BAF_{ℓ}^{fd}s, selecting the final baseline BAF, and calculating the national BAF (Steps 2 through 4 of the process) is provided in separate sections under each of the four BAF derivation procedures.

Figure 5-2. BAF Derivation for Nonionic Organic Chemicals



5.4.2 Selecting the BAF Derivation Procedure

This section describes the decisions that should be made to select one of the four available hierarchical procedures for deriving a national BAF for nonionic organic chemicals (Procedures #1 through #4 of Figure 5-1). As shown in Figure 5-1, two decision points exist in selecting the BAF derivation procedure. The first decision point requires knowledge of the chemical's hydrophobicity (i.e., the K_{ow} of the chemical). Guidance for selecting the K_{ow} for a chemical is provided in the Bioaccumulation TSD. The K_{ow} provides an initial basis for assessing whether biomagnification may be a concern for nonionic organic chemicals. The second decision point is based on the rate of metabolism for the chemical in the target organism. Guidance for assessing whether a high or low rate of metabolism is likely for a chemical of concern is provided below in Section 5.4.2.3. With the appropriate information for these two decision points, the BAF derivation procedure should be selected using the following guidelines.

5.4.2.1 Chemicals with Moderate to High Hydrophobicity

1. For the purposes of the 2000 Human Health Methodology, nonionic organic chemicals with log K_{ow} values equal to or greater than 4.0 should be classified as moderately to highly hydrophobic. For moderately to highly hydrophobic nonionic organic chemicals, available data indicate that exposure through the diet and other non-aqueous routes can become important in determining chemical residues in aquatic organisms (e.g., Russell et al., 1999; Fisk et al., 1998; Oliver and Niimi, 1983; Oliver and Niimi, 1988; Niimi, 1985; Swackhammer and Hites, 1988). Dietary and other non-aqueous exposure can become extremely important for those nonionic organic chemicals that are poorly metabolized by aquatic biota (e.g., certain PCB congeners, chlorinated pesticides, and polychlorinated dibenzo-p-dioxins and furans).

2. **Procedure #1** should be used to derive national BAFs for moderately to highly hydrophobic nonionic organic chemicals in cases where:

   (a) the rate of chemical metabolism by target aquatic organisms is expected to be sufficiently low such that biomagnification is of concern, or

   (b) the rate of chemical metabolism by target aquatic organisms is not sufficiently known.

   Procedure #1 accounts for non-aqueous exposure and the potential for biomagnification in aquatic food webs through the use of field-measured values for bioaccumulation (i.e., field measured BAF or BSAF) and FCMs when appropriate field data are unavailable. Guidance on deriving national BAFs using Procedure #1 is found below in Section 5.4.3.

3. **Procedure #2** should be used to derive the national BAFs for moderately to highly hydrophobic nonionic organic chemicals in cases where:

   (a) the rate of chemical metabolism by target aquatic organisms is expected to be sufficiently high such that biomagnification is not of concern.

Procedure #2 relaxes the requirement of using FCMs and eliminates the use of K_{ow}-based estimates of the BAF, two procedures that are most appropriate for poorly metabolized nonionic organic chemicals. Guidance on deriving national BAFs using Procedure #2 is found below in Section 5.4.4.

5.4.2.2 Chemicals with Low Hydrophobicity

1. For the purposes of these guidelines, nonionic organic chemicals with log K_{ow} values less than 4.0 should be classified as exhibiting low hydrophobicity. For nonionic organic chemicals that exhibit low hydrophobicity (i.e., log $K_{ow} < 4.0$), available information indicates that non-aqueous exposure to these chemicals is not likely to be important in determining chemical residues in aquatic organisms (e.g., Fisk et al., 1998; Gobas et al., 1993; Connolly and Pedersen, 1988; Thomann, 1989). For this group of chemicals, laboratory-measured BCFs and K_{ow}-predicted BCFs do not require adjustment with FCMs for determining the national BAF (Procedures #3 and #4), unless other appropriate data indicate differently.

   Other appropriate data include studies clearly indicating that non-aqueous exposure is important such that use of a BCF would substantially underestimate residues in aquatic organisms. In these cases, Procedure #1 should be used to derive the BAF for nonionic organic chemicals with log $K_{ow} < 4.0$. Furthermore, the data supporting the K_{ow} determination should be carefully reviewed for accuracy and appropriate interpretation, since the apparent discrepancy may be due to errors in determining K_{ow}.

2. **Procedure #3** should be used to derive national BAFs for nonionic organic chemicals of low hydrophobicity in cases where:

   (a) the rate of chemical metabolism by target aquatic organisms is expected to be negligible, such that tissue residues of the chemical of concern are not substantially reduced compared to an assumption of no metabolism, or

   (b) the rate of chemical metabolism by target aquatic organisms is not sufficiently known.

   Procedure #3 includes the use of K_{ow}-based estimates of the BCF to be used when lab or field data are absent. Guidance on deriving national BAFs using Procedure #3 is found below in Section 5.4.5.

3. **Procedure #4** should be used to derive national BAFs for nonionic organic chemicals of low hydrophobicity in cases where:

   (a) the rate of chemical metabolism by target aquatic organisms is expected to be sufficiently high, such that tissue residues of the chemical of concern are substantially reduced compared with an assumption of no metabolism.

Procedure #4 eliminates the option of using K_{ow}-based estimates of the BAF because the K_{ow} may over-predict accumulation when a chemical is metabolized substantially by an aquatic organism. Guidance on deriving national BAFs using Procedure #4 is found below in Section 5.4.6.

5.4.2.3 Assessing Metabolism

Currently, assessing the degree to which a chemical is metabolized by aquatic organisms is confounded by a variety of factors. First, conclusive data on chemical metabolism in aquatic biota are largely lacking. Such data include whole organism studies where the metabolic rates and breakdown products are quantified in fish and other aquatic organisms relevant to human consumption. However, the majority of information on metabolism is derived from *in vitro* liver microsomal preparations in which primary and secondary metabolites may be identified and their rates of formation may or may not be quantified. Extrapolating results from *in vitro* studies to the whole organism involves considerable uncertainty. Second, there are no generally accepted procedures for reliably predicting chemical metabolism by aquatic organisms in the absence of measured data. Third, the rate at which a chemical is metabolized by aquatic organisms can be species and temperature dependent. For example, PAHs are known to be metabolized readily by vertebrate aquatic species (primarily fish), although at rates much less than those observed for mammals. However, the degree of metabolism in invertebrate species is generally much less than the degree in vertebrate species (James, 1989). One hypothesis for this difference is that the invertebrate species lack the detoxifying enzymes and pathways that are present in many vertebrate species.

Given the current limitations on assessing the degree of chemical metabolism by aquatic organisms, the assessment of metabolism should be made on a case-by-case basis using a weight-of-evidence approach. When assessing a chemical's likelihood to undergo substantial metabolism in a target aquatic organism, the following data should be carefully evaluated:

(1) *in vivo* chemical metabolism data,
(2) bioconcentration and bioaccumulation data,
(3) data on chemical occurrence in target aquatic biota, and
(4) *in vitro* chemical metabolism data.

1. ***In vivo* Data.** *In vivo* data on metabolism in aquatic organisms are from studies of chemical metabolism using whole organisms. These studies are usually conducted using large fish from which blood, bile, urine, and individual tissues can be collected for the identification and quantification of metabolites formed over time. *In vivo* studies are considered the most useful for evaluating a chemical's degree of metabolism in an organism because both oxidative (Phase I) and conjugative (Phase II) metabolism can be assessed in these studies. Mass-balance studies, in which parent compound elimination is quantified separately from biotransformation and elimination of metabolites, allow calculation of conversion rate of parent to metabolite as well as metabolite elimination. This information might be used to estimate loss due to metabolism separately from that due to elimination of the parent compound for adjustment of K_{ow}-predicted BAFs. However, due to the analytical and experimental challenges these studies pose, data of

this type are limited. Less rigorous *in vivo* metabolism studies might include the use of metabolic blockers to demonstrate the influence of metabolism on parent compound kinetics. However, caution should be used in interpretation of absolute rates from these data due to the lack of specificity of mammalian derived blockers in aquatic species (Miranda et al., 1998).

2. **Bioconcentration or Bioaccumulation Data.** Data on chemical bioconcentration or bioaccumulation in aquatic organisms can be used indirectly for assessing metabolism. This assessment involves comparing acceptable lab-measured BCFs or field-measured BAFs (after converting to baseline values using procedures below) with the chemical's predicted value based on K_{ow}. The theoretical basis of bioconcentration and bioaccumulation for nonionic organic chemicals indicates that a chemical's baseline BCF should be similar to its K_{ow}-predicted value if metabolism is not occurring or is minimal (see the Bioaccumulation TSD). This theory also indicates that baseline BAFs should be similar to or higher than the K_{ow} for poorly metabolized organic chemicals, with highly hydrophobic chemicals often exhibiting higher baseline BAFs than K_{ow} values. Thus, if a chemical's baseline BCF or BAF is substantially lower than its K_{ow}, this may be an indication that the chemical is being metabolized by the aquatic organism of concern. Note, however, that this difference may also indicate problems in the experimental design or analytical chemistry, and that it may be difficult to discern the difference.

3. **Chemical Occurrence Data.** Although by no means definitive, data on the occurrence of chemicals in aquatic biota (i.e., residue studies) may offer another useful line of evidence for evaluating a chemical's likelihood to undergo substantial metabolism. Such studies are most useful if they have been conducted repeatedly over time and over wide geographical areas. Such studies might indicate a chemical is poorly metabolized if data show that the chemical is being biomagnified in the aquatic food web (i.e., higher lipid-normalized residues in successive trophic levels). Conversely, such studies might indicate a chemical is being metabolized substantially if residue data show a decline in residues with increasing trophic level. Again, other reasons for increases or decreases in concentrations with increasing trophic level might exist and should be carefully evaluated (e.g., incorrect food web assumptions, differences in exposure concentrations).

4. ***In vitro* Data.** *In vitro* metabolism data include data from studies where specific sub-cellular fractions (e.g., microsomal, cytosolic), cells, or tissues from an organism are tested outside the body (i.e., in test-tubes, cell- or tissue-culture). Compared with *in vivo* studies of chemical metabolism in aquatic organisms, *in vitro* studies are much more plentiful in the literature, with the majority of studies characterizing oxidative (Phase I) reactions de-coupled from conjugative (Phase II) metabolism. Cell, tissue, or organ level *in vitro* studies are less common but provide a more complete assessment of metabolism. While such studies are particularly useful for identifying the pathways, rates of formation, and metabolites formed, as well as the enzymes involved and differences in the temperature dependence of metabolism across aquatic species, they suffer from uncertainty when results are extrapolated to the whole organism. This uncertainty results from the fact that dosimetry (i.e., delivery of the toxicant to, and removal of metabolite

from, the target tissue) cannot currently be adequately reproduced in the laboratory or easily modeled.

When assessing chemical metabolism using the above information, the following guidelines apply.

a. A finding of substantial metabolism should be supported by two or more lines of evidence identified using the data described above.

b. At least one of the lines of evidence should be supported by either *in vivo* metabolism data or acceptable bioconcentration or bioaccumulation data.

c. A finding of substantial metabolism in one organism should not be extrapolated to another organism or another group of organisms unless data indicate similar metabolic pathways exist (or are very likely to exist) in both organisms. *In vitro* data may be particularly useful in cross-species extrapolations.

d. Finally, in situations where sufficient data are not available to properly assess the likelihood of significant metabolism in aquatic biota of concern, the chemical should be assumed to undergo little or no metabolism. This assumptions reflects a policy decision by EPA to err on the side of public health protection when sufficient information on metabolism is lacking.

5.4.3 Deriving National BAFs Using Procedure #1

This section contains guidance for calculating national BAFs for nonionic organic chemicals using Procedure #1 shown in Figure 5-1. The types of nonionic organic chemicals for which Procedure #1 is most appropriate are those that are classified as moderately to highly hydrophobic and subject to low (or unknown) rates of metabolism by aquatic biota (see Section 5.4.2 above). Non-aqueous contaminant exposure and subsequent biomagnification in aquatic food webs are of concern for chemicals that are classified in this category. Some examples of nonionic organic chemicals for which Procedure #1 is considered appropriate include:

- tetra-, penta- & hexachlorobenzenes;
- PCBs;
- octachlorostyrene;
- hexachlorobutadiene;
- endrin, dieldrin, aldrin;
- mirex, photomirex;
- DDT, DDE, DDD; and
- heptachlor, chlordane, nonachlor.

Under Procedure #1, the following four methods may be used in deriving a national BAF:

- using a BAF from an acceptable field study (i.e., a field-measured BAF);
- predicting a BAF from an acceptable field-measured BSAF;

- predicting a BAF from an acceptable laboratory-measured BCF and FCM; and
- predicting a BAF from an acceptable K_{ow} and FCM.

As shown in Figure 5-2, once the derivation procedure has been selected, the next steps in deriving a national BAF for a given trophic level include: calculating individual baseline BAF_{ℓ}^{fd}s (step 2), selecting the final baseline BAF_{ℓ}^{fd} (step 3), and calculating the national BAF from the final baseline BAF_{ℓ}^{fd} (step 4). Each of these three steps is discussed separately below.

5.4.3.1 Calculating Individual Baseline BAF_{ℓ}^{fd}s

Calculating an individual baseline BAF_{ℓ}^{fd} involves normalizing the field-measured BAF_T^t (or laboratory-measured BCF_T^t) which are based on total concentrations in tissue and water by the lipid content of the study organisms and the freely dissolved concentration in the study water. Both the lipid content in the organism and the freely dissolved concentration (as influenced by organic carbon in water) have been shown to be important factors that influence the bioaccumulation of nonionic organic chemicals (e.g., Mackay, 1982; Connolly and Pederson, 1988; Thomann, 1989, Suffet et al., 1994). Therefore, baseline BAF_{ℓ}^{fd}s (which are expressed on a freely dissolved and lipid-normalized basis) are considered more amenable to extrapolating between different species and bodies of water compared to BAFs expressed using the total concentration in the tissue and water. Because bioaccumulation can be strongly influenced by the trophic position of aquatic organisms (either due to biomagnification or physiological differences), extrapolation of baseline BAF_{ℓ}^{fd}s should not be performed between species of different trophic levels.

1. For each species for which acceptable data are available, calculate all possible baseline BAF_{ℓ}^{fd}s using each of the four methods shown above for Procedure #1.

2. Individual baseline BAF_{ℓ}^{fd}s should be calculated from field-measured BAF_T^ts, field-measured BSAFs, laboratory BCF_T^ts, and the K_{ow} according to the following procedures.

A. Baseline BAF_{ℓ}^{fd}s from Field-Measured BAFs

A baseline BAF_{ℓ}^{fd} should be calculated from each field-measured BAF_T^t using information on the lipid fraction in the tissue of concern for the study organism and the fraction of the total chemical that is freely dissolved in the study water.

1. **Baseline BAF_{ℓ}^{fd} Equation.** For each acceptable field-measured BAF_T^t, calculate a baseline BAF_{ℓ}^{fd} using the following equation:

$$\text{Baseline } BAF_{\ell}^{fd} = \left[\frac{\text{Measured } BAF_T^t}{f_{fd}} - 1 \right] \left(\frac{1}{f_{\ell}} \right) \qquad \text{(Equation 5-10)}$$

where:

Baseline BAF_{ℓ}^{fd}	=	BAF expressed on a freely dissolved and lipid-normalized basis
Measured BAF_T^t	=	BAF based on total concentration in tissue and water
f_{ℓ}	=	Fraction of the tissue that is lipid
f_{fd}	=	Fraction of the total chemical that is freely dissolved in the ambient water

The technical basis of Equation 5-10 is provided in the Bioaccumulation TSD. Guidance for determining each component of Equation 5-10 is provided below.

2. **Determining the Measured BAF_T^t.** The field-measured BAF_T^t shown in Equation 5-10 should be calculated based on the total concentration of the chemical in the appropriate tissue of the aquatic organism and the total concentration of the chemical in ambient water at the site of sampling. The equation to derive a measured BAF_T^t is:

$$\text{Measured } BAF_T^t = \frac{C_t}{C_w} \qquad \text{(Equation 5-11)}$$

where:

C_t	=	Total concentration of the chemical in the specified wet tissue
C_w	=	Total concentration of chemical in water

The data used to calculate a field-measured BAF_T^t should be reviewed thoroughly to assess the quality of the data and the overall uncertainty in the BAF value. The following general criteria apply in determining the acceptability of field-measured BAFs that are being considered for deriving national BAFs using Procedure #1.

a. Aquatic organisms used to calculate a field-measured BAF_T^t should be representative of aquatic organisms that are commonly consumed in the United States. An aquatic organism that is not commonly consumed in the United States can be used to calculate an acceptable field-measured BAF_T^t provided that the organism is considered to be a reasonable surrogate for a commonly consumed organism. Information on the ecology, physiology, and biology of the organism should be reviewed when assessing whether an organism is a reasonable surrogate of a commonly consumed organism.

b. The trophic level of the study organism should be determined by taking into account its life stage, diet, size, and the food web structure at the study location. Information from the study site (or similar sites) is preferred when evaluating trophic status. If such information is lacking, general information for assessing trophic status of aquatic organisms can be found in USEPA (2000a,b,c).

c. The percent lipid of the tissue used to determine the field-measured BAF_T^t should be either measured or reliably estimated to permit lipid-normalization of the chemical's tissue concentration.

d. The study from which the field-measured BAF_T^t is derived should contain sufficient supporting information from which to determine that tissue and water samples were collected and analyzed using appropriate, sensitive, accurate, and precise analytical methods.

e. The site of the field study should not be so unique that the BAF cannot be reasonably extrapolated to other locations where the BAF and resulting criteria will apply.

f. The water concentration(s) used to derive the BAF should reflect the average exposure of the aquatic organism that corresponds to the concentration measured in its tissue of concern. For nonionic organic chemicals, greater temporal and spatial averaging of chemical concentrations is required as the K_{ow} increases. In addition, as variability in water concentrations increase, greater temporal and spatial averaging is also generally required. Greater spatial averaging is also generally required for more mobile organisms.

g. The concentrations of particulate organic carbon and dissolved organic carbon in the study water should be measured or reliably estimated.

EPA is currently developing guidance for designing and conducting field studies for determining field-measured BAF_T^ts, including recommendations for minimum data requirements. A more detailed discussion of factors that should be considered when determining field-measured BAF_T^ts is provided in the Bioaccumulation TSD.

3. **Determining the Fraction Freely Dissolved (f_{fd}).** As illustrated by Equation 5-10, the fraction of the nonionic organic chemical that is freely dissolved in the study water is required for calculating a baseline BAF_ℓ^{fd} from a field-measured BAF_T^t. The freely dissolved fraction is the portion of the nonionic organic chemical that is not bound to particulate organic carbon or dissolved organic carbon. Together, the concentration of a nonionic organic chemical that is freely dissolved, bound to dissolved organic carbon, and bound to particulate organic carbon constitute its total concentration in water. As discussed further in the Bioaccumulation TSD, the freely dissolved fraction of a chemical is considered to be the best expression of the bioavailable form of nonionic organic chemicals to aquatic organisms (e.g., Suffet et al., 1994; USEPA, 1995b). Because the fraction of a nonionic organic chemical that is freely dissolved may vary among different bodies of water as a result of differences in dissolved and particulate organic carbon in the water, the bioavailability of the total chemical concentration in water is expected to vary from one body of water to another. Therefore, BAFs which are based on the freely dissolved concentration in water (rather than the total concentration in water) are considered to be more reliable for extrapolating and aggregating BAFs among different bodies of water. Currently, availability of BAFs based on measured freely dissolved

concentrations is very limited, partly because of difficulties in analytically measuring the freely dissolved concentration. Thus, if a BAF based on the total water concentration is reported in a given study, the fraction of the chemical that is freely dissolved should be predicted using information on the organic carbon content in the study water.

a. **Equation for Determining the Freely Dissolved Fraction.** If reliable measured data are unavailable to directly determine the freely dissolved fraction of the chemical in water, the freely dissolved fraction should be estimated using the following equation.

$$f_{fd} = \frac{1}{[1 + (POC \cdot K_{ow}) + (DOC \cdot 0.08 \cdot K_{ow})]} \qquad \text{(Equation 5-12)}$$

where:

POC	=	concentration of particulate organic carbon (kg/L)
DOC	=	concentration of dissolved organic carbon (kg/L)
K_{ow}	=	n-octanol water partition coefficient for the chemical

In Equation 5-12, K_{ow} is being used to estimate the partition coefficient to POC (i.e., K_{POC} in L/kg) and $0.08 \cdot K_{ow}$ is being used to estimate the partition coefficient to DOC (i.e., the K_{DOC} in L/kg). A discussion of the technical basis, assumptions, and uncertainty associated with the derivation and application of Equation 5-12 is provided in the Bioaccumulation TSD.

b. **POC and DOC Values.** When converting from the total concentration of a chemical to a freely dissolved concentration using Equation 5-12 above, the POC and DOC concentrations should be obtained from the original study from which the field-measured BAF is determined. If POC and DOC concentrations are not reported in the BAF study, reliable estimates of POC and DOC might be obtained from other studies of the same site used in the BAF study or closely related site(s) within the same water body. When using POC/DOC data from other studies of the same water body, care should be taken to ensure that environmental and hydrological conditions that might affect POC or DOC concentrations (i.e., runoff events, proximity to ground water or surface water inputs, sampling season) are reasonably similar to those in the BAF study. Additional information related to selecting POC and DOC values is provided in the Bioaccumulation TSD.

In some cases, BAFs are reported using the concentration of the chemical in filtered or centrifuged water. When converting these BAFs to a freely dissolved basis, the concentration of POC should be set equal to zero when using Equation 5-12. Particulates are removed from water samples by filtering or centrifuging the sample.

c. **Selecting K_{ow} Values.** A variety of techniques are available to measure or predict K_{ow} values. The reliability of these techniques depends to a large extent on the K_{ow} of the chemical. Because K_{ow} is an important input parameter for calculating the freely dissolved concentration of nonionic organic chemicals and for deriving BAFs using the other three methods of Procedure #1, care should be taken in selecting the most reliable K_{ow} value. The value of K_{ow} for use in estimating the freely dissolved fraction and other procedures used to derive national BAFs should be selected based on the guidance presented in the Bioaccumulation TSD.

4. **Determining the Fraction Lipid (f_ℓ).** Calculating a baseline BAF_ℓ^{fd} for a nonionic organic chemical using Equation 5-10 also requires that the total chemical concentration measured in the tissue used to determine the field-measured BAF_T^t be normalized by the lipid fraction (f_ℓ) in that same tissue. Lipid normalization of tissue concentrations reflects the assumption that BAFs (and BCFs) for nonionic organic chemicals are directly proportional to the percent lipid in the tissue upon which they are based. This assumption means that an organism with a two percent lipid content would be expected to accumulate twice the amount of a chemical at steady state compared with an organism with one percent lipid content, all else being equal. The assumption that aquatic organisms accumulate nonionic organic chemicals in proportion to their lipid content has been extensively evaluated in the literature (Mackay, 1982; Connell, 1988; Barron, 1990) and is generally accepted. Because the lipid content in aquatic organisms can vary both within and across species, BAFs that are expressed using the lipid-normalized concentration (rather than the total concentration in tissue) are considered to be the most reliable for aggregating multiple BAF values for a given species. Additional discussion of technical basis, assumptions, and uncertainties involved in lipid normalization is provided in the Bioaccumulation TSD.

a. The lipid fraction f_ℓ, is routinely reported in bioaccumulation studies involving nonionic organic chemicals. If the lipid fraction is not reported in the BAF study, it can be calculated using the following equation if the appropriate data are reported:

$$f_\ell = \frac{M_\ell}{M_t} \qquad \text{(Equation 5-13)}$$

where:

M_ℓ = Mass of lipid in specified tissue
M_t = Mass of specified tissue (wet weight)

b. Because lipid content can vary within an aquatic organism (and among tissues within that organism) due to several factors including the age and sex of the organism, changes in dietary composition, season of sampling and reproductive status, the lipid fraction used to calculate a baseline BAF_ℓ^{fd} should be measured in

the same tissue and organisms used to determine the field-measured BAF_T^t, unless comparability is demonstrated across organisms.

c. Experience has shown that different solvent systems used to extract lipids for analytical measurement can result in different quantities of lipids being extracted and measured in aquatic organisms (e.g., Randall et al.,1991, 1998). As a result, lipid measurements determined using different solvent systems might lead to apparent differences in lipid-normalized concentrations and lipid-normalized BAFs. The extent to which different solvent systems might affect lipid extractions (and lipid-normalized concentrations) is thought to vary depending on the solvent, chemical of concern, and lipid composition of the tissue being extracted. Guidance on measurement of lipid content, including the choice of solvent system and how different solvent systems may affect lipid content, is provided in the Bioaccumulation TSD.

B. Baseline BAF_ℓ^{fd} Derived from BSAFs

The second method of determining a baseline BAF_ℓ^{fd} for the chemical of concern in Procedure #1 involves the use of BSAFs. Although BSAFs may be used for measuring and predicting bioaccumulation directly from concentrations of chemicals in surface sediment, they may also be used to estimate BAFs (USEPA, 1995b; Cook and Burkhard, 1998). Since BSAFs are based on field data and incorporate effects of chemical bioavailability, food web structure, metabolism, biomagnification, growth, and other factors, BAFs estimated from BSAFs will incorporate the net effect of all these factors. The BSAF approach is particularly beneficial for developing water quality criteria for chemicals which are detectable in fish tissues and sediments, but are difficult to detect or measure precisely in the water column.

As shown by Equation 5-14 below, predicting baseline BAF_ℓ^{fd}s using BSAFs requires that certain types of data be used for the chemicals of interest (for which BAFs are to be determined) and reference chemicals (for which BAFs are measured) from a common sediment-water-organism data set. Differences between BSAFs for different organic chemicals are good measures of the relative bioaccumulation potentials of the chemicals. When calculated from a common organism-sediment sample set, chemical-specific differences in BSAFs reflect the net effect of biomagnification, metabolism, food chain, bioenergetics, and bioavailability factors on the degree of each chemical's equilibrium/disequilibrium between sediment and biota. At equilibrium, BSAFs are expected to be approximately 1.0. However, deviations from 1.0 (reflecting disequilibrium) are common due to: conditions where water is not at equilibrium with surface sediment; differences in organic carbon content of water and sediment; kinetic limitations for chemical transfer between sediments and water associated with specific biota; biomagnification; or biological processes such as growth or biotransformation. BSAFs are most useful (i.e., most predictable from one site to another) when measured under steady-state (or near steady-state) conditions. The use of non-steady-state BSAFs, such as found with new chemical loadings or rapid increases in loadings, increases uncertainty in this method for the relative degree of disequilibrium between the reference chemicals and the chemicals of interest. In general, the fact that concentrations of hydrophobic chemicals in sediment are less sensitive than concentrations in water to fluctuations in chemical loading and distribution makes the BSAF

method robust for estimating BAFs. Results from validation of the BAF procedure in Lake Ontario, the Fox River and Green Bay, Wisconsin, and the Hudson River, New York, demonstrate good agreement between observed and BSAF-predicted BAFs in the vast majority of comparisons made. Detailed results of the validation studies for the BSAF procedure are provided in the Bioaccumulation TSD.

Baseline BAF_{ℓ}^{fd}s should be calculated using acceptable BSAFs for chemicals of interest and appropriate sediment-to-water fugacity (disequilibrium) ratios $(\prod_{socw})_r/(K_{ow})_r$ for reference chemicals under the following guidelines.

1. **Baseline BAF_{ℓ}^{fd} Equation.** For each species with an acceptable field measured $(BSAF)_I$, a baseline BAF_{ℓ}^{fd} for the chemical of interest may be calculated using the following equation with an appropriate value of $(\prod_{socw})_r/(K_{ow})_r$:

$$(Baseline\ BAF_{\ell}^{fd})_i = (BSAF)_i \frac{(D_{i/r})\ (\prod_{socw})_r\ (K_{ow})_i}{(K_{ow})_r} \qquad \text{(Equation 5-14)}$$

where:

$(\text{Baseline } BAF_{\ell}^{fd})_I$	=	BAF expressed on a freely dissolved and lipid-normalized basis for chemical of interest "I"
$(BSAF)_I$	=	Biota-sediment accumulation factor for chemical of interest "I"
$(\prod_{socw})_r$	=	sediment organic carbon to water freely dissolved concentration ratio of reference chemical "r"
$(K_{ow})_I$	=	octanol-water partition coefficient for chemical of interest "I"
$(K_{ow})_r$	=	octanol-water partition coefficient for the reference chemical "r"
$D_{i/r}$	=	ratio between $\prod_{socw}/K_{ow}$ for chemicals "I" and "r" (normally chosen so that $D_{i/r} = 1$)

The technical basis, assumptions, and uncertainties associated with Equation 5-14 are provided in the Bioaccumulation TSD. Guidance for determining each component of Equation 5-14 is provided below.

2. **Determining Field-Measured BSAFs.** BSAFs should be determined by relating lipid-normalized concentrations of chemicals in an organism (C_{ℓ}) to organic carbon-normalized concentrations of the chemicals in surface sediment samples (C_{soc}) using the following equation:

$$BSAF = \frac{C_{\ell}}{C_{soc}} \qquad \text{(Equation 5-15)}$$

a. **Lipid-Normalized Concentration.** The lipid-normalized concentration of a chemical in an organism should be determined by:

$$C_{\ell} = \frac{C_t}{f_{\ell}} \qquad \text{(Equation 5-16)}$$

where:

C_t = Concentration of the chemical in the wet tissue (either whole organism or specified tissue) (µg/g)
f_{ℓ} = Fraction lipid content in the tissue

b. **Organic Carbon-Normalized Concentration.** The organic carbon-normalized concentration of a chemical in sediment should be determined by:

$$C_{soc} = \frac{C_s}{f_{oc}} \qquad \text{(Equation 5-17)}$$

where:

C_s = Concentration of chemical in sediment (µg/g sediment)
f_{oc} = Fraction organic carbon in sediment

The organic carbon-normalized concentrations of the chemicals in surface sediment samples should be associated with the average exposure environment of the organism.

3. **Sediment-to-Water Partition Coefficient** $(\prod_{socw})_r$. Sediment-to-water partition coefficients for reference chemicals should be determined by:

$$(\prod_{socw})_r = \frac{(C_{soc})_r}{(C_w^{fd})_r} \qquad \text{(Equation 5-18)}$$

where:

$(C_{soc})_r$ = Concentration of a reference chemical in sediment normalized to sediment organic carbon
$(C_w^{fd})_r$ = Concentration of the reference chemical freely dissolved in water

4. **Selecting Reference Chemicals.** Reference chemicals with $(\prod_{socw})$ / (K_{ow}) similar to that of the chemical of interest are preferred for this method. Theoretically, knowledge of the

difference between sediment-to-water fugacity ratios for two chemicals, "I" and "r" ($D_{i/r}$), could be used when reliable reference chemicals that meet the fugacity equivalence condition are not available. Similarity of $(\prod_{socw}) / (K_{ow})$ for two chemicals can be indicated on the basis of similar physical-chemical behavior in water (persistence, volatilization), similar mass loading histories, and similar concentration profiles in sediment cores.

Validation studies have demonstrated that choosing reference chemicals with well quantified concentrations in water is important because the uncertainty associated with measurement of barely detected chemicals is large (see the Bioaccumulation TSD). Similarity between K_{ow} values of the reference and target chemicals is generally desirable, although recent validation studies indicate that the accuracy of the method is not substantially decreased through use of reference chemicals with large differences in K_{ow}, as long as the chemicals are structurally similar and have similar persistence behavior in water and sediments.

5. The following data, procedural, and quality assurance requirements should be met for predicting baseline BAF_{ℓ}^{fd}s using field-measured BSAFs:

   a. Data on the reference chemicals and chemicals of interest should come from a common organism-water-sediment data set at a particular site.

   b. The chemicals of interest and reference chemicals should have similar physicochemical properties and persistence in water and sediment.

   c. The loadings history of the reference chemicals and chemicals of interest should be similar such that their expected sediment-water disequilibrium ratios $(\prod_{socw}/K_{ow})$ would not be expected to be substantially different (i.e., $D_{i/r} \sim 1$).

   d. The use of multiple reference chemicals is generally preferred for determining the value of $(\prod_{socw})_r$ so long as the concentrations are well quantified and the aforementioned conditions for selecting reference chemicals are met. In some cases, use of a single reference chemical may be necessary because of limited data.

   e. Samples of surface sediments (0-1 cm is ideal) should be from locations in which sediment is regularly deposited and is representative of average surface sediment in the vicinity of the organism.

   f. The K_{ow} value for the target and reference chemicals should be selected as described in the Bioaccumulation TSD.

   g. All other data quality and procedural guidelines described earlier for determining field-measured BAFs in Section 5.4.3.1(A) should be met.

Further details on the requirements for predicting BAFs from BSAF measurements, including the data, assumptions, and limitations of this approach are provided in the Bioaccumulation TSD.

C. Baseline BAF_{ℓ}^{fd} from a Laboratory-Measured BCF_T^t and FCM

The third method in Procedure #1 consists of using a laboratory-measured BCF_T^t (i.e., a BCF based on total concentrations in tissue and water) and FCMs to predict a baseline BAF_{ℓ}^{fd} for the chemical of concern. The BCF_T^t is used in conjunction with an FCM because non-aqueous routes of exposure and subsequent biomagnification is of concern for the types of chemicals applicable to Procedure #1. A laboratory-measured BCF inherently accounts for the effects of chemical metabolism that occurs in the organism used to calculate the BCF, but does not account for metabolism which may occur in other organisms of the aquatic food web.

1. **Baseline BAF_{ℓ}^{fd} Equation.** For each acceptable laboratory-measured BCF_T^t, calculate a baseline BAF_{ℓ}^{fd} using the following equation:

$$\text{Baseline } BAF_{\ell}^{fd} = (FCM) \cdot \left[\frac{\text{Measured } BCF_T^t}{f_{fd}} - 1 \right] \cdot \left(\frac{1}{f_{\ell}} \right) \qquad \text{(Equation 5-19)}$$

where:

Baseline BAF_{ℓ}^{fd}	=	BAF expressed on a freely dissolved and lipid-normalized basis
Measured BCF_T^t	=	BCF based on total concentration in tissue and water
f_{ℓ}	=	Fraction of the tissue that is lipid
f_{fd}	=	Fraction of the total chemical in the test water that is freely dissolved
FCM	=	The food chain multiplier either obtained from Table 5-1 by linear interpolation for the appropriate trophic level, or from appropriate field data

The technical basis for Equation 5-19 is provided in the Bioaccumulation TSD. Guidance for determining each component of Equation 5-19 is provided below.

2. **Determining the Measured BCF_T^t.** The laboratory-measured BCF_T^t shown in Equation 5-19 should be calculated using information on the total concentration of the chemical in the tissue of the organism and the total concentration of the chemical in the laboratory test water. The equation to derive a measured BCF_T^t is:

$$\text{Measured } BCF_T^t = \frac{C_t}{C_w} \qquad \text{(Equation 5-20)}$$

where:

C_t = Total concentration of the chemical in the specified wet tissue
C_w = Total concentration of chemical in the laboratory test water

The data used to calculate a laboratory-measured BCF_T^t should be reviewed thoroughly to assess the quality of the data and the overall uncertainty in the BCF value. The following general criteria apply in determining the acceptability of laboratory-measured BCF_T^t.

a. The test organism should not be diseased, unhealthy, or adversely affected by the concentration of the chemical because these attributes may alter accumulation of chemicals compared with healthy organisms.

b. The total concentration of the chemical in the water should be measured and should be relatively constant during the exposure period.

c. The organisms should be exposed to the chemical using a flow-through or renewal procedure.

d. The percent lipid of the tissue used to normalize the BCF_T^t should be either measured or reliably estimated to permit lipid normalization of chemical concentrations.

e. The concentrations of particulate organic carbon and dissolved organic carbon in the study water should be measured or reliably estimated.

f. Aquatic organisms used to calculate a laboratory-measured BCF_T^t should be representative of those aquatic organisms that are commonly consumed in the United States. An aquatic organism which is not commonly consumed in the United States can be used to calculate an acceptable laboratory-measured BCF_T^t provided that the organism is considered to be a reasonable surrogate for a commonly consumed organism. Information on the ecology, physiology, and biology of the organism should be reviewed when assessing whether an organism is a reasonable surrogate of a commonly consumed organism.

g. BCFs may be based on measurement of radioactivity from radiolabeled parent compounds only when the BCF is intended to include metabolites, when there is confidence that there is no interference due to metabolites of the parent compounds, or when studies are conducted to determine the extent of metabolism, thus allowing for a proper correction.

h. The calculation of the BCF_T^t should appropriately address growth dilution, which can be particularly important in affecting BCF_T^t determinations for poorly depurated chemicals.

I. Other aspects of the methodology used should be similar to those described by the American Society of Testing and Materials (ASTM, 1999) and USEPA *Ecological Effects Test Guidelines* (USEPA, 1996).

j. In addition, the magnitude of the K_{ow} and the availability of corroborating BCF data should be considered. For example, if the steady-state method is used for the BCF_T^t determination, exposure periods longer than 28 days will generally be required for highly hydrophobic chemicals to reach steady state between the water and the organism.

k. If a baseline BCF_ℓ^{fd} derived from a laboratory-measured BCF_T^t consistently increases or decreases as the chemical concentration increases in the test solutions for the test organisms, the BCF_T^t should be selected from the test concentration(s) that would most closely correspond to the 304(a) criterion. Note: a BCF_T^t should not be calculated from a control treatment.

3. **Selecting Food Chain Multipliers.** An FCM reflects a chemical's tendency to biomagnify in the aquatic food web. Values of FCMs greater than 1.0 are indicative of biomagnification and typically apply to organic chemicals with log K_{ow} values between 4.0 and 9.0. For a given chemical, FCMs tend to be greater at higher trophic levels, although FCMs for trophic level three can be higher than those for trophic level four.

Food chain multipliers used to derive baseline BAF_ℓ^{fd}s using Procedure #1 can be selected from model-derived or field-derived estimates.

a. **Model-Derived FCMs.** For nonionic organic chemicals appropriate for Procedure #1, EPA has calculated FCMs for various K_{ow} values and trophic levels using the bioaccumulation model of Gobas (1993). The FCMs shown in Table 5-1 were calculated using the Gobas model as the ratio of the baseline BAF_ℓ^{fd}s for trophic levels 2, 3, and 4 to the baseline BCF_ℓ^{fd}.

EPA recommends using the biomagnification model by Gobas (1993) to derive FCMs for nonionic organic chemicals for several reasons. First, the Gobas model includes both benthic and pelagic food chains, thereby incorporating exposure of organisms to chemicals from both the sediment and the water column. Second, the input data needed to run the model can be readily defined. Third, the predicted BAFs using the model are in agreement with field-measured BAFs for chemicals, even those with very high log K_{ow}s. Finally, the model predicts chemical residues in benthic organisms using equilibrium partitioning theory, which is consistent with EPA's equilibrium partitioning sediment guidelines (USEPA, 2000d).

The Gobas model requires input of specific data on the structure of the food chain and the water quality characteristics of the water body of interest. For calculating national BAFs, a mixed pelagic/benthic food web structure consisting of four trophic levels is assumed. Trophic level 1 is phytoplankton, trophic level 2 is

zooplankton, trophic level 3 is forage fish (e.g., sculpin and smelt), and trophic level 4 are predatory fish (e.g., salmonids). Additional assumptions are made regarding the composition of the aquatic species' diets (e.g., salmonids consume 10 percent sculpin, 50 percent alewives, and 40 percent smelt), the physical parameters of the aquatic species (e.g., lipid values), and the water quality characteristics (e.g., water temperature, sediment organic carbon).

A mixed pelagic/benthic food web structure has been assumed for the purpose of calculating FCMs because it is considered to be most representative of the types of food webs that occur in aquatic ecosystems. FCMs derived using the mixed pelagic/benthic structure are also about mid-range in magnitude between a 100% pelagic and 100% benthic driven food web (see the Bioaccumulation TSD). The validity of FCMs derived using the mixed pelagic/benthic food web structure has

Table 5-1
Food-Chain Multipliers for Trophic Levels 2, 3 and 4
(Mixed Pelagic and Benthic Food Web Structure and $\prod_{socw} / K_{OW} = 23$)

Log K_{OW}	Trophic Level 2	Trophic Level 3	Trophic Level 4	Log K_{OW}	Trophic Level 2	Trophic Level 3	Trophic Level 4
4.0	1.00	1.23	1.07	6.6	1.00	12.9	23.8
4.1	1.00	1.29	1.09	6.7	1.00	13.2	24.4
4.2	1.00	1.36	1.13	6.8	1.00	13.3	24.7
4.3	1.00	1.45	1.17	6.9	1.00	13.3	24.7
4.4	1.00	1.56	1.23	7.0	1.00	13.2	24.3
4.5	1.00	1.70	1.32	7.1	1.00	13.1	23.6
4.6	1.00	1.87	1.44	7.2	1.00	12.8	22.5
4.7	1.00	2.08	1.60	7.3	1.00	12.5	21.2
4.8	1.00	2.33	1.82	7.4	1.00	12.0	19.5
4.9	1.00	2.64	2.12	7.5	1.00	11.5	17.6
5.0	1.00	3.00	2.51	7.6	1.00	10.8	15.5
5.1	1.00	3.43	3.02	7.7	1.00	10.1	13.3
5.2	1.00	3.93	3.68	7.8	1.00	9.31	11.2
5.3	1.00	4.50	4.49	7.9	1.00	8.46	9.11
5.4	1.00	5.14	5.48	8.0	1.00	7.60	7.23
5.5	1.00	5.85	6.65	8.1	1.00	6.73	5.58
5.6	1.00	6.60	8.01	8.2	1.00	5.88	4.19
5.7	1.00	7.40	9.54	8.3	1.00	5.07	3.07
5.8	1.00	8.21	11.2	8.4	1.00	4.33	2.20
5.9	1.00	9.01	13.0	8.5	1.00	3.65	1.54
6.0	1.00	9.79	14.9	8.6	1.00	3.05	1.06
6.1	1.00	10.5	16.7	8.7	1.00	2.52	0.721
6.2	1.00	11.2	18.5	8.8	1.00	2.08	0.483
6.3	1.00	11.7	20.1	8.9	1.00	1.70	0.320
6.4	1.00	12.2	21.6	9.0	1.00	1.38	0.210
6.5	1.00	12.6	22.8				

been evaluated in several different ecosystems including Lake Ontario, the tidally influenced Bayou D'Inde in Louisiana, the Fox River and Green Bay, Wisconsin, and the Hudson River in New York. Additional details of the validation of EPA's national default FCMs and the assumptions, uncertainties, and input parameters for the model are provided in the Bioaccumulation TSD.

Although EPA uses the FCMs in Table 5-1 to derive its national 304(a) criteria, EPA recognizes that food webs of other waterbodies might differ from the assumptions used to calculate national BAFs. In these situations, States and authorized Tribes may wish to use alternate food web structures for calculating FCMs for use in setting State or Tribal water quality criteria. Additional guidance on the use of alternate food web structures for calculating State, Tribal, or site-specific criteria is provided in the Bioaccumulation TSD.

b. **Field-Derived FCMs.** In addition to model-derived estimates of FCMs, field data may also be used to derive FCMs. Currently, the use of field-derived FCMs is the only method recommended for estimating FCMs for inorganic and organometalic chemicals because appropriate model-derived estimates are not yet available (see Section 5.6). In contrast to the model-based FCMs described previously, field-derived FCMs account for any metabolism of the chemical of concern by the aquatic organisms used to calculate the FCM.

Field-derived FCMs should be calculated using lipid-normalized concentrations of the nonionic organic chemical in appropriate predator and prey species using the following equations.

$$FCM_{TL2} = BMF_{TL2} \quad \text{(Equation 5-21)}$$

$$FCM_{TL3} = (BMF_{TL3})\,(BMF_{TL2}) \quad \text{(Equation 5-22)}$$

$$FCM_{TL4} = (BMF_{TL4})\,(BMF_{TL3})\,(BMF_{TL2}) \quad \text{(Equation 5-23)}$$

where:

FCM = Food chain multiplier for designated trophic level (TL2, TL3, or TL4)
BMF = Biomagnification factor for designated trophic level (TL2, TL3, or TL4)

The basic difference between FCMs and BMFs is that FCMs relate back to trophic level one (or trophic level two as assumed by the Gobas (1993) model), whereas BMFs always relate back to the next lowest trophic level. For nonionic organic chemicals, BMFs can be calculated from tissue residue concentrations determined in biota at a site according to the following equations.

$$BMF_{TL2} = (C_{\ell, TL2}) / (C_{\ell, TL1}) \quad \text{(Equation 5-24)}$$

$$BMF_{TL3} = (C_{\ell, TL3}) / (C_{\ell, TL2}) \quad \text{(Equation 5-25)}$$

$$BMF_{TL4} = (C_{\ell, TL4}) / (C_{\ell, TL3}) \quad \text{(Equation 5-26)}$$

where:

C_ℓ = Lipid-normalized concentration of chemical in tissue of appropriate biota that occupy the specified trophic level (TL2, TL3, or TL4)

In addition to the acceptability guidelines pertaining to field-measured BAFs, the following procedural and quality assurance requirements apply to field-measured FCMs.

(1) Information should be available to identify the appropriate trophic levels for the aquatic organisms and appropriate predator-prey relationships for the site from which FCMs are being determined. General information on determining trophic levels of aquatic organisms can be found in USEPA 2000a,b,c.

(2) The aquatic organisms sampled from each trophic level should reflect the most important exposure pathways leading to human exposure via consumption of aquatic organisms. For higher trophic levels (e.g., 3 and 4), aquatic species should also reflect those that are commonly consumed by humans.

(3) The studies from which the FCMs are derived should contain sufficient supporting information from which to determine that tissue samples were collected and analyzed using appropriate, sensitive, accurate, and precise methods.

(4) The percent lipid should be either measured or reliably estimated for the tissue used to determine the FCM.

(5) The tissue concentrations should reflect average exposure over the approximate time required to achieve steady-state in the target species.

D. Baseline BAF_ℓ^{fd} from a K_{ow} and FCM

The fourth method in Procedure #1 consists of using a K_{ow} and an appropriate FCM for estimating the baseline BAF_ℓ^{fd}. In this method, the K_{ow} is assumed to be equal to the baseline BCF_ℓ^{fd}. Numerous investigations have demonstrated a linear relationship between the logarithm of the BCF and the logarithm of the octanol-water partition coefficient (K_{ow}) for organic chemicals for fish and other aquatic organisms. Isnard and Lambert (1988) list various regression equations that illustrate this linear relationship. When the regression equations are constructed using lipid-normalized BCFs, the slopes and intercepts are not significantly different from one and zero, respectively (e.g., de Wolf, et al., 1992). The underlying assumption for the linear relationship between the BCF and K_{ow} is that the bioconcentration process can be viewed as the partitioning of a chemical between the lipid of the aquatic organisms and water and that the K_{ow} is a useful surrogate for this partitioning process (Mackay, 1982). To account for biomagnification, Procedure #1 requires the K_{ow} value be used in conjunction with an appropriate FCM.

1. **Baseline BAF_{ℓ}^{fd} Equation.** For each acceptable K_{ow} value and FCM for the chemical of concern, calculate a baseline BAF_{ℓ}^{fd} using the following equation.

$$\text{Baseline } BAF_{\ell}^{fd} = (FCM) \cdot (K_{ow}) \qquad \text{(Equation 5-27)}$$

where:

Baseline BAF_{ℓ}^{fd} =		BAF expressed on a freely dissolved and lipid-normalized basis for a given trophic level
FCM	=	The food chain multiplier for the appropriate trophic level obtained from Table 5-1 by linear interpolation or from appropriate field data (used with Procedure #1 only)
K_{ow}	=	Octanol-water partition coefficient

The BCF-K_{ow} relationship has been developed primarily for nonionic organic chemicals that are not readily metabolized by aquatic organisms and thus is most appropriate for poorly-metabolized nonionic organic chemicals (i.e., Procedures #1 and #3 as depicted in Figure 5-1). For poorly-metabolized nonionic organic chemicals with large log K_{ow}s (i.e., > 6), reported log BCFs are often not equal to log K_{ow}. EPA believes that this nonlinearity is primarily due to not accounting for several factors which affect the BCF determination. These factors include not basing BCFs on the freely dissolved concentration in water, not accounting for growth dilution, not assessing BCFs at steady-state, inaccuracies in measurements of uptake and elimination rate constants, and complications from the use of solvent carriers in the exposure. Application of Equation 5-27 for predicting BAFs has been conducted in several different ecosystems including Lake Ontario, the tidally influenced Bayou D'Inde in Louisiana, the Fox River and Green Bay, Wisconsin, and the Hudson River in New York. Additional detail on the validation, technical basis, assumptions, and uncertainty associated with Equation 5-27 and is provided in the Bioaccumulation TSD.

2. **FCMs and K_{ow}s**. Food chain multipliers and K_{ow} values should be selected as described previously in Procedure #1.

5.4.3.2 Selecting Final Baseline BAF_{ℓ}^{fd}s

After calculating individual baseline BAF_{ℓ}^{fd}s using as many of the methods in Procedure #1 as possible, the next step is to determine a final baseline BAF_{ℓ}^{fd} for each trophic level from the individual baseline BAF_{ℓ}^{fd}s (see Figures 5-1 and 5-2). The final baseline BAF_{ℓ}^{fd} will be used in the last step to determine the national BAF for each trophic level. The final baseline BAF_{ℓ}^{fd} for each trophic level should be determined from the individual baseline BAF_{ℓ}^{fd}s by considering the data preference hierarchy defined by Procedure #1 and uncertainty in the data. The data preference hierarchy for Procedure #1 is (in order of preference):

1. a baseline BAF_{ℓ}^{fd} from an acceptable field-measured BAF (method 1)

2. a baseline BAF_{ℓ}^{fd} predicted from an acceptable field-measured BSAF (method 2),
3. a baseline BAF_{ℓ}^{fd} predicted from an acceptable BCF and FCM (method 3), or
4. a baseline BAF_{ℓ}^{fd} predicted from an acceptable K_{ow} and FCM (method 4).

This data preference hierarchy reflects EPA's preference for BAFs based on field-measurements of bioaccumulation (methods 1 and 2) over those based on laboratory-measurements and/or predictions of bioaccumulation (methods 3 and 4). However, this data preference hierarchy should not be considered inflexible. Rather, it should be used as a guide for selecting the final baseline BAF_{ℓ}^{fd}s when the uncertainty is similar among two or more baseline BAF_{ℓ}^{fd}s derived using different methods. The following steps and guidelines should be followed for selecting the final baseline BAF_{ℓ}^{fd}s using Procedure #1.

1. **Calculate Species-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method where more than one acceptable baseline BAF_{ℓ}^{fd} is available for a given species, calculate a species-mean baseline BAF_{ℓ}^{fd} as the geometric mean of all available individual baseline BAF_{ℓ}^{fd}s. When calculating a species-mean baseline BAF_{ℓ}^{fd}, individual baseline BAF_{ℓ}^{fd}s should be reviewed carefully to assess the uncertainty in the BAF values. For highly hydrophobic chemicals applicable to Procedure #1, particular attention should be paid to whether sufficient spatial and temporal averaging of water and tissue concentrations was likely achieved in the BAF, BSAF, or BCF study. Highly uncertain baseline BAF_{ℓ}^{fd}s should not be used. Large differences in individual baseline BAF_{ℓ}^{fd}s for a given species (e.g., greater than a factor of 10) should be investigated further. In such cases, some or all of the baseline BAF_{ℓ}^{fd}s for a given species might not be used. Additional discussion on evaluating acceptability of BAF values is provided in the Bioaccumulation TSD.

2. **Calculate Trophic-Level-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method where more than one acceptable species-mean baseline BAF_{ℓ}^{fd} is available within a given trophic level, calculate a trophic-level-mean baseline BAF_{ℓ}^{fd} as the geometric mean of acceptable species-mean baseline BAF_{ℓ}^{fd}s in that trophic level. Trophic-level-mean baseline BAF_{ℓ}^{fd}s should be calculated for trophic levels two, three, and four because available data on U.S. consumers of fish and shellfish indicate significant consumption of organisms in these trophic levels.

3. **Select a Final Baseline BAF_{ℓ}^{fd} for Each Trophic Level.** For each trophic level, select the final baseline BAF_{ℓ}^{fd} using best professional judgment by considering: (1) the data preference hierarchy shown previously, (2) the relative uncertainty in the trophic-level-mean baseline BAF_{ℓ}^{fd}s derived using different methods, and (3) the weight of evidence among the four methods.

    a. In general, when more than one trophic-level-mean baseline BAF_{ℓ}^{fd} is available for a given trophic level, the final trophic-level-mean baseline BAF_{ℓ}^{fd} should be selected from the most preferred BAF method defined by the data preference hierarchy for Procedure #1.

    b. If uncertainty in a trophic-level-mean baseline BAF based on a higher tier (more preferred) method is judged to be substantially greater than a trophic-level-mean

baseline BAF from a lower tier method, and the weight of evidence among the various methods suggests that a BAF value from lower tier method is likely to be more accurate, then the final baseline BAF_{ℓ}^{fd} should be selected using a trophic level-mean baseline BAF_{ℓ}^{fd} from a lower tier method.

c. When considering the weight of evidence among the various BAF methods, greater confidence in the final baseline BAF_{ℓ}^{fd} is generally assigned when BAFs from a greater number of methods are in agreement for a given trophic level. However, lack of agreement among methods does not necessarily indicate less confidence if such disagreements can be adequately explained. For example, if the chemical of concern is metabolized by aquatic organisms represented by a BAF value, one would expect disagreement between a field-measured BAF (the highest priority data) and a predicted BAF using a K_{ow} and model-derived FCM. Thus, field-measured BAFs should generally be given the greatest weight among methods because they reflect direct measures of bioaccumulation and incorporate any metabolism which might occur in the organism and its food web.

d. The above steps should be performed for each trophic level until a final baseline BAF_{ℓ}^{fd} is selected for trophic levels two, three, and four.

5.4.3.3 Calculating National BAFs

The last step in deriving a national BAF for each trophic level is to convert the final baseline BAF_{ℓ}^{fd} determined in the previous step to a BAF that reflects conditions to which the national 304(a) criteria will apply (Figure 5-2). Since a baseline BAF_{ℓ}^{fd} is by definition normalized by lipid content and expressed on a freely dissolved basis, it needs to be adjusted to reflect the lipid fraction of aquatic organisms commonly consumed in the U.S. and the freely dissolved fraction expected in U.S. bodies of water. Converting a final baseline BAF_{ℓ}^{fd} to a national BAF requires information on: (1) the percent lipid of the aquatic organisms commonly consumed by humans, and (2) the freely dissolved fraction of the chemical of concern that would be expected in the ambient waters of interest. For each trophic level, a national BAF should be determined from a final baseline BAF_{ℓ}^{fd} according to the following guidelines.

1. **National BAF Equation.** For each trophic level, calculate a national BAF using the following equation.

$$\text{National BAF}_{(TL\ n)} = [(\text{Final Baseline BAF}_{\ell}^{fd})_{TL\ n} \cdot (f_{\ell})_{TL\ n} + 1] \cdot (f_{fd}) \qquad \text{(Equation 5-28)}$$

where:

Final Baseline BAF_{ℓ}^{fd} = Final trophic-level-mean baseline BAF expressed on a freely dissolved and lipid-normalized basis for trophic level "n"

$f_{\ell(TL\ n)}$	=	Lipid fraction of aquatic species consumed at trophic level "n"
f_{fd}	=	Fraction of the total chemical in water that is freely dissolved

The technical basis of Equation 5-28 is provided in the Bioaccumulation TSD. Guidance for determining each component of Equation 5-28 is provided below.

2. **Determining the Final Baseline BAF_{ℓ}^{fd}.** The final trophic-level-mean baseline BAF_{ℓ}^{fd}s used in this equation are those which have been determined using the guidance presented in Section 5.4.3.2 for selecting the final baseline BAF_{ℓ}^{fd}s.

3. **Lipid Content of Commonly Consumed Aquatic Species.** As illustrated by Equation 5-28, the percent lipid of the aquatic species consumed by humans is needed to accurately characterize the potential exposure to a chemical from ingestion of aquatic organisms.

   a. **National Default Lipid Values.** For the purposes of calculating a national 304(a) criterion, the following national default values for lipid fraction should be used: 1.9% (for trophic level two organisms), 2.6% (for trophic level three organisms), and 3.0% (for trophic level four organisms).

      These national default values for lipid content reflect national per capita average patterns of fish consumption in the United States. Specifically, they were calculated using the consumption-weighted mean lipid content of commonly consumed fish and shellfish as identified by the USDA Continuing Survey of Food Intake by Individuals (CSFII) for 1994 through 1996. This same national survey data was used to derive national default values of fish consumption. To maintain consistency with the fish consumption assumptions, only freshwater and estuarine organisms were included in the derivation of the national default lipid values. Additional details on the technical basis, assumptions, and uncertainty in the national default values of lipid fraction are provided in the Bioaccumulation TSD.

      Although national default lipid values are used by EPA to set national 304(a) criteria, EPA encourages States and authorized Tribes to use local or regional data on lipid content of consumed aquatic species when adopting criteria into their water quality standards because local or regional consumption patterns (and lipid content) can differ from national consumption patterns. Additional guidance on developing site-specific values of lipid content, including a database of lipid content for many commonly consumed aquatic organisms, is found in the Bioaccumulation TSD.

4. **Freely Dissolved Fraction.** The third piece of information required for deriving a national BAF is the freely dissolved fraction of the chemical of concern that is expected

in waters of the United States. As noted previously, expressing BAFs on the freely dissolved concentration in water allows a common basis for averaging BAFs from several studies. However, for use in criteria development, these BAFs should be converted back to values based on the total concentration in the water to be consistent with monitored water column and effluent concentrations, which are typically based on total concentrations of chemicals in the water. This should be done by multiplying the freely dissolved baseline BAF_{ℓ}^{fd} by the fraction of the freely dissolved chemical expected in water bodies of the United States where criteria are to be applied, as shown in Equation 5-29.

$$f_{fd} = \frac{1}{[1 + (POC \cdot K_{ow}) + (DOC \cdot 0.08 \cdot K_{ow})]} \qquad \text{(Equation 5-29)}$$

where:

POC	=	national default value for the particulate organic carbon concentration (kg/L)
DOC	=	national default value for the dissolved organic carbon concentration (kg/L)
K_{ow}	=	n-octanol water partition coefficient for the chemical

Equation 5-29 is identical to Equation 5-12, which was used to determine the freely dissolved fraction for deriving baseline BAF_{ℓ}^{fd}s from field-measured BAFs. However, the POC and DOC concentrations used in Equation 5-29 reflect those values that are expected in U.S. bodies of water, not the POC and DOC values in the study water used to derive the BAF. Guidance for determining each component of Equation 5-29 follows.

a. **National Default Values of POC and DOC.** For estimating the freely dissolved fraction of the chemical of concern that is expected in U.S. water bodies, national default values of 0.5 mg/L (5×10^{-7} kg/L) for POC and 2.9 mg/L (2.9×10^{-6} kg/L) for DOC should be used. These values are 50th percentile values (medians) based on an analysis of over 110,000 DOC values and 85,000 POC values contained in EPA's STORET database from 1980 through 1999. These default values reflect a combination of values for streams, lakes and estuaries across the United States. Additional details on the technical basis, assumptions, and uncertainty in the derivation and application of the national default values of POC and DOC are provided in the Bioaccumulation TSD.

   Although national default values of POC and DOC concentrations are used by EPA to set national 304(a) criteria as described by this document, EPA encourages States and authorized Tribes to use local or regional data on POC and DOC when adopting criteria into their water quality standards. EPA encourages States and Tribes to consider local or regional data on POC and DOC because local or regional conditions may result in differences in POC or DOC

concentrations compared with the values used as national defaults. Additional guidance on developing local or regional values of POC and DOC, including a database of POC and DOC values segregated by waterbody type, is found in the Bioaccumulation TSD.

b. **K_{ow} Value.** The value selected for the K_{ow} of the chemical of concern should be the same value used in earlier calculations (e.g., for calculating baseline BAF_{ℓ}^{fd}s and FCMs). Guidance for selecting the K_{ow} value is found in the Bioaccumulation TSD.

5.4.4 Deriving National BAFs Using Procedure #2

This section provides guidance for calculating national BAFs for nonionic organic chemicals using Procedure #2 shown in Figure 5-1. The types of nonionic organic chemicals for which Procedure #2 is most appropriate are those that are classified as moderately to highly hydrophobic and subject to high rates of metabolism by aquatic biota (see Section 5.4.2 above). Non-aqueous contaminant exposure and subsequent biomagnification in aquatic food webs are not generally of concern for chemicals that are classified in this category. As a result, FCMs are not used in this procedure. In addition, K_{ow} -based predictions of bioconcentration are not used in this procedure since the K_{ow} /BCF relationship is primarily based on poorly metabolized chemicals. Some nonionic organic chemicals for which Procedure #2 is probably appropriate include certain PAHs which are believed to be metabolized substantially by fish (e.g., benzo[a]pyrene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene and chrysene/triphenylene; USEPA, 1980; Burkhard and Lukasewycz, 2000).

According to Procedure #2, the following three methods can be used in deriving a national BAF:

- using a BAF from an acceptable field study (i.e., a field-measured BAF) (method 1),
- predicting a BAF from an acceptable BSAF (method 2), and
- predicting a BAF from an acceptable BCF (method 3).

Each of these three methods relies on measured data for assessing bioaccumulation and therefore, includes the effects of chemical metabolism by the study organism in the BAF estimate. The field-measured BAF and BSAF methods also incorporate any metabolism which occurs in the aquatic food web.

As shown in Figure 5-2, the next steps in deriving a national BAF after selecting the derivation procedure are: (1) calculating individual baseline BAF_{ℓ}^{fd}s, (2) selecting the final baseline BAF_{ℓ}^{fd}s, and (3) calculating the national BAFs. Each of these three steps is discussed separately below.

5.4.4.1 Calculating Individual Baseline BAF_{ℓ}^{fd}s

As described previously in Procedure #1, calculating individual baseline BAF_{ℓ}^{fd}s involves normalizing the measured BAF_T^t or BCF_T^t (which are based on the total chemical in water and

tissue) by the lipid content of the study organisms and the freely dissolved fraction of the chemical in the study water. Converting measured BAF_T^t (or BCF_T^t) values to baseline BAF_ℓ^{fd} (or BCF_ℓ^{fd}) values is designed to account for variation in measured BAF_T^ts that is caused by differences in lipid content of study organisms and differences in the freely dissolved fraction of chemical in study waters. Therefore, baseline BAF_ℓ^{fd}s are considered more amenable for extrapolating and averaging BAFs across different species and different study waters compared with total BAF_T^ts.

1. For each species where acceptable data are available, calculate all possible baseline BAF_ℓ^{fd}s using each of the three methods shown above for Procedure #2.

2. Individual baseline BAF_ℓ^{fd}s should be calculated from field-measured BAF_T^ts, field-measured BSAFs, and laboratory BCF_T^ts according to the following procedures.

A. Baseline BAF_ℓ^{fd} from Field-Measured BAFs

1. Except where noted below, a baseline BAF_ℓ^{fd} should be calculated from a field-measured BAF_T^t using the guidance and equations outlined in Section 5.4.3.1(A) for determining baseline BAF_ℓ^{fd}s from field-measured BAFs in Procedure #1.

2. Because nonionic organic chemicals applicable to Procedure #2 have relatively high rates of metabolism in aquatic organisms, they will tend to reach steady state more quickly than nonionic organic chemicals with similar K_{ow} values but which undergo little or no metabolism. Therefore, less temporal averaging of chemical concentrations would generally be required for determining field-measured BAF_T^ts with highly metabolizable chemicals compared with chemicals that are poorly metabolized by aquatic biota.

B. Baseline BAF_{ℓ}^{fd} Derived from Field-measured BSAFs

1. A baseline BAF_{ℓ}^{fd} should be calculated from a field-measured BSAF using the guidance and equations outlined in Section 5.4.3.1(B) for determining baseline BAF_{ℓ}^{fd}s from field-measured BSAFs in Procedure #1.

C. Baseline BAF_{ℓ}^{fd} from a Laboratory-Measured BCF

1. Except where noted below, a baseline BAF_{ℓ}^{fd} should be calculated from a laboratory-measured BCF_T^t using the guidance and equations outlined in Section 5.4.3.1(c) for determining baseline BAF_{ℓ}^{fd}s from a laboratory-measured BCF and FCM in Procedure #1.

2. Because biomagnification is not an overriding concern for nonionic organic chemicals applicable to Procedure #2, food chain multipliers are not used in the derivation of a baseline BAF_{ℓ}^{fd} from a laboratory-measured BCF_T^t .

5.4.4.2 Selecting Final Baseline BAF_{ℓ}^{fd}s

After calculating individual, baseline BAF_{ℓ}^{fd}s using as many of the methods in Procedure #2 as possible, the next step is to determine a final baseline BAF_{ℓ}^{fd} for each trophic level from the individual baseline BAF_{ℓ}^{fd}s. The final baseline BAF_{ℓ}^{fd} will be used in the last step to determine the national BAF for each trophic level. A final baseline BAF_{ℓ}^{fd} for each trophic level should be determined from the individual baseline BAF_{ℓ}^{fd}s by considering the data preference hierarchy defined by Procedure #2 and uncertainty in the data. The data preference hierarchy for Procedure #2 is (in order of preference):

1. a baseline BAF_{ℓ}^{fd} from an acceptable field-measured BAF (method 1),
2. a baseline BAF_{ℓ}^{fd} from an acceptable field-measured BSAF (method 2), or
3. a baseline BAF_{ℓ}^{fd} from an acceptable laboratory-measured BCF (method 3).

This data preference hierarchy reflects EPA's preference for BAFs based on field-measurements of bioaccumulation (methods 1 and 2) over those based on laboratory-measurements (method 3). However, as explained in Procedure #1, this data preference hierarchy should not be considered inflexible. Rather, it should be used as a guide for selecting the final baseline BAF_{ℓ}^{fd}s when the underlying uncertainty is similar among two or more baseline BAF_{ℓ}^{fd}s derived using different methods. Although biomagnification is not generally a concern for chemicals subject to Procedure #2, trophic level differences in bioaccumulation might be substantial to the extent that the rate of chemical metabolism by organisms in different trophic levels differs. For example, certain PAHs have been shown to be metabolized to a much greater extent by some fish compared with some invertebrate species (James, 1989). Therefore, final baseline BAF_{ℓ}^{fd}s for chemicals applicable to Procedure #2 should be determined on a trophic-level-specific basis according to the following guidelines.

1. The final baseline BAF_{ℓ}^{fd}s in Procedure #2 should be selected according to the same steps described in Procedure #1 but with the substitution of the data preference hierarchy described above for Procedure #2. Specifically, the species-mean baseline BAF_{ℓ}^{fd}s,

trophic-level-mean baseline BAF_{ℓ}^{fd}s, and the final baseline BAF_{ℓ}^{fd}s should be determined according to the guidelines presented in Procedure #1 (Section 5.4.3.2, Steps 1, 2, and 3).

5.4.4.3 Calculating the National BAFs

As described in Procedure #1, the last step in deriving national BAFs for nonionic organic chemicals is to convert the final baseline BAF_{ℓ}^{fd}s determined in the previous step to BAFs which reflect conditions to which the national 304(a) criteria will apply (Figure 5-2).

1. For trophic levels two, three, and four, national BAFs should be calculated from the final baseline BAF_{ℓ}^{fd}s using the same equation and procedures described previously in Procedure #1 (see Section 5.4.3.3 entitled "Calculating the National BAFs").

5.4.5 Deriving National BAFs Using Procedure #3

This section provides guidance for calculating national BAFs for nonionic organic chemicals using Procedure #3 shown in Figure 5-1. The types of nonionic organic chemicals for which Procedure #3 is most appropriate are those that are classified as low in hydrophobicity (i.e., log K_{ow} values less than 4.0) and subject to low (or unknown) rates of metabolism by aquatic biota (see Section 5.4.2 above). Non-aqueous contaminant exposure and subsequent biomagnification in aquatic food webs are not generally of concern for chemicals that are classified in this category (Fisk et al., 1998; Gobas et al., 1993; Connolly and Pedersen, 1988; Thomann, 1989). As a result, FCMs are not used in this procedure.

According to Procedure #3, the following three methods can be used in deriving a national BAF:

- using a BAF from an acceptable field study (i.e., a field-measured BAF),
- predicting a BAF from an acceptable laboratory-measured BCF, and
- predicting a BAF from an acceptable K_{ow}.

After selecting the derivation procedure, the next steps in deriving a national BAF at a given trophic level for nonionic organic chemicals are: (1) calculating individual baseline BAF_{ℓ}^{fd}s, (2) selecting the final baseline BAF_{ℓ}^{fd}, and (3) calculating the national BAF (Figure 5-2). Each of these three steps is discussed separately below.

5.4.5.1 Calculating Individual Baseline BAF_{ℓ}^{fd}s

Calculating individual baseline BAF_{ℓ}^{fd}s involves normalizing each measured BAF_{T}^{t} or BCF_{T}^{t} (which are based on the total chemical in water and tissue) by the lipid content of the study organism and the freely dissolved fraction of the chemical in the study water. For additional discussion of the technical basis for calculating baseline BAF_{ℓ}^{fd}s, see Section 5.4.3.1 in Procedure #1.

1. For each species where acceptable data are available, calculate all possible baseline BAF_{ℓ}^{fd}s using each of the three methods shown above for Procedure #3.

2. An individual baseline BAF_{ℓ}^{fd} should be calculated from field-measured BAF_{T}^{t}s, laboratory-measured BCF_{T}^{t}s, and K_{ow} values according to the following procedures.

A. Baseline BAF_{ℓ}^{fd} from Field-Measured BAFs

1. Except where noted below, a baseline BAF_{ℓ}^{fd} should be calculated from a field-measured BAF_{T}^{t} using the guidance and equations outlined in Section 5.4.3.1(A) in Procedure #1.

2. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log $K_{ow} < 4.0$), nonionic organic chemicals applicable to Procedure #3 are expected to remain almost entirely in the freely dissolved form in natural waters with dissolved and particulate organic carbon concentrations typical of most field BAF studies. Therefore, the freely dissolved fraction should be assumed to be equal to 1.0, unless the concentrations of DOC and POC are very high in the field BAF study. For studies with very high DOC or POC concentrations, (e.g., about 100 mg/L or higher for DOC or 10 mg/L or higher for POC), the freely dissolved fraction may be substantially lower than 1.0 and therefore should be calculated using Equation 5-12.

3. **Temporal Averaging of Concentrations.** Also due to their low hydrophobicity, nonionic organic chemicals appropriate to Procedure #3 will also tend to reach steady state quickly compared with those chemicals to which Procedure #1 applies. Therefore, the extent of temporal averaging of tissue and water concentrations is typically much less than that required for highly hydrophobic chemicals to which Procedure #1 is applied. In addition, field studies used to calculate BAFs for these chemicals should have sampled water and tissue at similar points in time because tissue concentrations respond more rapidly to changes in water concentrations. EPA will be providing additional guidance on appropriate BAF study designs for nonionic organic chemicals (including those appropriate to Procedure #3) in its forthcoming guidance document on conducting field BAF and BSAF studies.

B. Baseline BAF_{ℓ}^{fd} from a Laboratory-Measured BCF

1. Except where noted below, a baseline BAF_{ℓ}^{fd} should be calculated from a laboratory-measured BCF_{T}^{t} using the guidance and equations outlined in Section 5.4.3.1(c) of Procedure #1.

2. **Food Chain Multipliers.** Because biomagnification is not an overriding concern for the minimally hydrophobic chemicals applicable to Procedure #3, FCMs are not used in the derivation of a baseline BAF_{ℓ}^{fd} from a laboratory-measured BCF_{T}^{t}.

3. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log $K_{ow} < 4.0$), nonionic organic chemicals to which Procedure #3 is applied are expected to remain

almost entirely in the freely dissolved form in waters containing dissolved and particulate organic carbon concentrations typical of laboratory BCF studies. Therefore, the freely dissolved fraction should usually be assumed equal to 1.0. The freely dissolved fraction will be substantially less than 1.0 only in situations where unusually high concentrations of DOC and POC are present in the laboratory BCF study (e.g., above about 100 mg/L for DOC or about 10 mg/L for POC). In this situation, the freely dissolved fraction should be calculated according to Equation 5-12.

C. Baseline BAF_{ℓ}^{fd} from a K_{ow}

1. Except where noted below, a baseline BAF_{ℓ}^{fd} should be calculated from an acceptable K_{ow} using the guidance and equations outlined in Section 5.4.3.1(D) in Procedure #1.

2. Because biomagnification is not an overriding concern for nonionic organic chemicals with low hydrophobicity (i.e., log $K_{ow} < 4.0$), food chain multipliers are not used in Procedure #3 for deriving the baseline BAF_{ℓ}^{fd} from a K_{ow}.

5.4.5.2 Selecting Final Baseline BAF_{ℓ}^{fd}s

After calculating individual baseline BAF_{ℓ}^{fd}s using as many of the methods in Procedure #3 as possible, the next step is to determine a final baseline BAF_{ℓ}^{fd} for each trophic level from the individual baseline BAF_{ℓ}^{fd}s (Figure 5-2). The final baseline BAF_{ℓ}^{fd} will be used in the last step to determine the national BAF for each trophic level. The final baseline BAF_{ℓ}^{fd} for each trophic level should be determined from the individual baseline BAF_{ℓ}^{fd}s by considering the data preference hierarchy defined by Procedure #3 and uncertainty in the data. The data preference hierarchy for Procedure #3 is (in order of preference):

1. a baseline BAF_{ℓ}^{fd} from an acceptable field-measured BAF or laboratory-measured BCF, or
2. a baseline BAF_{ℓ}^{fd} predicted from an acceptable K_{ow} value.

This data preference hierarchy reflects EPA's preference for BAFs that are based on measured data (field-measured BAFs and laboratory-measured BCFs) over BAFs based on predictive methods (K_{ow}). This data preference hierarchy should be used as a guide for selecting the final baseline BAF_{ℓ}^{fd}s when the uncertainty is similar among two or more baseline BAF_{ℓ}^{fd}s derived using different methods. Since bioaccumulation via dietary uptake and subsequent biomagnification generally are not of concern for chemicals subject to Procedure #3, field-measured BAFs and laboratory-measured BCFs are considered equally in determining the national BAF.

Final baseline BAF_{ℓ}^{fd}s should be selected for each trophic level using the following steps and guidelines.

1. **Calculate Species-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method (i.e., field-measured BAF, BAF from a lab-measured BCF, or BAF from a K_{ow}) where more than one

acceptable baseline BAF_{ℓ}^{fd} is available for a given species, calculate a species-mean baseline BAF_{ℓ}^{fd} according to the guidance described previously in Procedure #1.

2. **Calculate Trophic-Level-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method where more than one acceptable species-mean baseline BAF_{ℓ}^{fd} is available within a given trophic level, calculate the trophic-level-mean baseline BAF_{ℓ}^{fd} as the geometric mean of acceptable species-mean baseline BAF_{ℓ}^{fd}s in that trophic level.

3. **Select a Final Baseline BAF_{ℓ}^{fd} for Each Trophic Level.** For each trophic level, select the final baseline BAF_{ℓ}^{fd} using best professional judgment by considering: (1) the data preference hierarchy, (2) the relative uncertainties among trophic-level-mean baseline BAF_{ℓ}^{fd}s derived using different methods, and (3) the weight of evidence among the three methods.

   a. In general, when more than one trophic-level-mean baseline BAF_{ℓ}^{fd} is available within a given trophic level, the final baseline BAF_{ℓ}^{fd} should be selected from the most preferred BAF method defined by the data preference hierarchy for Procedure #3. Within the first data preference tier, field-measured BAFs and laboratory-measured BCFs are considered equally desirable for deriving a final trophic-level-mean baseline BAF_{ℓ}^{fd} using Procedure #3. If a trophic-level-mean baseline BAF_{ℓ}^{fd} is available from both a field-measured BAF and a laboratory-measured BCF, the final baseline BAF_{ℓ}^{fd} should be selected using the trophic-level-mean baseline BAF_{ℓ}^{fd} or BCF_{ℓ}^{fd} with the least overall uncertainty.

   b. If uncertainty in a trophic-level-mean baseline BAF_{ℓ}^{fd} based on a higher tier (more preferred) method is judged to be substantially greater than a trophic-level-mean baseline BAF_{ℓ}^{fd} from a lower tier method, then the final baseline BAF_{ℓ}^{fd} should be selected using a trophic-level-mean baseline BAF_{ℓ}^{fd} from a lower tier method.

   c. The above steps should be performed for each trophic level until a final baseline BAF_{ℓ}^{fd} is selected for trophic level two, three, and four.

5.4.5.3 Calculating the National BAFs

As described in Procedure #1, the last step in deriving a national BAF for a given trophic level for nonionic organic chemicals is to convert the final baseline BAF_{ℓ}^{fd} determined in the previous step to a BAF that reflect conditions to which the national 304(a) criterion will apply (Figure 5-2). Each national BAF should be determined from a final baseline BAF_{ℓ}^{fd} according to the following guidelines.

1. **National BAF Equation.** Except where noted below, national BAFs for trophic levels two, three, and four should be calculated from the final, trophic-level-mean baseline BAF_{ℓ}^{fd}s using Equation 5-28 and associated guidance described in Procedure #1 (see Section 5.4.3.3).

2. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log K_{ow} < 4.0), a freely dissolved fraction of 1.0 should be assumed for calculating national BAFs for nonionic organic chemicals using Procedure #3. A freely dissolved fraction of 1.0 should be assumed because at a log K_{ow} of less than 4.0, nonionic organic chemicals are expected to remain over 99 percent in the freely dissolved form at POC and DOC concentrations corresponding to national default values for U.S. bodies of water (i.e., 0.5 mg/L and 2.9 mg/L, respectively).

5.4.6 Deriving National BAFs Using Procedure #4

This section provides guidance for calculating national BAFs for nonionic organic chemicals using Procedure #4 shown in Figure 5-1. The types of nonionic organic chemicals for which Procedure #4 is most appropriate are those that are classified as having low hydrophobicity and subject to high rates of metabolism by aquatic biota (see Section 5.4.2 above). Non-aqueous contaminant exposure and subsequent biomagnification in aquatic food webs are not generally of concern for chemicals that are classified in this category. As a result, FCMs are not used in this procedure. In addition, K_{ow} -based predictions of bioconcentration are not used in this procedure since the K_{ow} /BCF relationship is primarily based on poorly metabolized chemicals. One example of a nonionic organic chemical for which Procedure #4 appears appropriate is butyl benzyl phthalate in fish. Using radiolabeling techniques with confirmation by chromatographic analysis, Carr et al. (1997) present evidence that indicates butyl benzyl phthalate is extensively metabolized in sunfish. Carr et al. (1997) also report measured BCFs (and subsequently lipid-normalized BCFs) which are substantially below predicted BCFs based on log $K_{ow.}$ In a study of chlorinated anilines (which would be essentially un-ionized at ambient pH), de Wolf et al. (1992) reported measured BCFs substantially lower than those predicted based on K_{ow}. The authors suggested that biotransformation (metabolism) involving the amine (NH_2) was responsible for the lower measured BCFs.

According to Procedure #4, the following two methods can be used in deriving a national BAF:

- using a BAF from an acceptable field study (i.e., a field-measured BAF), and
- predicting a BAF from an acceptable BCF.

After selecting the derivation procedure, the next steps in deriving a national BAF for a given trophic level for nonionic organic chemicals are: (1) calculating individual baseline BAF_{ℓ}^{fd}s, (2) selecting the final baseline BAF_{ℓ}^{fd}, and (3) calculating the national BAF (Figure 5-2). Each of these three steps is discussed separately below.

5.4.6.1 Calculating Individual Baseline BAF_{ℓ}^{fd}s

Calculating individual baseline BAF_{ℓ}^{fd}s involves normalizing the measured BAF_T^t or BCF_T^t (which are based on the total chemical in water and tissue) by the lipid content of the study organism and the freely dissolved fraction of the chemical in the study water. For additional discussion of the technical basis for calculating baseline BAF_{ℓ}^{fd}s, see Section 5.4.3.1 in Procedure #1.

1. For each species where acceptable data are available, calculate all possible baseline BAF_{ℓ}^{fd}s using each of the two methods shown above for Procedure #4.

2. Individual baseline BAF_{ℓ}^{fd}s should be calculated from field-measured BAF_{T}^{t}s and laboratory-measured BCF_{T}^{t}s according to the following procedures.

A. Baseline BAF_{ℓ}^{fd} from Field-Measured BAFs

1. A baseline BAF_{ℓ}^{fd} should be calculated from a field-measured BAF_{T}^{t} using the guidance and equations outlined in Section 5.4.3.1(A) in Procedure #1.

2. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log $K_{ow} < 4.0$), nonionic organic chemicals applicable to Procedure #4 are expected to remain almost entirely in the freely dissolved form in natural waters with dissolved and particulate organic carbon concentrations typical of most field BAF studies. Therefore, the freely dissolved fraction should be assumed equal to 1.0 unless the concentrations of DOC and POC are very high in the field BAF study. For studies with very high DOC or POC concentrations, (e.g., about 100 mg/L or higher for DOC or 10 mg/L or higher for POC), the freely dissolved fraction may be substantially lower than 1.0 and therefore should be calculated using Equation 5-12.

3. **Temporal Averaging of Concentrations.** Also due to their low hydrophobicity, nonionic organic chemicals appropriate to Procedure #4 will also tend to reach steady-state quickly compared with those chemicals to which Procedure #1 applies. Therefore, the extent of temporal averaging of tissue and water concentrations is typically much less than that required for highly hydrophobic chemicals to which Procedure #1 is applied. In addition, field studies used to calculate BAFs for these chemicals should have sampled water and tissue at similar points in time because tissue concentrations should respond rapidly to changes in water concentrations. EPA will be providing additional guidance on appropriate BAF study designs for nonionic organic chemicals (including those appropriate to Procedure #4) in its forthcoming guidance document on conducting field BAF and BSAF studies.

B. Baseline BAF_{ℓ}^{fd} from a Laboratory-Measured BCF

1. Except where noted below, a baseline BAF_{ℓ}^{fd} should be calculated from a laboratory-measured BCF_{T}^{t} using the guidance and equations outlined in Section 5.4.3.1(c) of Procedure #1.

2. **Food Chain Multipliers.** Because biomagnification is not an important concern for the minimally hydrophobic chemicals applicable to Procedure #4, FCMs are not used in the derivation of a baseline BAF_{ℓ}^{fd} from a laboratory-measured BCF_{T}^{t}.

3. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log $K_{ow} < 4.0$), nonionic organic chemicals to which Procedure #4 is applied are expected to remain

almost entirely in the freely dissolved form in waters containing dissolved and particulate organic carbon concentrations typical of laboratory BCF studies. Therefore, the freely dissolved fraction should usually be assumed to be equal to 1.0. The freely dissolved fraction will be substantially less than 1.0 only in situations where unusually high concentrations of DOC and POC are present in the lab BCF study (e.g., above about 100 mg/L for DOC or about 10 mg/L for POC). In this situation, the freely dissolved fraction should be calculated according to Equation 5-12.

5.4.6.2 Selecting Final Baseline BAF_{ℓ}^{fd}s

After calculating individual baseline BAF_{ℓ}^{fd}s using as many of the methods in Procedure #4 as possible, the next step is to determine a final baseline BAF_{ℓ}^{fd} for a given trophic level from the individual baseline BAF_{ℓ}^{fd}s (Figure 5-2). The final baseline BAF_{ℓ}^{fd} will be used in the last step to determine the national BAF for each trophic level. A final baseline BAF_{ℓ}^{fd} should be determined for each trophic level from the individual baseline BAF_{ℓ}^{fd}s by considering the data preference hierarchy defined by Procedure #4 and uncertainty in the data. The data preference hierarchy for Procedure #4 is:

1. a baseline BAF_{ℓ}^{fd} from an acceptable field-measured BAF or predicted from an acceptable laboratory-measured BCF.

Since bioaccumulation via dietary uptake and subsequent biomagnification generally are not of concern for chemicals subject to Procedure #4, field-measured BAFs and laboratory-measured BCFs are considered equally in determining the national BAF.

Final baseline BAF_{ℓ}^{fd}s should be selected for each trophic level using the following steps and guidelines.

1. **Calculate Species-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method (i.e., field-measured BAF or a BAF from a lab-measured BCF) where more than one acceptable baseline BAF_{ℓ}^{fd} is available for a given species, calculate a species-mean baseline BAF_{ℓ}^{fd} according to the guidance described previously in Procedure #1.

2. **Calculate Trophic-Level-Mean Baseline BAF_{ℓ}^{fd}s.** For each BAF method where more than one acceptable species-mean baseline BAF_{ℓ}^{fd} is available within a given trophic level, calculate the trophic-level-mean baseline BAF_{ℓ}^{fd} as the geometric mean of acceptable species-mean baseline BAF_{ℓ}^{fd}s for that trophic level.

3. **Select a Final Baseline BAF_{ℓ}^{fd} for Each Trophic Level.** For each trophic level, select the final baseline BAF_{ℓ}^{fd} using best professional judgment by considering: (1) the data preference hierarchy, and (2) the relative uncertainties among trophic-level-mean BAFs derived using different methods.

    a. As discussed above, field-measured BAFs and laboratory-measured BCFs are considered equally desirable for deriving a final trophic-level-mean baseline

BAF_{ℓ}^{fd} using Procedure #4. If a trophic-level-mean baseline BAF_{ℓ}^{fd} is available from both a field-measured BAF and a laboratory-measured BCF, the final baseline BAF_{ℓ}^{fd} should be selected using the trophic-level-mean baseline BAF_{ℓ}^{fd} or BCF_{ℓ}^{fd} with the least overall uncertainty.

b. The above steps should be performed for each trophic level until a final baseline BAF_{ℓ}^{fd} is selected for trophic levels two, three, and four.

5.4.6.3 Calculating National BAFs

As described in Procedure #1, the last step in deriving a national BAF for a given trophic level for nonionic organic chemicals is to convert the final baseline BAF_{ℓ}^{fd} determined in the previous step to a BAF that reflects conditions to which the national 304(a) criterion will apply (Figure 5-2). Each national BAF should be determined from a final baseline BAF_{ℓ}^{fd} according to the following guidelines.

1. **National BAF Equation.** Except where noted below, national BAFs for trophic-levels two, three, and four should be calculated from the final, trophic-level-mean baseline BAF_{ℓ}^{fd}s using the same equation and procedures described previously in Procedure #1 (see Section 5.4.3.3 in Procedure #1).

2. **Freely Dissolved Fraction**. Due to their low hydrophobicity (i.e., log $K_{ow} < 4.0$), a freely dissolved fraction of 1.0 should be assumed for calculating national BAFs for nonionic organic chemicals using Procedure #4. A freely dissolved fraction of 1.0 should be assumed because at a log K_{ow} value of less than 4.0, nonionic organic chemicals are expected to remain over 99 percent in the freely dissolved form at POC and DOC concentrations corresponding to national default values for U.S. bodies of water (i.e., 0.5 mg/L and 2.9 mg/L, respectively).

5.5 NATIONAL BIOACCUMULATION FACTORS FOR IONIC ORGANIC CHEMICALS

This section contains guidelines for deriving national BAFs for ionic organic chemicals (i.e., organic chemicals which undergo significant ionization in water). As defined in Section 5.3.5, ionic organic chemicals contain functional groups which can either readily donate protons (e.g., organic acids with hydroxyl, carboxylic, and sulfonic groups) or readily accept protons (e.g., organic bases with amino and aromatic heterocyclic nitrogen groups). Some examples of ionic organic compounds include:

- chlorinated phenols (e.g., 2,4,6-trichlorophenol, pentachlorophenol),
- chlorinated phenoxyalkanoic acids (e.g., 2,4-dichlorophenoxyacetic acid [2,4-D]),
- nitrophenols (e.g., 2-nitrophenol, 2,4,6-trinitrophenol),
- cresols (e.g., 2,4-dinitro-*o*-cresol [DNOC]),
- pyridines (e.g., 2,4-dimethypyidine),
- aliphatic and aromatic amines (e.g., trimethylamine, aniline), and

- linear alkylbenzenesulfonate (LAS) surfactants.

Ionic organic chemicals are considered separately for deriving national BAFs because the anionic or cationic species of these chemicals behave much differently in the aquatic environment compared with their neutral (un-ionized) counterparts. The neutral species of ionic organic chemicals are thought to behave in a similar manner as nonionic organic compounds (e.g., partitioning to lipids and organic carbon as a function of hydrophobicity). However, the ionized (cationic, anionic) species exhibit a considerably more complex behavior involving multiple environmental partitioning mechanisms (e.g., ion exchange, electrostatic, and hydrophobic interactions) and a dependency on pH and other factors including ionic strength and ionic composition (Jafvert et al., 1990; Jafvert 1990; Schwarzenbach, et al., 1993). As a consequence, methods to predict the environmental partitioning of organic cations and anions are less developed and validated compared with methods for nonionic organic chemicals (Spacie, 1994; Suffet et al., 1994).

Given the current limitations in the state of the science for predicting the partitioning and bioaccumulation of the ionized species of ionic organic chemicals, procedures for deriving national BAFs for these chemicals differ depending on the extent to which the fraction of the total chemical is likely to be represented by the ionized (cationic, anionic) species in U.S. surface waters. When a significant fraction of the total chemical concentration is expected to be present as the ionized species in water, procedures for deriving the national BAF rely on empirical (measured) methods (i.e., Procedures #5 and 6 in Section 5.6). When an insignificant fraction of the total chemical is expected to be present as the ionized species (i.e., the chemical exists essentially in the neutral form), procedures for deriving the national BAF will follow those established for nonionic organic chemicals (e.g., Procedures #1 through #4 in Section 5.4). The following guidelines apply for assessing the occurrence of cationic and anionic forms at typical environmental pH ranges.

1. For the ionic organic chemical of concern, the dissociation constant, pK_a, should be compared to the range of pH values expected in fresh and estuarine waters of the U.S. At pH equal to the pK_a, 50% of the organic acid or base is expected to be present in the ionized species. The pH values for U.S. fresh and estuarine waters typically range between 6 and 9, although somewhat higher and lower values can occur in some bodies of water (e.g., acidic bogs and lakes, highly alkaline and eutrophic systems, etc.).

2. For organic acids, the chemical will exist almost entirely in its un-ionized form when pH is about 2 or more units below the pK_a. For organic bases, the chemical will exist almost entirely in its un-ionized form when pH is about 2 or more units above the pK_a. In these cases, the aqueous behavior of the chemical would be expected to be similar to nonionic organic chemicals. Therefore, national BAF should usually be derived using Procedures #1 through #4 in Section 5.4.

3. When pH is greater than the pK_a minus 2 for organic acids (or less than the pKa plus 2 for organic bases), the fraction of the total chemical that is expected to exist in its ionized form can become significant (i.e., $\geq$1% in the ionized). In these cases, the national BAF should usually be derived using Procedures #5 and #6 in Section 5.6.

4. In general, most organic acids (e.g., pentachlorophenol and silvex), exist primarily in the ionized form in ambient waters because their pK_a's (4.75 and 3.07, respectively) are much smaller than the pH of the ambient waters. Conversely, most organic bases, (e.g., aniline) exist mostly in the un-ionized form in ambient waters because their pK_a's (4.63 for aniline) are much smaller than the pH of the ambient waters.

5. The above guidelines are intended to be a general guide for deriving national BAFs for ionic organic chemicals, not an inflexible rule. Modifications to these guidelines should be considered on a case-by-case basis, particularly when such modifications are strongly supported by measured bioaccumulation or bioconcentration data. For example, initial models have been developed for predicting the solid and organic-phase partitioning of certain organic acids (e.g., Jafvert 1990, Jafvert et al., 1990). As these or other models become more fully developed and appropriately validated in the future, they should be considered in the development of national BAFs. In addition, since pH is a controlling factor for dissociation and subsequent partitioning of ionic organic chemicals, consideration should be given to expressing BAFs or BCFs as a function of pH (or other factors) where sufficient data exist to reliably establish such relationships.

5.6 NATIONAL BIOACCUMULATION FACTORS FOR INORGANIC AND ORGANOMETALLIC CHEMICALS

This section contains guidelines for deriving national BAFs for inorganic and organometallic chemicals as defined in Section 5.3.5. The derivation of BAFs for inorganic and organometallic chemicals differs in several ways from procedures for nonionic organic chemicals. First, lipid normalization of chemical concentrations in tissues does not generally apply for inorganic and organometallic chemicals. Thus, BAFs and BCFs cannot be extrapolated from one tissue to another based on lipid-normalized concentrations as is done for nonionic organic chemicals. Second, the bioavailability of inorganics and organometallics in water tends to be chemical-specific and thus, the techniques for expressing concentrations of nonionic organic chemicals based on the freely dissolved form do not generally apply. Third, at the present time there are no generic bioaccumulation models that can be used to predict BAFs for inorganic and organometallic chemicals as a whole, unlike the existence of K_{ow}-based models for nonionic organic chemicals. While some chemical-specific bioaccumulation models have been developed for inorganic and organometallic chemicals (e.g., Mercury Cycling Model by Hudson et. al, 1994), those models currently tend to require site-specific data for input to the model and are restricted to site-specific applications. As the models become more fully developed and validated in the future, they should be considered on a case-by-case basis in conjunction with the following procedures for deriving national BAFs.

5.6.1 Selecting the BAF Derivation Procedure

As shown in Figure 5-1, national BAFs can be derived using two procedures for inorganic and organometallic chemicals (Procedures #5 and #6). The choice of the BAF derivation procedure depends on whether or not the chemical undergoes biomagnification in aquatic food webs.

1. For many inorganic and organometallic chemicals, biomagnification does not occur and the BCF will be equal to the BAF. For these types of chemicals, Procedure #5 should be used to derive the national BAF. Procedure #5 considers BAFs and BCFs to be of equal value in determining the national BAF and does not require the use of FCMs with BCF measurements. Guidance for deriving BAFs using Procedure #5 is provided in Section 5.6.3.

2. For some inorganic and organometallic chemicals (e.g., methylmercury), biomagnification does occur and Procedure #6 should be used to determine the national BAF. Procedure #6 gives general preference to the use of field-measured BAFs over laboratory-measured BCFs and requires FCMs to be used with BCF measurements for predicting BAFs. Guidance for deriving BAFs using Procedure #6 is provided in Section 5.6.4.

3. Determining whether or not biomagnification occurs for inorganic and organometallic chemicals requires chemical-specific data on measured concentrations of the chemical in aquatic organisms and their prey. Concentrations in aquatic organisms that increase substantially at successive trophic levels of a food web suggest that biomagnification is

occurring. Concentrations in aquatic organisms that remain about the same or decrease at successive trophic levels of a food web suggest that biomagnification is not occurring. When comparing tissue concentrations for assessing biomagnification, care should be taken to ensure that the aquatic organisms chosen actually represent functional predator-prey relationships and that all major prey species are considered in the comparisons.

5.6.2 Bioavailability

The chemical-specific nature of inorganic and organometallic bioavailability is likely due in part to chemical-specific differences in several factors which affect bioavailability and bioaccumulation. These factors include differences in the mechanisms for chemical uptake by aquatic organisms (e.g., passive diffusion, facilitated transport, active transport), differences in sorption affinities to biotic and abiotic ligands, and differences in chemical speciation in water. Some inorganic and organometallic chemicals exist in multiple forms and valence states in aquatic ecosystems that can differ in their bioavailability to aquatic organisms and undergo conversions between forms. For example, selenium can exist in various forms in aquatic ecosystems, including inorganic selenite($^{+4}$) and selenate($^{+6}$) oxyanions, elemental selenium (^{0}) under reducing conditions (primarily in sediments), and organoselenium compounds of selenide ($^{-2}$). Dominant forms of mercury in natural, oxic waters include inorganic ($^{+2}$) mercury compounds and methylmercury; the latter is generally considered to be substantially more bioavailable than inorganic mercury compounds to higher trophic level organisms. Although a generic analogue to the "freely dissolved" conversion for nonionic organic chemicals does not presently exist for inorganic and organometallic chemicals as a whole, the occurrence and bioavailability of different forms of these chemicals should be carefully considered when deriving national BAFs.

1. If data indicate that: (1) a particular form (or multiple forms) of the chemical of concern largely governs its bioavailability to target aquatic organisms, and (2) BAFs are more reliable when derived using the bioavailable form(s) compared with using other form(s) of the chemical of concern, then BAFs and BCFs should be based on the appropriate bioavailable form(s).

2. Because different forms of many inorganic and organometallic chemicals may interconvert once released to the aquatic environment, regulatory and mass balance considerations typically require an accounting of the total concentration in water. In these cases, sufficient data should be available to enable conversion between total concentrations and the other (presumably more bioavailable) forms in water.

5.6.3 Deriving BAFs Using Procedure #5

This section contains guidance for calculating national BAFs for inorganic and organometallic chemicals using Procedure #5 as shown in Figure 5-1. The types of inorganic and organometallic chemicals for which Procedure #5 is appropriate are those that are not likely to biomagnify in aquatic food webs (see Section 5.1 above). In Procedure #5, two methods are available to derive the national BAF for a given trophic level:

- using a BAF from an acceptable field study (i.e., field-measured BAF), or
- predicting a BAF from an acceptable laboratory-measured BCF.

Individual BAFs should be determined from field-measured BAFs or laboratory-measured BCFs according to the following guidelines.

5.6.3.1 Determining Field-Measured BAFs

1. Except where noted below, field-measured BAFs should be determined using the guidance provided in Section 5.4.3.1(A) of Procedure #1.

2. As described previously, conversion of field-measured BAFs to baseline BAF_{ℓ}^{fd}s based on lipid-normalized and freely-dissolved concentrations does not apply for inorganic and organometallic chemicals. Therefore, the guidance and equations provided in Procedure #1 which pertain to converting field-measured BAFs to baseline BAF_{ℓ}^{fd}s and subsequently to national BAFs do not generally apply to inorganic chemicals. As discussed in Section 5.6.2 above, an analogous procedure in concept might be required for converting total BAFs to BAFs based on the most bioavailable form(s) for some inorganic and organometallic chemicals of concern. Such procedures should be applied on a chemical-specific basis.

3. BAFs should be expressed on a wet-weight basis; BAFs reported on a dry-weight basis can be used only if they are converted to a wet-weight basis using a conversion factor that is measured or reliably estimated for the tissue used in the determination of the BAF.

4. BAFs should be based on concentrations in the edible tissue(s) of the biota unless it is demonstrated that whole-body BAFs are similar to edible tissue BAFs. For some finfish and shellfish species, whole body is considered to be the edible tissue.

5. The concentrations of an inorganic or organometallic chemical in a bioaccumulation study should be greater than normal background levels and greater than levels required for normal nutrition of the test species if the chemical is a micronutrient, but below levels that adversely affect the species. Bioaccumulation of an inorganic or organometallic chemical that is essential to the nutrition of aquatic organisms might be overestimated if concentrations are at or below normal background levels due to selective accumulation by the organisms to meet their nutritional requirements.

5.6.3.2 Determining Laboratory-Measured BCFs

1. Except where noted below, BAFs should be predicted from laboratory-measured BCFs using the guidance provided in Section 5.4.3.1(c) of Procedure #1.

2. As described previously, conversion of laboratory-measured BCFs to baseline BCF_{ℓ}^{fd}s based on lipid-normalized and freely dissolved concentrations does not apply for inorganic and organometallic chemicals. Therefore, the guidance and equations provided in Procedure #1 which pertain to converting laboratory-measured BCFs to baseline BCF_{ℓ}^{fd}s and subsequently to national BCFs do not generally apply to inorganic and organometallic chemicals. As discussed in Section 5.6.2 above, an analogous procedure in concept might be required for converting total BCFs to BCFs based on the most bioavailable form(s) of some inorganic and organometallic chemicals of concern. Such procedures should be applied on a chemical-specific basis. In addition, the use of FCMs with BCFs does not apply to chemicals applicable to Procedure #5.

3. BCFs should be expressed on a wet-weight basis; BCFs reported on a dry-weight basis can be used only if they are converted to a wet-weight basis using a conversion factor that is measured or reliably estimated for the tissue used in the determination of the BCF.

4. BCFs should be based on concentrations in the edible tissue(s) of the biota unless it is demonstrated that whole-body BCFs are similar to edible tissue BCFs. For some finfish and shellfish species, whole body is considered to be the edible tissue.

5. The concentrations of an inorganic or organometallic chemical in a bioconcentration test should be greater than normal background levels and greater than levels required for normal nutrition of the test species if the chemical is a micronutrient, but below levels that adversely affect the species. Bioaccumulation of an inorganic or organometallic chemical that is essential to the nutrition of aquatic organisms might be overestimated if concentrations are at or below normal background levels due to selective accumulation by the organisms to meet their nutritional requirements.

5.6.3.3 Determining the National BAFs

After calculating individual BAFs using as many of the methods in Procedure #5 as possible, the next step is to determine national BAFs for each trophic level from the individual BAFs. The national BAFs will be used to determine the national 304(a) criteria. The national BAFs should be determined from the individual BAFs by considering the data preference hierarchy defined for Procedure #5 and uncertainty in the data. The data preference hierarchy for Procedure #5 is:

1. a BAF from an acceptable field-measured BAF or predicted from an acceptable laboratory-measured BCF.

Since bioaccumulation via dietary uptake and subsequent biomagnification are not of concern for chemicals subject to Procedure #5, field-measured BAFs and laboratory-measured

BCFs are considered equally in determining the national BAFs. The national BAFs should be selected for each trophic level using the following steps and guidelines.

1. **Calculate Species-Mean BAFs.** For each BAF method where more than one acceptable field-measured BAF (or a BAF predicted from a BCF) is available for a given species, calculate the species-mean BAF as the geometric mean of all acceptable individual measured or BCF-predicted BAFs. When calculating species-mean BAFs, individual measured or BCF-predicted BAFs should be reviewed carefully to assess uncertainties in the BAF values. Highly uncertain BAFs should not be used. Large differences in individual BAFs for a given species (e.g., greater than a factor of 10) should be investigated further and in such cases, some or all of the BAFs for a given species might not be used. Additional discussion on evaluating the acceptability of BAF and BCF values is provided in the Bioaccumulation TSD.

2. **Calculate Trophic-Level-Mean BAFs.** For each BAF method where more than one acceptable species-mean BAF is available within a given trophic level, calculate the trophic-level-mean BAF as the geometric mean of acceptable species-mean BAFs in that trophic level. Trophic-level-mean BAFs should be calculated for trophic levels two, three and four because available data on U.S. consumers of fish and shellfish indicate significant consumption of organisms in these trophic levels.

3. **Select a Final National BAF for Each Trophic Level.** For each trophic level, select the final national BAF using best professional judgment by considering: (1) the data preference hierarchy in Procedure #5, and (2) the relative uncertainties among trophic level-mean BAFs derived using different methods.

    a. As discussed above, field-measured BAFs and laboratory-measured BCFs are considered equally desirable for deriving a final national BAF using Procedure #5. If a trophic-level-mean BAF is available from both a field-measured BAF and a laboratory-measured BCF, the final national BAF should be selected using the trophic-level-mean BAF with the least overall uncertainty.

    b. The above steps should be performed for each trophic level until a national BAF is selected for trophic levels two, three, and four.

5.6.4 Deriving BAFs Using Procedure #6

This section contains guidance for calculating national BAFs for inorganic and organometallic chemicals using Procedure #6 as shown in Figure 5-1. The types of inorganic and organometallic chemicals for which Procedure #6 is appropriate are those that are considered likely to biomagnify in aquatic food webs (see Section 5.6.1 above). Methylmercury is an example of an organometallic chemical to which Procedure #6 applies. In Procedure #6, two methods are available to derive the national BAF:

- using a BAF from an acceptable field study (i.e., field-measured BAF), or

- predicting a BAF from an acceptable laboratory-measured BCF and a FCM.

Individual BAFs should be determined from field-measured BAFs or laboratory-measured BCFs and FCMs according to the following guidelines.

5.6.4.1 Determining Field-Measured BAFs

1. Field-measured BAFs should be determined using the guidance provided in Section 5.6.3.1 of Procedure #5.

5.6.4.2 Determining Laboratory-Measured BCFs

1. Except where noted below, BAFs should be predicted from laboratory-measured BCFs using the guidance provided in Section 5.6.3.2 of Procedure #5.

2. Because biomagnification is of concern for chemicals applicable to Procedure #6, BAFs should be predicted from laboratory-measured BCF using FCMs. Currently, there are no generic models from which to predict FCMs for inorganic or organometallic chemicals. Therefore, FCMs should be determined using field data as described in the section entitled: "Field-Derived FCMs" in Section 5.4.3.1(c) of Procedure #1. Unlike nonionic organic chemicals, field-derived FCMs for inorganic and organometallic chemicals are not based on lipid-normalized concentrations in tissues. For calculating FCMs for inorganic and organometallic chemicals, concentrations in tissues should be based on the consistent use of either wet-weight or dry-weight concentrations in edible tissues. FCMs should be derived for trophic levels two, three, and four.

5.6.4.3 Determining the National BAF

After calculating individual BAFs using as many of the methods in Procedure #6 as possible, the next step is to determine national BAFs for each trophic level from the individual BAFs. The national BAFs will be used to determine the national 304(a) criteria. The national BAFs should be determined from the individual BAFs by considering the data preference hierarchy defined for Procedure #6 and uncertainty in the data. The data preference hierarchy for Procedure #6 is (in order of preference):

1. a BAF from an acceptable field-measured BAF, or
2. a predicted BAF from an acceptable laboratory-measured BCF and FCM.

This data preference hierarchy reflects EPA's preference for field-measured BAFs over BAFs predicted from a laboratory-measured BCF and FCM, because field-measured BAFs are direct measures of bioaccumulation and biomagnification in aquatic food webs. BAFs predicted from laboratory-measured BCFs and FCMs indirectly account for biomagnification through the use of the FCM. For each trophic level, the national BAFs should be determined using the following steps and guidelines.

1. **Calculate Species-Mean BAFs.** For each BAF method where more than one acceptable field-measured BAF or BAF predicted using a BCF and FCM is available, calculate a species-mean BAF according to the guidance described previously in Procedure #5.

2. **Calculate Trophic Level-Mean BAFs.** For each BAF method where more than one acceptable species-mean BAF is available within a given trophic level, calculate the trophic level-mean BAF according to guidance described previously in Procedure #5.

3. **Select a Final National BAF for Each Trophic Level.** For each trophic level, select the final national BAF using best professional judgment by considering: (1) the data preference hierarchy in Procedure #6, and (2) the relative uncertainties among trophic level-mean BAFs derived using different methods.

    a. When a trophic-level mean BAF is available using both methods for a given trophic level (i.e., a field-measured BAF and a BAF predicted from a BCF and FCM), the national BAF should usually be selected using the field-measured BAF which is the preferred BAF method in the data preference hierarchy in Procedure #6.

    b. If uncertainty in the trophic-level mean BAF derived using field-measured BAFs is considered to be substantially greater than a trophic-level mean BAF derived using a BCF and FCM, the national BAF for that trophic level should be selected from the second tier (BCF · FCM) method.

    c. The above steps should be performed for each trophic level until a national BAF is selected for trophic levels two, three, and four.

5.7 REFERENCES

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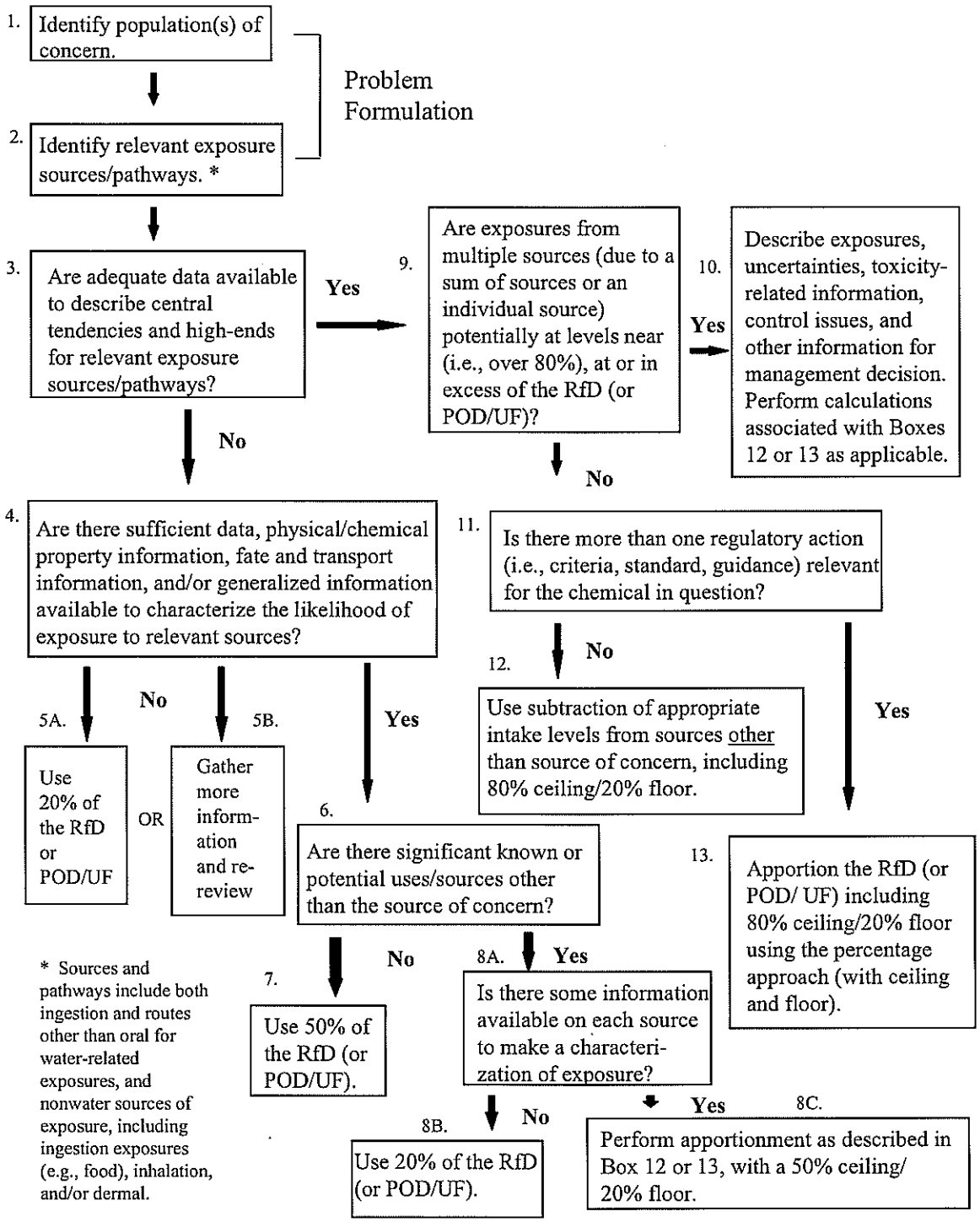
Attachment 4

Figure 4-1

Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment



Attachment 5

United States Environmental Protection Agency

Office of Water
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INTERIM
Drinking Water Health Advisory: Perfluorooctanoic Acid (PFOA) CASRN 335-67-1

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Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Office of Science and Technology
Health and Ecological Criteria Division
Washington, DC 20460

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- Office of Water
- Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics
- Office of Land and Emergency Management
- Office of Policy
- Office of Children's Health Protection
- Office of Research and Development

Contents

Tables

Abbreviations and Acronyms

AIX	anion exchange
ANSI	American National Standards Institute
AWWA	American Water Works Association
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
Br-DBP	brominated disinfection byproduct
bw or BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CCL	Contaminant Candidate List
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CSF	cancer slope factor
DBP	disinfection byproduct
DOM	dissolved organic matter
DQO	data quality objective
DWI	drinking water intake
DWI-BW	body weight-adjusted drinking water intake
E	human exposure
EBCT	empty bed contact time
EF	exposure factor
EFH	Exposure Factors Handbook
EPA	U.S. Environmental Protection Agency
Eq.	equation
FCID	Food Commodity Intake Database
GAC	granular activated carbon
HA	Health Advisory
HECD	Health and Ecological Criteria Division
HESD	Health Effects Support Document
HI	hazard index
HQ	hazard quotient
iHA	interim Health Advisory
i	mixture component chemical
IRIS	Integrated Risk Information System
$L/(m^2 \cdot hr)$	liters per square meter per hour
LC/MS/MS	liquid chromatography/ tandem mass spectrometry
LOAEL	lowest-observed-adverse-effect level
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg/kg bw-day	milligrams per kilogram body weight per day
mg/L	milligrams per liter
m/hr	meters per hour
MPa	megapascal
MRL	minimum reporting level
NF	nanofiltration
ng/L	nanograms per liter
NHANES	National Health and Nutrition Examination Survey

NOAEL	no-observed-adverse-effect level
NOM	natural organic matter
NPDWR	National Primary Drinking Water Regulation
OGWDW	Office of Ground Water and Drinking Water
ORD	Office of Research and Development
OST	Office of Science and Technology
OW	Office of Water
PAC	powdered activated carbon
PBPK	physiologically-based pharmacokinetic
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutane sulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
pK_a	acid dissociation constant
POD	point of departure
POD_{HED}	point of departure human equivalent dose
ppq	parts per quadrillion
ppt	parts per trillion
PWS	public water system
QC	quality control
RfD	reference dose
RfV	reference value
RO	reverse osmosis
RPF	relative potency factor
RSC	relative source contribution
SAB	Science Advisory Board
SAB PFAS Panel	Science Advisory Board Per- and Polyfluoroalkyl Substances Review Panel
SDWA	Safe Drinking Water Act
SNUR	Significant New Use Rule
TSCA	Toxic Substances Control Act
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	lowest-observed-adverse-effect level-to-no-observed-adverse-effect level extrapolation uncertainty factor
UF_S	subchronic-to-chronic exposure duration extrapolation uncertainty factor
µg/L	micrograms per liter

1.0 Introduction: Background and Scope of Interim Health Advisory

The Safe Drinking Water Act (SDWA) (42 U.S.C. § § 300f - 300j-27) authorizes the U.S. Environmental Protection Agency (EPA) to develop drinking water Health Advisories (HAs).[1] HAs are national non-enforceable, non-regulatory drinking water concentration levels of a specific contaminant at or below which exposure for a specific duration is not anticipated to lead to adverse human health effects.[2] HAs are intended to provide information that tribal, state, and local government officials and managers of public water systems (PWSs) can use to determine whether actions are needed to address the presence of a contaminant in drinking water. HA documents reflect the best available science and include HA values as well as information on health effects, analytical methodologies for measuring contaminant levels, and treatment technologies for removing contaminants from drinking water. EPA's lifetime HAs identify levels to protect all Americans, including sensitive populations and life stages, from adverse health effects resulting from exposure throughout their lives to contaminants in drinking water.

Interim or provisional HA values can be developed to provide information in response to an urgent or rapidly developing situation. EPA has developed an interim lifetime noncancer HA (iHA) for perfluorooctanoic acid (PFOA) to replace the 2016 lifetime HA of 0.07 micrograms per liter (µg/L) (70 parts per trillion [ppt]) because analyses of more recent health effects studies show that PFOA can impact human health at exposure levels much lower than reflected by the 2016 PFOA lifetime HA. EPA has developed an interim rather than a final HA for PFOA because the input values used to derive the iHA are currently draft values and EPA has identified a pressing need to provide information to public health officials prior to their finalization.

In 2009, EPA developed a provisional HA for PFOA (U.S. EPA, 2009a) based on the best information available at that time. Also, PFOA was included on the third and fourth drinking water Contaminant Candidate Lists (CCLs)[3] (U.S. EPA, 2009b, 2016a). After PFOA was listed on the third CCL in 2009, EPA initiated development of a Health Effects Support Document (HESD) for PFOA to assist officials and PWS managers in protecting public health when PFOA is present in drinking water. The HESD was published in 2016 after peer review (U.S. EPA, 2016b). EPA developed a final HA for PFOA (U.S. EPA, 2016c) based on data and analyses in the 2016 HESD and agency guidance on exposure and risk assessment.

In March 2021, EPA published a final determination to regulate PFOA with a National Primary Drinking Water Regulation (NPDWR) under SDWA (U.S. EPA, 2021a). NPDWRs include legally-enforceable Maximum Contaminant Levels (MCLs) and/or treatment technique requirements that apply to PWSs. To support the development of the NPDWR, EPA developed the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for*

[1] SDWA § 1412(b)(1)(F) authorizes EPA to "publish health advisories (which are not regulations) or take other appropriate actions for contaminants not subject to any national primary drinking water regulation." www.epa.gov/sites/default/files/2020-05/documents/safe_drinking_water_act-title_xiv_of_public_health_service_act.pdf

[2] This document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community. This document is not enforceable against any person and does not have the force and effect of law. No part of this document, nor the document as a whole, constitutes final agency action that affects the rights and obligations of any person. EPA may change any aspects of this document in the future.

[3] The CCL is a list (published every five years) of contaminants that are not currently subject to any National Primary Drinking Water Regulation (NPDWR) but are known or anticipated to occur in PWSs and may require future regulation under SDWA.

Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water (U.S. EPA, 2021b) (hereafter referred to as "draft PFOA document") which includes an updated health effects assessment of the peer-reviewed literature, cancer classification, draft chronic reference dose (RfD), and draft relative source contribution (RSC) value. The development of the draft noncancer chronic RfD for PFOA was performed by a cross-agency per- and polyfluoroalkyl substances (PFAS) Science Working Group to support the PFAS NPDWR. In November 2021, EPA announced the Science Advisory Board (SAB) PFAS Review Panel's (SAB PFAS Panel's) review (U.S. EPA, 2021c) of the draft PFOA document along with three other draft documents supporting the NPDWR (U.S. EPA, 2022a).

The 2021 data and analyses described in the draft PFOA document indicate that PFOA exposure levels at which adverse health effects have been observed are much lower than previously understood when EPA issued an HA for PFOA in 2016. As a result, EPA announced in 2021[4] that it would move quickly to update the 2016 HA for PFOA to reflect the latest, best available science as well as input from the SAB PFAS Panel. An updated PFOA HA is consistent with EPA's commitments for action on PFAS described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021d).

In April 2022, the SAB PFAS Panel made public a draft report of its review of the draft PFOA document (U.S. EPA, 2022a) which indicated general support for the draft conclusions but recommended additional analyses be performed prior to finalizing the RfD and RSC. Because the RfD in the draft PFOA document is much lower than the RfD used to derive the 2016 HA, there is a pressing need to provide updated information on the current best available science to public health officials prior to finalization of the health effects assessment. Therefore, EPA has decided to issue an iHA using the draft chronic RfD and RSC values. Additionally, EPA derived multiple candidate cancer slope factors (CSFs) in the draft PFOA document but did not yet select one overall draft CSF; therefore, EPA has not derived an updated interim 10^{-6} cancer risk concentration for PFOA in this iHA document. As noted in the draft PFOA document, the candidate CSFs derived from the more recent human and animal studies indicate that PFOA is a more potent carcinogen than was described in the 2016 HA document. An initial evaluation of the multiple candidate CSFs indicates that resulting 10^{-6} cancer risk concentrations are either comparable to or greater than the lifetime noncancer iHA value for PFOA. EPA is currently reviewing and evaluating the available information to derive a CSF for PFOA as part of the NPDWR.

After receiving SAB's final report, EPA will fully address SAB feedback and recommendations, which could lead EPA to draw different conclusions than are reflected in the draft PFOA document and this iHA document. EPA anticipates proposing a NPDWR in fall 2022 and finalizing the NPDWR in fall 2023. EPA may update or remove the iHA for PFOA upon finalization of the NPDWR.

1.1 PFOA General Information and Uses

PFOA is a synthetic fluorinated organic chemical that has been manufactured and used in a variety of industries since the 1940s (U.S. EPA, 2018). It repels water and oil, is chemically and thermally stable, and exhibits surfactant properties. Based on these properties, it has been used in

[4] *EPA Advances Science to Protect the Public from PFOA and PFOS in Drinking Water* [Press release], Nov 16, 2021: https://www.epa.gov/newsreleases/epa-advances-science-protect-public-pfoa-and-pfos-drinking-water

the manufacture of many materials, including cosmetics, paints, polishes, and nonstick coatings on fabrics, paper, and cookware. It is very persistent in the human body and the environment (Calafat et al., 2007, 2019). More information about PFOA's uses and properties can be found in the 2016 HA document for PFOA (U.S. EPA, 2016c) and the draft PFOA document (U.S. EPA, 2021b).

In 2006, EPA invited eight major companies to commit to working toward the elimination of their production and use of PFOA (and chemicals that degrade to PFOA) and elimination of these chemicals from emissions and products by the end of 2015.[5] All eight companies have since phased out manufacturing PFOA. PFOA is included in EPA's Toxic Substances Control Act (TSCA) Significant New Use Rule (SNUR) issued in January 2015, which ensures that EPA will have an opportunity to review any efforts to reintroduce the chemical into the marketplace and take action, as necessary, to address potential concerns (U.S. EPA, 2015). Limited existing uses of PFOA-related chemicals, including as a component of anti-reflective coatings in the production of semiconductors, were excluded from the regulations (U.S. EPA, 2021e).

1.2 Occurrence in Water and Exposure to Humans

1.2.1 Occurrence in Water

EPA requires sampling at drinking water systems under the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are known or suspected to be found in drinking water and do not have health-based standards under SDWA. A new UCMR is issued every five years. The first four UCMRs required monitoring of all large public drinking water systems (> 10,000 people) and a subset of smaller systems serving < 10,000 people. The third UCMR (UCMR 3), conducted from 2013–2015, is currently the best available source of national occurrence data for PFOA in drinking water (U.S. EPA, 2017a, 2021a,b,f). A total of 379 samples from 117 PWSs (out of 36,972 total samples from 4,920 PWSs) had detections of PFOA (i.e., greater than or equal to the minimum reporting level [MRL][6] of 0.02 µg/L). PFOA concentrations for these detections ranged from 0.02 µg/L (the MRL) to 0.349 µg/L (median concentration of 0.03 µg/L; 90th percentile concentration of 0.07 µg/L).

In 2016, EPA recommended that when PFOA and perfluorooctane sulfonic acid (PFOS) co-occur at the same time and location in drinking water sources, a conservative and health-protective approach is to consider the sum of the concentrations. An analysis of the UCMR 3 data showed that 508 samples from 162 PWSs (out of 36,972 samples from 4,920 PWSs) had detections of PFOA and/or PFOS (i.e., at or above the MRL of 0.02 µg/L for PFOA or 0.04 µg/L for PFOS). The sum of reported PFOA and/or PFOS concentrations ranged from 0.02 to 7.22 µg/L. Although it is not possible to determine the full extent of PFOA and/or PFOS occurrence based on UCMR 3 detections, sites where elevated levels of PFOA and/or PFOS were detected during UCMR 3 monitoring may have taken steps to mitigate exposure including installing treatment systems and/or blending water from multiple sources, or remediating known sources of contamination (U.S. EPA, 2021a).

[5] *Fact Sheet: 2010/2015 PFOA Stewardship Program* available at https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program

[6] The MRL refers to the quantitation level selected by EPA to ensure reliable and consistent results. It is the minimum quantitation level that can be achieved with 95 percent confidence by capable analysts at 75 percent or more of the laboratories using a specified analytical method (U.S. EPA, 2021g).

The fifth UCMR (UCMR 5) will require monitoring for 29 PFAS, including PFOA, using EPA methods 533 (U.S. EPA, 2019a) and 537.1 (U.S. EPA, 2020). UCMR 5 monitoring will take place from 2023–2025 and will include all large PWSs serving > 10,000 people, all systems serving 3,300–10,000 people (subject to the availability of appropriations), and a subset of smaller systems serving < 3,300 people (U.S. EPA, 2021g). EPA established an MRL for PFOA of 0.004 µg/L under UCMR 5, which is 5-fold lower than the MRL used in UCMR 3.

Some states have conducted monitoring for PFOA in drinking water (by selecting sampling locations randomly, and/or sampling from targeted locations). PFOA has been detected in the finished drinking water of at least 20 states (ADEM, 2021; AZDEQ, 2021; CADDW, 2021; CDPHE, 2020; DE ODW, 2021; GAEPD, 2021; ILEPA, 2021; KYDEP, 2019; MAEEA, 2021; MDE, 2021; MEDEP, 2020; MI EGLE, 2021; NCDEQ, 2021; NHDES, 2021; NJDEP, 2021; OHDOH, 2020; PADEP, 2021; RIDOH, 2020; SCDHEC, 2020; VTDEC, 2021).

1.2.2 Exposure in Humans

As noted in the draft PFOA document (U.S. EPA, 2021b), the Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOA has been detected in up to 98% of serum samples collected in biomonitoring studies that are representative of the U.S. general population; however, blood levels of PFOA declined by more than 60% between 1999 and 2014, presumably due to restrictions on PFOA commercial usage in the United States. (CDC, 2017). NHANES biomonitoring data from 1999–2000 reveal a mean serum PFOA concentration of 5.21 µg/L (95% confidence interval [CI]) of 4.72–5.74 µg/L) and a 90th percentile serum PFOA concentration of 9.4 µg/L (95% CI 8.2–11.1 µg/L) across 1,562 samples representative of the U.S. population. For 2013–2014, mean and 90th percentile serum PFOA concentrations were 1.94 µg/L (95% CI 1.76–2.14 µg/L) and 4.27 µg/L (95% CI 3.57–5.17 µg/L), respectively (2,165 samples) (CDC, 2021). In 2017–2018, the mean serum PFOA concentration was 1.42 µg/L (95% CI 1.33–1.52 µg/L) and the 90th percentile serum PFOA concentration was 2.97 µg/L (95% CI 2.77–3.37 µg/L) across 1,929 samples (CDC, 2021). For additional information about PFOA exposure in humans, see Sections 3.3 and 5.0 of U.S. EPA (2021b).

1.3 Source of Toxicity Information for Interim Health Advisory Development

The lifetime noncancer iHA for PFOA is derived from draft values (i.e., chronic RfD and RSC) and relies on the best available science as derived in the draft PFOA document (U.S. EPA, 2021b), which is currently undergoing peer review by the SAB PFAS Panel. To develop the updated toxicity information in the draft PFOA document, a systematic review and evidence-mapping approach was utilized to identify, screen, and evaluate health effects data for PFOA. A literature search was performed to identify studies on the health effects of PFOA exposure in animals and humans published since the 2016 HESD and HA for PFOA. The search results were screened for relevancy, and literature identified as relevant underwent study quality evaluation and data extraction (please see U.S. EPA [2021b] for more details). Evidence for each health outcome was analyzed and synthesized, and overall judgments about the strength of the evidence were developed. The best available health effects information identified and analyzed using systematic review was then used in the derivation of the chronic RfD. This systematic review process has been peer reviewed and is used by EPA's Office of Research and Development

(ORD) Integrated Risk Information System (IRIS) program, as summarized in the draft PFOA document (U.S. EPA, 2021b). Similarly, a systematic review approach was used to identify, screen, and evaluate exposure information to develop the RSC based on the best available science.

1.4 Exposure Factor Information

An exposure factor (EF), such as body weight-adjusted drinking water intake (DWI-BW), is one of the input values for deriving a drinking water HA. EFs are factors related to human activity patterns, behavior, and characteristics that help determine an individual's exposure to a contaminant. EPA's *Exposure Factors Handbook* (EFH)[7] is a resource for conducting exposure assessments and provides EFs based on information from publicly available, peer-reviewed studies. Chapter 3 of the EFH presents EFs in the form of drinking water intake values (DWIs) and DWI-BWs for various populations or life stages within the general population (U.S. EPA, 2019b). The use of EFs in HA calculations is intended to protect sensitive populations within the general population from adverse effects resulting from exposure to a contaminant.

When developing HAs, the goal is to protect all ages of the general population including potentially sensitive populations such as children. The approach to select the EF for drinking water HA derivation includes a step to identify potentially sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can prevent identification of the most sensitive population (e.g., not all windows of exposure or health outcomes have been assessed for PFOA), the critical effect and point-of-departure (e.g., human equivalent benchmark dose [BMD]) that form the basis for the RfD can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In such cases, EPA can select the corresponding EFs for that sensitive population or life stage from the EFH (U.S. EPA, 2019b) for use in HA derivation. When multiple potentially sensitive populations or life stages are identified based on the critical effect or other health effects data (from animal or human studies), EPA selects the population or life stage with the greatest DWI-BW because it is the most health protective. For deriving lifetime HA values, the RSC corresponding to the selected sensitive life stage is also determined when data are available (see Section 2.2). In the absence of information indicating a potentially sensitive population or life stage, the EF corresponding to all ages of the general population may be selected.

To derive a chronic HA, EPA typically uses a DWI normalized to body weight (i.e., DWI-BW in L of water consumed/kg bw-day) for all ages of the general population or for a sensitive population or life stage, when identified. The Joint Institute for Food Safety and Applied Nutrition's Food Commodity Intake Database (FCID) Consumption Calculator Tool[8] includes the EFs from EPA's EFH and can also be used to estimate DWIs and DWI-BWs for specific populations, life stages, or age ranges. EPA uses the 90th percentile DWI-BW to ensure that the

[7] Available at https://www.epa.gov/expobox/about-exposure-factors-handbook. The latest edition of the EFH was released in 2011, but since October 2017, EPA has begun to release chapter updates individually.

[8] Joint Institute for Food Safety and Applied Nutrition's FCID Commodity Consumption Calculator is available at https://fcid.foodrisk.org/percentiles

HA is protective of the general population as well as sensitive populations or life stages (U.S. EPA, 2000a, 2016c). In 2019, EPA updated its EFs for DWI and DWI-BW based on newly available science (U.S. EPA, 2019b).

1.5 Approach for Lifetime Health Advisory Calculation

The following equation (Eq. 1) is used to derive an interim or final lifetime noncancer HA. A lifetime noncancer HA is designed to be protective of noncancer effects over a lifetime of exposure and is typically based on a chronic *in vivo* experimental animal toxicity study and/or human epidemiological data.

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 1)}$$

Where:

DWI-BW = the 90th percentile DWI for the selected population, adjusted for body weight, in units of L/kg bw-day. The DWI-BW considers both direct and indirect consumption of tap water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee).

RfD = chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime.

RSC = relative source contribution—the percentage of the total oral exposure attributed to drinking water sources where the remainder of the exposure is allocated to all other routes or sources (U.S. EPA, 2000a).

2.0 Interim Health Advisory Derivation: PFOA

A lifetime noncancer iHA was derived for PFOA. The DWI-BW selected to derive the iHA is for 0- to < 5-year-old children because PFOA exposure was measured in 5-year-old children in the critical study, and it is reasonable to expect that PFOA exposure levels were similar from birth through age 5 (see Section 2.2). Since a DWI-BW for 0- to < 5-year-old children was used, the iHA for PFOA is expected to be protective of children and adults of all ages in the general population; however, available data on the most sensitive population or life stage are limited.

Short-term iHAs (e.g., one- or ten-day iHAs) were not derived for PFOA because the draft PFOA document did not derive an RfD for short-term exposure. Additionally, EPA considers the lifetime iHA for PFOA to be applicable to short-term as well as lifetime risk assessment scenarios because the critical health effect on which the draft chronic RfD used to calculate the iHA is based (i.e., deficient antibody response to tetanus vaccine in children) resulted from PFOA exposure during a developmental life stage. EPA's risk assessment guidelines indicate that adverse effects can result from even brief exposure during a critical period of development (U.S. EPA, 1991). Therefore, the lifetime iHA for PFOA (calculated in Section 2.4) and the draft chronic RfD from which it is derived (see Table 1) are considered applicable to short-term PFOA exposures via drinking water.

In accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the draft PFOA document (U.S. EPA, 2021b) classified PFOA as *likely to be carcinogenic to humans* based on evidence of kidney and testicular cancer in humans and Leydig cell tumors, pancreatic acinar cell tumors, and hepatocellular adenomas in rats. The draft report of the SAB Panel's review of the draft PFOA document (U.S. EPA, 2022a) indicated general agreement with this classification, but an interim 10^{-6} cancer risk concentration for PFOA was not derived because the selection of a CSF is ongoing. Candidate draft CSFs from human and animal studies were identified in the draft PFOA document, but one was not selected as the preferred draft CSF for derivation of a 10^{-6} cancer risk concentration (U.S. EPA, 2021b). An initial evaluation of the candidate CSFs shows that they would result in 10^{-6} cancer risk concentrations that are either comparable to or greater (i.e., less health-protective) than the iHA value for PFOA.

2.1 Toxicity

Table 1 reports the draft chronic RfD derived in the draft PFOA document (U.S. EPA, 2021b) that was used to develop the lifetime iHA for PFOA.

Table 1. Draft Chronic RfD, Critical Effect, and Critical Study Used to Develop the Lifetime iHA for PFOA.

Source	For the Lifetime iHA for PFOA			
	RfD (mg/kg bw-day)	**PFOA Exposure in Critical Study**	**Critical Effect**	**Principal and Associated Studies (Study Type)**
Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water [Draft] (U.S. EPA, 2021b)	1.5×10^{-9}	PFOA measured in serum of 5-year-old children	Developmental immune health outcome (suppression of tetanus vaccine response in 7-year-old children)	Grandjean et al., 2012; Budtz-Jorgensen and Grandjean, 2018 (epidemiological study)

Note: mg/kg bw-day = milligrams per kilogram body weight per day.

Decreased serum anti-tetanus antibody concentration in children, which was associated with increased serum PFOA concentrations (Budtz-Jorgensen and Grandjean, 2018; Grandjean et al., 2012), was selected as the critical effect for draft chronic RfD derivation. As noted in the draft PFOA document (U.S. EPA, 2021b), selection of this draft critical effect is expected to be protective of all other adverse health effects in humans because this adverse effect of decreased immune response to vaccination was observed after exposure during a sensitive developmental life stage, and it yields the lowest point of departure (POD) human equivalent dose (POD_{HED}) among the candidate $PODs_{HED}$. Other candidate RfDs were derived based on other health effects (e.g., development/growth) observed in epidemiology studies; all of the candidate RfDs are

associated with low daily oral exposure doses, ranging from ~10^{-6} to 10^{-9} milligrams per kilogram body weight per day (mg/kg bw-day) (U.S. EPA, 2021b; Table 23).

The selected draft POD_{HED} for the critical effect was derived by performing BMD modeling (see Appendix B1 of U.S. EPA, 2021b) on the measured PFOA serum concentrations at age five reported in the critical study, which yielded an internal serum concentration POD in milligrams per liter (mg/L). This internal serum concentration POD was then converted to an external dose (POD_{HED}) in mg/kg bw-day using the updated physiologically-based pharmacokinetic (PBPK) model developed by Verner et al. (described in Section 4.1.3.2 of U.S. EPA, 2021b). Specifically, the POD_{HED} was calculated as the external dose (*in utero* through age five) that results in the internal serum concentration measured at five years of age in the critical study. (Note that the model predicted slightly different values for male and female children; the lower POD_{HED} was selected to be more health protective.) An intraspecies uncertainty factor (UF_H) of 10 was applied to the selected draft POD_{HED} to account for variability in the response within the human population in accordance with methods described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002). EPA applied a value of 1 for the remaining four uncertainty factors (UFs): interspecies UF (UF_A), because the critical effect was observed in humans and there is no need to account for uncertainty associated with animal-to-human extrapolation; lowest-observed-adverse-effect level (LOAEL)-to-no-observed-adverse-effect level (NOAEL) extrapolation UF (UF_L), because a benchmark dose lower confidence limit (BMDL) instead of a LOAEL was used as the basis for POD_{HED} derivation; subchronic-to-chronic exposure duration extrapolation UF (UF_S), because the critical effect on the developing immune system in children was observed after exposure during gestation and/or early childhood, a sensitive period that can lead to severe effects without lifetime exposure; and a database UF (UF_D), because the database of animal and human studies on the effects of PFOA is comprehensive (see the draft PFOA document [U.S. EPA, 2021b] for further details). Thus, the total or composite UF (UF_C) used to derive the PFOA RfD was 10.

2.2 Exposure Factors

To identify potentially sensitive populations, EPA considered the sensitive life stage of exposure associated with the critical effect on which the draft chronic RfD was based. The critical study that was selected for draft chronic RfD derivation (see Table 1) established an association in children between PFOA serum concentration (measured at age five, after three of four tetanus vaccinations) and decreased anti-tetanus antibody concentration (measured at age seven, approximately two years after all four tetanus vaccinations) (Budtz-Jorgensen and Grandjean, 2018). Based on limited available data to inform the critical PFOA exposure window for this critical developmental immune effect, the serum PFOA concentrations measured in 5-year-old children in this study are assumed to represent PFOA exposure from birth to the time of measurement. EPA acknowledges that the DWI-BW varies between ages 0 and 5 years (U.S. EPA, 2019b); however, the available data do not permit a more precise identification of the most sensitive or critical PFOA exposure window for the developmental immune outcome because studies with different exposure intervals have not been performed.

EPA calculated and considered DWI-BWs for other potentially sensitive age ranges indicated by the critical study data (e.g., 0 to < 7 years, 1 to < 5 years, 1 to < 7 years; Table 2). The DWI-BW for children aged 0 to < 5 years was selected among the DWI-BWs (see Table 2) because it is the greatest value and therefore the most health-protective. EPA also considered the use of a DWI-

BW for formula-fed infants (i.e., infants fed primarily or solely with water-reconstituted infant formula) because their DWI-BW is higher (U.S. EPA, 2019b) and the infant life stage occurs within the 0- to < 5-year age range. However, a greater RSC would be used for formula-fed infants than for 0- to < 5-year-olds, which would result in a less health-protective iHA value (see Section 2.3). Therefore, EPA selected the DWI-BW for 0- to < 5-year-olds.

Table 2. EPA Exposure Factors for Drinking Water Intake for Candidate Sensitive Populations Based on the Critical Effect and Study.

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Children aged 0 to < 5 yrs	**0.0701**	90th percentile direct and indirect consumption of community water, consumers-only population, two-day average[a]	*Exposure Factors Handbook*, Chapter 3 (U.S. EPA, 2019b), NHANES 2005–2010[b]
Children aged 0 to < 7 yrs	0.0553		
Children aged 1 to < 5 yrs	0.0447		
Children aged 1 to < 7 yrs	0.0426		

Notes: yrs = years; L/kg bw-day = liters of water consumed per kilogram body weight per day. The DWI-BW used to calculate the iHA is in bold.

[a] Community water = water from PWSs; consumers-only population = quantity of water consumed per person in a population composed only of individuals who consumed water during a specified period.

[b] DWI-BWs are based on NHANES 2005−2010 data which is also reported in the EFH. DWI-BWs for the age ranges in this table were calculated using the FCID Commodity Consumption Calculator (available at https://fcid.foodrisk.org/percentiles).

2.3 Relative Source Contribution

When calculating HA values, EPA applies an RSC which represents the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee or tea, as well as from transfer to dietary items prepared with the local drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD is allocated to other potential exposure sources (U.S. EPA, 2000a); for PFOA, other potential exposure sources include food and food contact materials, consumer products (e.g., personal care products), ambient and indoor air, and indoor dust. The purpose of the RSC is to ensure that the level of a contaminant (e.g., the HA value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD (U.S. EPA, 2000a).

To determine the RSC, EPA follows the Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a). EPA conducted a broad literature search in 2019 to identify and evaluate information on sources of human PFAS (including PFOA) exposure to inform RSC determination, and subsequently updated the search through March 2021 (see U.S. EPA [2021b] for more details on the literature search methodologies and results). This literature search focused on real-world occurrences (measured concentrations) primarily in media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil). The initial search identified 3,622 peer-

reviewed papers that matched search criteria (U.S. EPA, 2021b). Despite the U.S. phase-out of production, EPA has found widespread PFOA contamination in water, sediments, and soils. Exposure to PFOA can occur through food (including fish and shellfish), water, house dust, and contact with consumer products. The search did not identify adequate exposure information across potential exposure sources and specific to children aged 0 to < 5 years that could be used to quantify exposure and inform RSC derivation. The findings indicate that many other sources of PFOA exposure beyond drinking water ingestion exist (e.g., food, indoor dust), but that data are insufficient to allow for quantitative characterization of the different exposure sources. EPA's Exposure Decision Tree approach states that when there is insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20% (U.S. EPA, 2000a). This means that 20% of the exposure equal to the RfD is allocated to drinking water, and the remaining 80% is attributed to all other potential exposure sources.

2.4 Derivation of Health Advisory Value: Interim Lifetime Noncancer HA

The lifetime iHA for PFOA is calculated as follows:

$$\textbf{Lifetime iHA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 1)}$$

$$\textbf{Lifetime iHA} = \left(\frac{0.0000000015 \ \frac{\text{mg}}{\text{kg bw-day}}}{0.0701 \frac{\text{L}}{\text{kg bw-day}}}\right) * 0.2$$

$$\textbf{Lifetime iHA} = 0.000000004 \ \frac{\text{mg}}{\text{L}}$$

$$= 0.000004 \ \frac{\mu\text{g}}{\text{L}}$$

$$= 0.004 \ \frac{\text{ng}}{\text{L}}$$

Based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, the lifetime iHA can be applied to short-term scenarios because the critical effect identified for PFOA is a developmental effect that can potentially result from short-term PFOA exposure during a critical period of development (U.S. EPA, 1991). EPA concludes that the lifetime iHA of 0.004 nanograms per liter (ng/L) (or 4 parts per quadrillion [ppq]) for PFOA can be applied to both short-term and chronic risk assessment scenarios.

3.0 Analytical Methods

EPA developed the following liquid chromatography/tandem mass spectrometry (LC/MS/MS) analytical methods to quantitatively monitor drinking water for targeted PFAS that include PFOA: EPA Method 533 (U.S. EPA, 2019a) and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020).

EPA Method 533 monitors for 25 select PFAS with published measurement accuracy and precision data for PFOA in reagent water, finished ground water, and finished surface water. For further details about the procedures for this analytical method, please see *Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry* (U.S. EPA, 2019a).

EPA Method 537.1 (an update to EPA Method 537 [U.S. EPA, 2009c]) monitors for 18 select PFAS with published measurement accuracy and precision data for PFOA in reagent water, finished ground water, and finished surface water. For further details about the procedures for this analytical method, please see *Method 537.1, Version 2.0, Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)* (U.S. EPA, 2020).

Drinking water analytical laboratories have different performance capabilities dependent upon their instrumentation (manufacturer, age, usage, routine maintenance, operating configuration, etc.) and analyst experience. Some laboratories will effectively generate accurate, precise, quantifiable results at lower concentrations than others. Organizations leading efforts that include the collection of data need to establish data quality objectives (DQOs) to meet the needs of their program. These DQOs should consider establishing reasonable quantitation limits that laboratories can routinely meet, without recurring quality control (QC) failures that will necessitate repeating sample analyses, increase costs, and potentially reduce laboratory capacity. Establishing a quantitation limit that is too high may result in important lower-concentration results being overlooked.

EPA's approach to establishing DQOs within the UCMR program serves as an example. EPA established MRLs for UCMR 5,[9] and requires laboratories approved to analyze UCMR samples to demonstrate that they can make quality measurements at or below the established MRLs. EPA calculated the UCMR 5 MRLs using quantitation-limit data from multiple laboratories participating in an MRL-setting study. The laboratories' quantitation limits represent their lowest concentration for which future recovery is expected, with 99% confidence, to be between 50 and 150%.

The UCMR 5-derived and promulgated MRL for PFOA is 0.004 µg/L (4 ng/L).

4.0 Treatment Technologies

This section summarizes the available drinking water treatment technologies that have been demonstrated to remove PFOA from drinking water, but it is not meant to provide specific operational guidance or design criteria. Sorption-based treatment processes such as granular activated carbon (GAC), powdered activated carbon (PAC), and anion exchange (AIX), as well as high-pressure membrane processes such as nanofiltration (NF) and reverse osmosis (RO), have been shown to successfully remove PFOA from drinking water to below the 0.004 µg/L MRL for UCMR 5 (Bartell et al., 2010; Hölzer et al., 2009). These treatment processes may have additional benefits on finished water quality by removing other contaminants and disinfection byproduct (DBP) precursors. Care should be taken when introducing one of these processes into

[9] Information about UCMR 5 is available at https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule

a well-functioning treatment train, as there can be interactions with other treatment processes. Care should also be taken for system operators unfamiliar with proper operation and potential hazards. General information and published PFAS treatment data for these processes may be found in EPA's Drinking Water Treatability Database (U.S. EPA, 2022b).

Non-treatment PFOA management practices such as changing source waters, source water protection, or consolidation are also viable PFOA drinking water reduction options. One resource for protecting source water from PFAS, including PFOA, is the *PFAS − Source Water Protection Guide and Toolkit* (ASDWA, 2020), which shares effective strategies for addressing PFAS contamination risk in source waters. Source water protection is particularly important since PFOA can withstand biotic and abiotic degradation mechanisms except in unique situations that cannot be controlled *in situ* or result in complete defluorination (Huang and Jaffe, 2019; Rahman et al., 2014), indicating that PFOA is persistent and thus, natural attenuation is not a valid PFOA management strategy.

4.1 Sorption Technologies

Sorption technologies remove substances present in liquids by accumulation onto a solid phase (Crittenden et al., 2012). The two main sorption technologies that have been successfully used for full-scale PFOA removal are activated carbon and AIX. Activated carbon has been successfully applied in contactors as GAC or in powdered as well as slurry forms (PAC). Key considerations in choosing sorption technologies include influent water quality and desired effluent quality. Influent water quality can greatly impact the ability of sorption technologies to treat drinking water. Desired water quality can drive both operational and capital expenditures. When using a technology requiring a contactor, sizing the contactor is an important consideration that should include a pilot study. Pilot scale testing is highly recommended to ensure the treatment performance will be maximized for given source waters. EPA's *ICR Manual for Bench- and Pilot-Scale Treatment Studies* (U.S. EPA, 1996) contains guidance on conducting pilot studies for contactors which are used for GAC and AIX. Contactor efficacy can be compromised by particulate, organic, and inorganic constituents.

Both GAC and AIX can typically be regenerated when treatment performance reaches an unacceptable level. The choice between regeneration and replacement is a key planning decision. Regeneration can be on- or off-site. On-site regeneration typically requires a higher spatial footprint and capital outlay. Given water quality and other considerations, regenerated media can become totally exhausted or "poisoned" with other contaminants not removed during the regeneration process and must be replaced. However, most AIX resins in current use for PFOA technologies are single-use resins and not designed to be regenerated.

Two common interferences with sorption technologies relevant to PFAS are preloading (when a non-targeted compound is removed ahead of the targeted contaminant and prevents the targeted contaminant from accessing the sorption site) and competitive sorption (when one compound inhibits the removal of another by direct competition). The interferences can result in slowed sorption kinetics and reduced sorption capacities. It is also important to note that sorption technologies are largely reversible. PFAS in general, and PFOA specifically, can detach from sorbents and re-enter drinking water under certain conditions. In addition, direct competition with stronger sorbing constituents can lead to effluent PFOA concentrations temporarily exceeding influent concentration (known as chromatographic peaking). This has been

documented in full-scale treatment plants (Appleman et al., 2013; Eschauzier et al., 2012; McCleaf et al., 2017; Takagi et al., 2011). Common PFOA competitors for binding sites on sorptive media include natural or dissolved organic matter (NOM/DOM) which lowers treatment efficacy (McNamara et al., 2018; Park et al., 2020; Pramanik et al., 2015; Yu et al., 2012). Preloading may be controlled in the design phase through pretreatment processes. For more information about managing preloading, see AWWA (2018a). Competitive sorption may be controlled by changing or regeneration of the sorptive media at appropriate intervals.

4.1.1 Activated Carbon

Activated carbon is a highly porous media with high internal surface areas (U.S. EPA, 2017b). Activated carbon can be made from a variety of materials. Designs that work with carbon made from one source material activated in a specific way may not be optimized for other carbon types. There is some indication that of the common trace capacity tests, higher methylene blue numbers are most correlated with higher PFOA removal (Sörengård et al., 2020). Installing activated carbon as a treatment method may also have ancillary benefits on finished water quality, particularly regarding DBP control, other contaminants, as well as taste-and-odor compounds.

Activated carbon tends to remove non-polar, larger compounds more easily from water than smaller, more polar compounds. Adsorption of acids and bases on activated carbon is pH-dependent. Adsorption of neutral forms, as opposed to anionic forms, is generally stronger, so lowering the pH increases PFOA sorption. However, the acid dissociation constant (pKa) of PFOA is about 3.8 (Burns et al., 2008) and lowering the pH may not be practical operationally.

Before the addition of activated carbon to an existing treatment train, there are issues which should be considered. For instance, activated carbon may change system pH or release leachable metals (particularly arsenic and antimony) especially when new carbon media is first used without acid washing. These effects are typically mitigated through an acid wash or forward flushing. Activated carbon may also impact disinfection efficacy depending on process placement and requires consideration to mitigate its effects; for more information, please see the American Water Works Association (AWWA) GAC standard (American National Standards Institute (ANSI)/AWWA B604-18; AWWA, 2018a) or the AWWA published standard for PAC (ANSI/AWWA B600-16; AWWA, 2016). Activated carbon can also shift the bromide-to-total organic carbon ratio and increase brominated (Br)-DBP concentrations (Krasner et al., 2016); however, despite increased Br-DBP, studies have indicated a decreased overall DBP concentration and risk (Wang et al., 2019). DBPs may be mitigated through NOM (DBP precursor) removal; please see Zhang et al. (2015) for additional information.

4.1.1.1 Granular Activated Carbon

PFOA can be effectively removed from water by using GAC; contactors are normally placed as a post-filter step. Key design criteria include empty bed contact time (EBCT), superficial velocity, and carbon type. Typical EBCTs for PFOA removal are 10–20 minutes and superficial linear velocities are normally 5–15 meters per hour (m/hr). Normal height-to-diameter ratios are around 1.5 to 2.0; lower ratios can cause problems with too-shallow beds and require more space, and higher ratios can induce greater head drops. AWWA has published a GAC standard

(ANSI/AWWA B604-18; AWWA, 2018a) and a standard for GAC reactivation (ANSI/AWWA B605-18; AWWA, 2018b).

4.1.1.2 Powdered Activated Carbon

PAC is the same material as GAC, but it has a smaller particle size and is applied differently. PAC is typically dosed intermittently although it can be employed continuously if there are spatial constraints restricting contactor use. PAC dosage and type, along with dosing location, contact time, and water quality, often influence process cost as well as treatment efficiency (Heidari et al., 2021). For more information on employing PAC, please see the Drinking Water Treatability Database (U.S. EPA, 2022b).

While relatively unstudied in PFAS, increasing PAC dose with other contaminants increases removal to a point, after which it starts to decrease. Jar testing is typically used to empirically determine the optimal PAC dosage; doses between 45 and 100 mg/L are generally suitable for PFOA (Dudley, 2012; Hopkins et al., 2018; Sun et al., 2016). Standardized jar testing procedures have been published (ASTM International, 2019; AWWA, 2011). The AWWA published standard for PAC is ANSI/AWWA B600-16 (AWWA, 2016).

PAC can pose additional safety considerations including depleting oxygen in confined or partially enclosed areas, fire hazards including spontaneous combustion when stored with hydrocarbons or oxidants, and inhalation hazards and must be managed accordingly. PAC is also a good electrical conductor and can create dangerous conditions when it accumulates (AWWA, 2016). These dangers can be effectively mitigated through occupational safety programs such as confined space or fire safety programs. Please see AWWA (2016) for more information.

4.1.2 Ion Exchange

Ion exchange involves the exchange of an aqueous ion (e.g., contaminant) for an ion on an exchange resin. Once the resin has exchanged all its ions for contaminants, it can either be replaced (single use) or regenerated (i.e., restoring its ions for further use).

Different resin types preferentially bind certain ions over others; therefore, resin selection is an important consideration. As PFOA will predominantly exist in an anionic form in water and is a strong acid (U.S. EPA, 2021h), strongly basic AIX resins will be the most relevant for PFOA. Regenerating PFOA-saturated resins has been accomplished effectively with a brine of > 20% sodium chloride and ammonium chloride. Sodium hydroxide may be added to the sodium chloride solution to combat organic fouling; this is referred to as ‘brine squeeze’ and helps in solubilizing NOM and unplugging pores (Dixit et al., 2021). Regenerated media can be “poisoned,” meaning that a non-target ion not removed by the in-place regeneration procedures eventually crowds out available active sites. When this happens or if media is not regenerated, it must be disposed of appropriately. Once PFAS-contaminated spent brine is recovered, it must be treated or disposed of. Resin regeneration may not be practical for water utilities from safety and/or cost perspectives (Liu and Sun, 2021).

In some situations, AIX may outperform activated carbon for removing PFOA from drinking water (Liu and Sun, 2021). Key design parameters for GAC also apply to AIX, and they can be operated similarly. AIX typically uses 2-to-5-minute EBCTs, allowing for lower capital costs and a smaller footprint; compared to GAC, smaller height-to-diameter ratios are typically used in

exchange columns. However, AIX resin is typically more costly compared to GAC which may increase overall operational costs. Columns used in pilot studies are scaled directly to full-scale if loading rates and EBCTs are kept constant (Crittenden et al., 2012).

Before the addition of AIX to an existing treatment train, there are effects which must be considered. For instance, AIX can increase water corrosivity and/or release amines and will increase concentrations of the counter-ion used (typically chloride). These effects may usually be mitigated through prior planning which may include corrosion control adjustments; for more information about corrosion control, see U.S. EPA (2016d). Additionally, PFOA-saturated resin regeneration creates an additional PFOA waste stream which will require appropriate handling. For more information about AIX, please see Crittenden et al. (2012), Dixit et al. (2021), Tanaka (2015), Tarleton (2014), and the EPA Drinking Water Treatability Database (U.S. EPA, 2022b).

4.2 High-Pressure Membranes

NF and RO are high-pressure processes where water is forced across a membrane. The water that transverses the membrane is known as permeate or produce, and has few solutes left in it; the remaining water is known as concentrate, brine, retentate, or reject water and forms a waste stream with concentrated solutes. NF has a less dense active layer than RO, which enables lower operating pressures but also makes it less effective at removing contaminants. Higher operating pressures and initial flux generally enhance removal. Temperature and pH are also significant parameters affecting performance. In general, organic NF membranes have lower operating costs and easier processing than inorganic membranes while maintaining appropriate robustness for PFOA treatment (Jin et al., 2021). NF and RO tend to take up less space than sorptive separation technologies; however, both NF and RO also tend to have higher operating expenses, use a significant amount of energy, and generate concentrate waste streams which require disposal. Generally, NF and RO require pre- and post-treatment processes. Higher expenses typically associated with NF and RO are only rarely competitive from an economic perspective for removing a specific contaminant; however, for waters requiring significant treatment and where concentrate disposal options are reasonably available, NF and RO may be the best option.

PFOA removal fluxes are generally 20–80 liters per square meter per hour (L/[m^2·hr]) at 0.2–1.2 megapascal (MPa) operating pressure (Mastropietro et al., 2021) with removal from 90% to > 99% (Jin et al., 2021). Temperature can dramatically impact flux; it is common to normalize flux to a specific reference temperature for operational purposes (U.S. EPA, 2005b). It is also common to normalize flux to pressure ratios to identify productivity changes attributable to fouling (U.S. EPA, 2005b). It is important to note that water may traverse the membranes from outside-in or inside-out; different system configurations operating at the same flux produce differing quantities of finished water. This means that membrane systems with differing configurations cannot be directly compared based on flux. Total flow per module and cost per module are more important decision support indicators for capital planning. Unlike low-pressure membranes, NF and RO systems are not manufactured as proprietary equipment and membranes from one manufacturer are typically interchangeable with those from others (U.S. EPA, 2005b).

High-pressure membranes may have effects when added onto a well-functioning treatment train. For instance, high-pressure membranes may remove beneficial minerals and increase corrosivity. Increased water corrosivity may need to be addressed through corrosion control treatment

modifications and water may require remineralization. For more information, see AWWA (2007) and U.S. EPA (2016d).

4.3 Point-of-Use Devices for Individual Household PFOA Removal

Although the focus of this treatment technologies section is the different available options for removal of PFOA at drinking water treatment plants, centralized treatment technologies can also often be used in a decentralized fashion as point-of-entry (where the distribution system meets a service connection) or point-of-use (at a specific tap or application) treatment in cases where centralized treatment is impractical or individual consumers wish to further reduce their individual household risks. Many home drinking water treatment units are certified by independent third-party accreditation organizations using ANSI standards to verify contaminant removal claims. NSF International has developed protocols for NSF/ANSI Standards 53 (sorption) and 58 (RO) that establish minimum requirements for materials, design, construction, and performance of point-of-use systems. Previously, NSF P473 was designed to certify PFOA reduction technologies below EPA's 2016 HA of 70 ppt for PFOA; in 2019, these standards were retired and folded into NSF/ANSI 53 and 58. PFOA removal by faucet filters has reportedly averaged 84%, whereas pitcher filters had an average of 67% removal, refrigerator filters 71%, single-stage under-sink filters 56%, two-stage filters > 99%, and RO filters > 92%. Some filters can remove PFOA to below the 0.004 µg/L UCMR 5 reporting limit (Herkert et al., 2020). Boiling water is not an effective point-of-use PFOA treatment, as it will concentrate PFOA.

4.4 Treatment Technologies Summary

Non-treatment PFOA management options, such as changing source waters, source water protection, or consolidation are viable strategies for reducing PFOA concentrations in finished drinking water. Should treatment be necessary, GAC, PAC, AIX, NF, and RO are the best means for removing PFOA from drinking water and can be used in central treatment plants or in point-of-use applications. These treatment processes are separation technologies and produce waste streams with PFOA, and all processes may have unintended effects on the existing treatment trains. PFOA treatment technologies often require pre- as well as post-treatment and may help remove other unwanted contaminants and DBP precursors. Boiling water will concentrate PFOA and should not be considered as an emergency action.

5.0 Consideration of Noncancer Health Risks from PFAS Mixtures

EPA recently released a *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (U.S. EPA, 2021i) that is currently undergoing SAB PFAS Panel review. That draft document describes a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on current, available EPA chemical mixtures approaches and methods (U.S. EPA, 2000b). Examples are presented for three approaches—Hazard Index (HI), Relative Potency Factor (RPF), and Mixture BMD—to demonstrate application to PFAS mixtures. To use these approaches, specific input values and information for each PFAS are needed or can be developed. These approaches may help to inform PFAS evaluation(s) by federal, state, and tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential noncancer human health hazards and risks associated with PFAS mixtures.

The HI approach, for example, could be used to assess the potential noncancer risk of a mixture of four component PFAS for which HAs, either final or interim, are available from EPA (PFOA, PFOS, GenX chemicals [hexafluoropropylene oxide dimer acid and its ammonium salt], and perfluorobutane sulfonic acid [PFBS]). In the HI approach described in the draft framework (U.S. EPA, 2021i), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (U.S. EPA, 1986). The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Eq. 2). In the context of PFAS in drinking water, a mixture PFAS HI can be calculated when health-based water concentrations (e.g., HAs, Maximum Contaminant Level Goals [MCLGs]) for a set of PFAS are available or can be calculated. In this example, HQs are calculated by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HA (e.g., expressed as ng/L) (Eqs. 3, 4). The component chemical HQs are then summed across the PFAS mixture to yield the mixture PFAS HIs based on interim and final HAs.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i} \quad \text{(Eq. 2)}$$

$$HI = HQ_{PFOA} + HQ_{PFOS} + HQ_{GenX} + HQ_{PFBS} \quad \text{(Eq. 3)}$$

$$HI = \left(\frac{[PFOA_{water}]}{[PFOA_{iHA}]}\right) + \left(\frac{[PFOS_{water}]}{[PFOS_{iHA}]}\right) + \left(\frac{[GenX_{water}]}{[GenX_{HA}]}\right) + \left(\frac{[PFBS_{water}]}{[PFBS_{HA}]}\right) \quad \text{(Eq. 4)}$$

Where:

HI = hazard index

n = the number of component (i) PFAS

HQ_i = hazard quotient for component (i) PFAS

E_i = human exposure for component (i) PFAS

RfV_i = human health-based toxicity value for component (i) PFAS

HQ_{PFAS} = hazard quotient for a given PFAS

$[PFAS_{water}]$ = concentration for a given PFAS in water

$[PFAS_{HA}]$ = HA value, interim or final, for a given PFAS

In cases when the mixture PFAS HI is greater than 1, this indicates an exceedance of the health protective level and indicates potential human health risk for noncancer effects from the PFAS mixture in water. When component health-based water concentrations (in this case, HAs) are below the analytical method detection limit, as is the case for PFOA and PFOS, such individual component HQs exceed 1, meaning that any detectable level of PFOA or PFOS will result in an HI greater than 1 for the whole mixture. Further analysis could provide a refined assessment of the potential for health effects associated with the individual PFAS and their contributions to the potential joint toxicity associated with the mixture. For more details of the approach and illustrative examples of the RPF approach and Mixture BMD approaches, please see U.S. EPA (2021i).

6.0 Interim Health Advisory Characterization

The purpose of developing the lifetime iHA for PFOA is to reflect the best available scientific information which indicates that PFOA can lead to adverse noncancer health effects at exposure levels that are much lower than previously understood (U.S. EPA, 2016c). The PFOA iHA of 0.004 ng/L is considered applicable to both short-term and chronic risk assessment scenarios because the critical effect identified for PFOA can result from developmental exposure and leads to long-term adverse health effects. Therefore, short-term PFOA exposure during a critical period of development may lead to adverse health effects across life stages.

In 2019, EPA initiated an updated literature search and analysis of health effects information for PFOA to better characterize the health hazards and risks of exposure using information published since EPA developed the 2016 HA for PFOA (draft PFOA document; U.S. EPA, 2021b). The draft PFOA document includes an updated cancer classification, draft chronic RfD, and draft RSC. The draft PFOA document is currently undergoing review by the SAB PFAS Panel as part of EPA's process for developing a NPDWR for PFOA under SDWA. The draft report of the SAB PFAS Panel's review (U.S. EPA, 2022a) is supportive of the draft conclusions; however, the SAB PFAS Panel is recommending analyses that may impact the final RfD, CSF, and RSC. Because the iHA is based on draft values, it is subject to change. Additionally, the candidate draft CSFs calculated in the draft PFOA document indicate that PFOA is a more potent carcinogen than described in the 2016 HA for PFOA. However, because the draft PFOA document presented multiple candidate CSFs from the available human and animal studies and did not select one draft CSF, EPA did not derive an updated 10^{-6} cancer risk concentration for PFOA for this iHA document. Furthermore, an initial evaluation of the multiple candidate CSFs indicates that the resulting 10^{-6} cancer risk concentrations are either greater than or in the same range as the iHA value.

EPA expects to propose an MCLG and NPDWR for PFOA in the fall of 2022 and to promulgate a final MCLG and NPDWR by the fall of 2023 after considering public comment. EPA will complete its revisions to address the final SAB report's comments in the proposed PFOA MCLG and NPDWR. EPA may update or remove the iHA for PFOA at that time. Based, however, on the updated systematic review of the best available science on PFOA exposure and health effects and taking into consideration the work EPA is doing now to address SAB comments, the health-based drinking water values for PFOA (HA and MCLG) are anticipated to remain below the current UCMR 5 analytical MRL (0.004 µg/L or 4 ng/L).

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Attachment 6

United States Environmental Protection Agency

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Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Office of Science and Technology
Health and Ecological Criteria Division
Washington, DC 20460

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- Office of Water
- Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics
- Office of Land and Emergency Management
- Office of Policy
- Office of Children's Health Protection
- Office of Research and Development

Contents

Tables

Abbreviations and Acronyms

AIX	anion exchange
ANSI	American National Standards Institute
AWWA	American Water Works Association
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
Br-DBP	brominated disinfection byproduct
bw or BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CCL	Contaminant Candidate List
CDC	Centers for Disease Control and Prevention
CDR	Chemical Data Reporting
CI	confidence interval
CSF	cancer slope factor
DBP	disinfection byproduct
DOM	dissolved organic matter
DQO	data quality objective
DWI	drinking water intake
DWI-BW	body weight-adjusted drinking water intake
E	human exposure
EBCT	empty bed contact time
EF	exposure factor
EFH	Exposure Factors Handbook
EPA	U.S. Environmental Protection Agency
Eq.	equation
FCID	Food Commodity Intake Database
GAC	granular activated carbon
HA	Health Advisory
HECD	Health and Ecological Criteria Division
HESD	Health Effects Support Document
HI	hazard index
HQ	hazard quotient
iHA	interim Health Advisory
i	mixture component chemical
IRIS	Integrated Risk Information System
$L/(m^2 \cdot hr)$	liters per square meter per hour
lbs	pounds
LC/MS/MS	liquid chromatography/ tandem mass spectrometry
LOAEL	lowest-observed-adverse-effect level
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg/kg bw-day	milligrams per kilogram body weight per day
mg/L	milligrams per liter
m/hr	meters per hour
MPa	megapascal
MRL	minimum reporting level
NF	nanofiltration

ng/L	nanograms per liter
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NOM	natural organic matter
NPDWR	National Primary Drinking Water Regulation
OGWDW	Office of Ground Water and Drinking Water
ORD	Office of Research and Development
OST	Office of Science and Technology
OW	Office of Water
PAC	powdered activated carbon
PBPK	physiologically-based pharmacokinetic
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutane sulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
pK_a	acid dissociation constant
POD	point of departure
POD_{HED}	point of departure human equivalent dose
ppq	parts per quadrillion
ppt	parts per trillion
PWS	public water system
QC	quality control
RfD	reference dose
RfV	reference value
RO	reverse osmosis
RPF	relative potency factor
RSC	relative source contribution
SAB	Science Advisory Board
SAB PFAS Panel	Science Advisory Board Per- and Polyfluoroalkyl Substances Review Panel
SDWA	Safe Drinking Water Act
SNUR	Significant New Use Rule
TSCA	Toxic Substances Control Act
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	lowest-observed-adverse-effect level-to-no-observed-adverse-effect level extrapolation uncertainty factor
UF_S	subchronic-to-chronic exposure duration extrapolation uncertainty factor
µg/L	micrograms per liter

1.0 Introduction: Background and Scope of Interim Health Advisory

The Safe Drinking Water Act (SDWA) (42 U.S.C. § § 300f - 300j-27) authorizes the U.S. Environmental Protection Agency (EPA) to develop drinking water Health Advisories (HAs).[1] HAs are national non-enforceable, non-regulatory drinking water concentration levels of a specific contaminant at or below which exposure for a specific duration is not anticipated to lead to adverse human health effects.[2] HAs are intended to provide information that tribal, state, and local government officials and managers of public water systems (PWSs) can use to determine whether actions are needed to address the presence of a contaminant in drinking water. HA documents reflect the best available science and include HA values as well as information on health effects, analytical methodologies for measuring contaminant levels, and treatment technologies for removing contaminants from drinking water. EPA's lifetime HAs identify levels to protect all Americans, including sensitive populations and life stages, from adverse health effects resulting from exposure throughout their lives to contaminants in drinking water.

Interim or provisional HA values can be developed to provide information in response to an urgent or rapidly developing situation. EPA has developed an interim lifetime noncancer HA (iHA) for perfluorooctane sulfonic acid (PFOS) to replace the 2016 lifetime HA of 0.07 micrograms per liter (µg/L) (70 parts per trillion [ppt]) because analyses of more recent health effects studies show that PFOS can impact human health at exposure levels much lower than reflected by the 2016 PFOS lifetime HA. EPA has developed an interim rather than a final HA for PFOS because the input values used to derive the iHA are currently draft values and EPA has identified a pressing need to provide information to public health officials prior to their finalization.

In 2009, EPA developed a provisional HA for PFOS (U.S. EPA, 2009a) based on the best information available at that time. Also, PFOS was included on the third and fourth drinking water Contaminant Candidate Lists (CCLs)[3] (U.S. EPA, 2009b, 2016a). After PFOS was listed on the third CCL in 2009, EPA initiated development of a Health Effects Support Document (HESD) for PFOS to assist officials and PWS managers in protecting public health when PFOS is present in drinking water. The HESD was published in 2016 after peer review (U.S. EPA, 2016b). EPA developed a final HA for PFOS (U.S. EPA, 2016c) based on data and analyses in the 2016 HESD and agency guidance on exposure and risk assessment.

In March 2021, EPA published a final determination to regulate PFOS with a National Primary Drinking Water Regulation (NPDWR) under SDWA (U.S. EPA, 2021a). NPDWRs include legally-enforceable Maximum Contaminant Levels (MCLs) and/or treatment technique requirements that apply to PWSs. To support the development of the NPDWR, EPA developed

[1] SDWA § 1412(b)(1)(F) authorizes EPA to "publish health advisories (which are not regulations) or take other appropriate actions for contaminants not subject to any national primary drinking water regulation." www.epa.gov/sites/default/files/2020-05/documents/safe_drinking_water_act-title_xiv_of_public_health_service_act.pdf

[2] This document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community. This document is not enforceable against any person and does not have the force and effect of law. No part of this document, nor the document as a whole, constitutes final agency action that affects the rights and obligations of any person. EPA may change any aspects of this document in the future.

[3] The CCL is a list (published every five years) of contaminants that are not currently subject to any National Primary Drinking Water Regulation (NPDWR) but are known or anticipated to occur in PWSs and may require future regulation under SDWA.

the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b) (hereafter referred to as "draft PFOS document") which includes an updated health effects assessment of the peer-reviewed literature, draft chronic reference dose (RfD), and draft relative source contribution (RSC) value. The development of the draft noncancer chronic RfD for PFOS was performed by a cross-agency per- and polyfluoroalkyl substances (PFAS) Science Working Group to support the PFAS NPDWR. In November 2021, EPA announced the Science Advisory Board (SAB) PFAS Review Panel's (SAB PFAS Panel's) review (U.S. EPA, 2021c) of the draft PFOS document along with three other draft documents supporting the NPDWR (U.S. EPA, 2022a).

The 2021 data and analyses described in the draft PFOS document indicate that PFOS exposure levels at which adverse health effects have been observed are much lower than previously understood when EPA issued an HA for PFOS in 2016. As a result, EPA announced in 2021[4] that it would move quickly to update the 2016 HA for PFOS to reflect the latest, best available science as well as input from the SAB PFAS Panel. An updated PFOS HA is consistent with EPA's commitments for action on PFAS described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021d).

In April 2022, the SAB PFAS Panel made public a draft report of its review of the draft PFOS document (U.S. EPA, 2022a), which indicated general support for the draft conclusions but recommended additional analyses be performed prior to finalizing the RfD and RSC. Because the RfD in the draft PFOS document is much lower than the RfD used to derive the 2016 HA, there is a pressing need to provide updated information on the current best available science to public health officials prior to finalization of the health effects assessment. Therefore, EPA has decided to issue an iHA using the draft chronic RfD and RSC values. An updated 10^{-6} cancer risk concentration was not derived in this iHA document because the draft PFOS document concluded that, based on EPA guidelines (U.S. EPA, 2005a), the available human and animal studies provide *suggestive evidence of carcinogenic potential* (U.S. EPA, 2021b). Given the identified uncertainties in the available evidence (see Section 2.0 for further information), the draft PFOS document concluded that these data did not support a quantitative characterization of cancer risk associated with PFOS exposure.

After receiving SAB's final report, EPA will fully address SAB feedback and recommendations, which could lead EPA to draw different conclusions than are reflected in the draft PFOS document and this iHA document. EPA anticipates proposing a NPDWR in fall 2022 and finalizing the NPDWR in fall 2023. EPA may update or remove the iHA for PFOS upon finalization of the NPDWR.

1.1 PFOS General Information and Uses

PFOS is a synthetic fluorinated organic chemical that has been manufactured and used in a variety of industries since the 1940s (U.S. EPA, 2018). It repels water and oil, is chemically and thermally stable, and exhibits surfactant properties. Based on these properties, it has been used in the manufacture of many materials, including cosmetics, paints, polishes, and nonstick coatings on fabrics, paper, and cookware. It is very persistent in the human body and the environment (Calafat

[4] *EPA Advances Science to Protect the Public from PFOA and PFOS in Drinking Water* [Press release], Nov 16, 2021: https://www.epa.gov/newsreleases/epa-advances-science-protect-public-pfoa-and-pfos-drinking-water

et al., 2007, 2019). More information about PFOS's uses and properties can be found in the 2016 HA document for PFOS (U.S. EPA, 2016c) and the draft PFOS document (U.S. EPA, 2021b).

In 2000, the principal manufacturer of PFOS agreed to a voluntary phase-out of PFOS production and use. This phase-out was completed in 2002 (U.S. EPA, 2007). PFOS is included in EPA's Toxic Substances Control Act (TSCA) Significant New Use Rule (SNUR) issued in December 2002, which ensures that EPA will have an opportunity to review any efforts to reintroduce PFOS into the marketplace and take action, as necessary, to address potential concerns (U.S. EPA, 2002a). Limited existing uses of PFOS-related chemicals, including as an anti-erosion additive in fire-resistant aviation hydraulic fluids and as a component of anti-reflective coating in the production of semiconductors, were excluded from the regulation (U.S. EPA, 2013). PFOS was not reported as manufactured (or imported) in the United States as part of the 2006, 2012, or 2016 TSCA Chemical Data Reporting (CDR) effort, which requires reporting if a certain production volume threshold is met at any single site (the threshold for PFOS was 25,000 pounds [lbs] in 2006 and 2012, and 2,500 lbs in 2016).[5] PFOS manufacture or importation has not been reported to EPA as part of this collection effort since 2002.

1.2 Occurrence in Water and Exposure to Humans

1.2.1 Occurrence in Water

EPA requires sampling at drinking water systems under the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are known or suspected to be found in drinking water and do not have health-based standards under SDWA. A new UCMR is issued every five years. The first four UCMRs required monitoring of all large public drinking water systems (> 10,000 people) and a subset of smaller systems serving < 10,000 people. The third UCMR (UCMR 3), conducted from 2013–2015, is currently the best available source of national occurrence data for PFOS in drinking water (U.S. EPA, 2017a, 2021a,b,e). A total of 292 samples from 95 PWSs (out of 36,972 total samples from 4,920 PWSs) had detections of PFOS (i.e., greater than or equal to the minimum reporting level [MRL][6] of 0.04 µg/L). PFOS concentrations for these detections ranged from 0.04 µg/L (the MRL) to 7 µg/L (median concentration of 0.06 µg/L; 90th percentile concentration of 0.25 µg/L).

In 2016, EPA recommended that when PFOS and perfluorooctanoic acid (PFOA) co-occur at the same time and location in drinking water sources, a conservative and health-protective approach is to consider the sum of the concentrations. An analysis of the UCMR 3 data showed that 508 samples from 162 PWSs (out of 36,971 samples from 4,920 PWSs) had detections of PFOA and/or PFOS (i.e., at or above the MRL of 0.02 µg/L for PFOA or 0.04 µg/L for PFOS). The sum of reported PFOA and/or PFOS concentrations ranged from 0.02 to 7.22 µg/L. Although it is not possible to determine the full extent of PFOS and/or PFOA occurrence based on UCMR 3 detections, sites where elevated levels of PFOS and/or PFOA were detected during UCMR 3 monitoring may have taken steps to mitigate exposure including installing treatment systems

[5] The TSCA CDR requires manufacturers (including importers) to provide EPA with information on the production and use of chemicals if they meet certain production volume thresholds. For more information, see www.epa.gov/chemical-data-reporting.
[6] The MRL refers to the quantitation level selected by EPA to ensure reliable and consistent results. It is the minimum quantitation level that can be achieved with 95 percent confidence by capable analysts at 75 percent or more of the laboratories using a specified analytical method (U.S. EPA, 2021f).

and/or blending water from multiple sources, or remediating known sources of contamination (U.S. EPA, 2021a).

The fifth UCMR (UCMR 5) will require monitoring for 29 PFAS, including PFOS, using EPA methods 533 (U.S. EPA, 2019a) and 537.1 (U.S. EPA, 2020). UCMR 5 monitoring will take place from 2023–2025 and will include all large public drinking water systems serving > 10,000 people, all systems serving 3,300–10,000 people (subject to the availability of appropriations), and a subset of smaller systems serving < 3,300 people (U.S. EPA, 2021f). EPA established an MRL for PFOS of 0.004 µg/L under UCMR 5, which is 10-fold lower than the MRL used in UCMR 3.

Some states have conducted monitoring for PFOS in drinking water (by selecting sampling locations randomly, and/or sampling from targeted locations). PFOS has been detected in the finished drinking water of at least 19 states (ADEM, 2021; AZDEQ, 2021; CADDW, 2021; CDPHE, 2020; GAEPD, 2021; ILEPA, 2021; KYDEP, 2019; MAEEA, 2021; MDE, 2021; MEDEP, 2020; MI EGLE, 2021; NCDEQ, 2021; NHDES, 2021; NJDEP, 2021; OHDOH, 2020; PADEP, 2021; RIDOH, 2020; SCDHEC, 2020; VTDEC, 2021).

1.2.2 Exposure in Humans

As noted in the draft PFOS document (U.S. EPA, 2021b), the Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOS has been detected in up to 98% of serum samples collected in biomonitoring studies that are representative of the U.S. general population; however, blood levels of PFOS declined by more than 80% between 1999 and 2014, presumably due to restrictions on PFOS commercial usage in the United States (CDC, 2017). NHANES biomonitoring data from 1999–2000 reveal a mean serum PFOS concentration of 30.4 µg/L (95% confidence interval [CI] of 27.1–33.9 µg/L) and a 90th percentile serum PFOS concentration of 57 µg/L (95% CI 50.2–71.7 µg/L) across 1,562 samples representative of the U.S. population. For 2013–2014, mean and 90th percentile serum PFOS concentrations were 4.99 µg/L (95% CI 4.5–5.52 µg/L) and 13.9 µg/L (95% CI 11.9–15.5 µg/L), respectively (2,165 samples) (CDC, 2021). In 2017–2018, the mean serum PFOS concentration was 4.25 µg/L (95% CI 3.90–4.62 µg/L) and the 90th percentile serum PFOS concentration was 11.5 µg/L (95% CI 10.0–13.1 µg/L) across 1,929 samples (CDC, 2021). For additional information about PFOS exposure in humans, see sections 3.3 and 5.0 of U.S. EPA (2021b).

1.3 Source of Toxicity Information for Interim Health Advisory Development

The lifetime noncancer iHA for PFOS is derived from draft values (i.e., chronic RfD and RSC) and relies on the best available science as derived in the draft PFOS document (U.S. EPA, 2021b), which is currently undergoing peer review by the SAB PFAS Panel. To develop the updated toxicity information in the draft PFOS document, a systematic review and evidence-mapping approach was utilized to identify, screen, and evaluate health effects data for PFOS. A literature search was performed to identify studies on the health effects of PFOS exposure in animals and humans published since the 2016 HESD and HA for PFOS. The search results were screened for relevancy, and literature identified as relevant underwent study quality evaluation and data extraction (please see U.S. EPA [2021b] for more details). Evidence for each health outcome was analyzed and synthesized, and overall judgments about the strength of the evidence

were developed. The best available health effects information identified and analyzed using systematic review was then used in the derivation of the chronic RfD. This systematic review process has been peer reviewed and is used by EPA's Office of Research and Development (ORD) Integrated Risk Information System (IRIS) program, as summarized in the draft PFOS document (U.S. EPA, 2021b). Similarly, a systematic review approach was used to identify, screen, and evaluate exposure information to develop the RSC based on the best available science.

1.4 Exposure Factor Information

An exposure factor (EF), such as body weight-adjusted drinking water intake (DWI-BW), is one of the input values for deriving a drinking water HA. EFs are factors related to human activity patterns, behavior, and characteristics that help determine an individual's exposure to a contaminant. EPA's *Exposure Factors Handbook* (EFH)[7] is a resource for conducting exposure assessments and provides EFs based on information from publicly available, peer-reviewed studies. Chapter 3 of the EFH presents EFs in the form of drinking water intake values (DWIs) and DWI-BWs for various populations or life stages within the general population (U.S. EPA, 2019b). The use of EFs in HA calculations is intended to protect sensitive populations within the general population from adverse effects resulting from exposure to a contaminant.

When developing HAs, the goal is to protect all ages of the general population including potentially sensitive populations such as children. The approach to select the EF for drinking water HA derivation includes a step to identify potentially sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can prevent identification of the most sensitive population (e.g., not all windows of exposure or health outcomes have been assessed for PFOS), the critical effect and point-of-departure (e.g., human equivalent benchmark dose [BMD]) that form the basis for the RfD can provide some information about potentially sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In such cases, EPA can select the corresponding EFs for that sensitive population or life stage from the EFH (U.S. EPA, 2019b) for use in HA derivation. When multiple potentially sensitive populations or life stages are identified based on the critical effect or other health effects data (from animal or human studies), EPA selects the population or life stage with the greatest DWI-BW because it is the most health protective. For deriving lifetime HA values, the RSC corresponding to the selected sensitive life stage is also determined when data are available (see Section 2.2). In the absence of information indicating a potentially sensitive population or life stage, the EF corresponding to all ages of the general population may be selected.

To derive a chronic HA, EPA typically uses a DWI normalized to body weight (i.e., DWI-BW in L of water consumed/kg bw-day) for all ages of the general population or for a sensitive population or life stage, when identified. The Joint Institute for Food Safety and Applied

[7] Available at https://www.epa.gov/expobox/about-exposure-factors-handbook. The latest edition of the EFH was released in 2011, but since October 2017, EPA has begun to release chapter updates individually.

Nutrition's Food Commodity Intake Database (FCID) Consumption Calculator Tool[8] includes the EFs from EPA's EFH and can also be used to estimate DWIs and DWI-BWs for specific populations, life stages, or age ranges. EPA uses the 90th percentile DWI-BW to ensure that the HA is protective of the general population as well as sensitive populations or life stages (U.S. EPA, 2000a, 2016c). In 2019, EPA updated its EFs for DWI and DWI-BW based on newly available science (U.S. EPA, 2019b).

1.5 Approach for Lifetime Health Advisory Calculation

The following equation (Eq. 1) is used to derive an interim or final lifetime noncancer HA. A lifetime noncancer HA is designed to be protective of noncancer effects over a lifetime of exposure and is typically based on a chronic *in vivo* experimental animal toxicity study and/or human epidemiological data.

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 1)}$$

Where:

DWI-BW = the 90th percentile DWI for the selected population, adjusted for body weight, in units of L/kg bw-day. The DWI-BW considers both direct and indirect consumption of tap water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee).

RfD = chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime.

RSC = relative source contribution—the percentage of the total oral exposure attributed to drinking water sources where the remainder of the exposure is allocated to all other routes or sources (U.S. EPA, 2000a).

2.0 Interim Health Advisory Derivation: PFOS

A lifetime noncancer iHA was derived for PFOS. The DWI-BW selected to derive the iHA is for 0- to < 5-year-old children because PFOS exposure was measured in 5-year-old children in the critical study, and it is reasonable to expect that PFOS exposure levels were similar from birth through age 5 (see Section 2.2). Since a DWI-BW for 0- to < 5-year-old children was used, the iHA for PFOS is expected to be protective of children and adults of all ages in the general population; however, available data on the most sensitive population or life stage are limited.

Short-term iHAs (e.g., one- or ten-day iHAs) were not derived for PFOS because the draft PFOS document did not derive an RfD for short-term exposure. Additionally, EPA considers the lifetime iHA for PFOS to be applicable to short-term as well as lifetime risk assessment scenarios because the critical health effect on which the draft chronic RfD used to calculate the

[8] Joint Institute for Food Safety and Applied Nutrition's FCID Commodity Consumption Calculator is available at https://fcid.foodrisk.org/percentiles

iHA is based (i.e., deficient antibody response to diphtheria vaccine in children) resulted from PFOS exposure during a developmental life stage. EPA's risk assessment guidelines indicate that adverse effects can result from even brief exposure during a critical period of development (U.S. EPA, 1991). Therefore, the lifetime iHA for PFOS (calculated in Section 2.4) and the draft chronic RfD from which it is derived (see Table 1) are considered applicable to short-term PFOS exposures via drinking water.

As noted in the draft PFOS document (U.S. EPA, 2021b), there is *suggestive evidence of carcinogenic potential* of PFOS based on EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Epidemiological study results suggest a potential association between PFOS exposure and bladder or prostate cancers as discussed in the 2016 HESD for PFOS (U.S. EPA, 2016b). More recent epidemiological studies examining the association between PFOS and breast cancer show mixed results, and study characteristics (e.g., small sample sizes, narrow exposure levels) limit the ability to draw stronger conclusions about PFOS and breast cancer. The single available chronic duration cancer bioassay in animals reported increased incidences of liver, thyroid, and mammary gland tumors in rats, but a dose-response pattern was not observed. As noted in the draft PFOS document (U.S. EPA, 2021b), a draft cancer slope factor (CSF) was not derived for PFOS. This is consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) which state that when the available evidence is suggestive for carcinogenicity, a quantitative risk estimate is generally not derived unless there exists a well-conducted study that could facilitate an understanding of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In the draft PFOS document, EPA concluded that the available human and animal studies for PFOS are not sufficient to establish a reasonable understanding of the magnitude and uncertainty of potential risks for PFOS exposure and tumor incidence, and therefore do not justify a quantitative cancer assessment (U.S. EPA, 2021b). Since a draft CSF was not developed for PFOS, an interim 10^{-6} cancer risk concentration was not derived.

2.1 Toxicity

Table 1 reports the draft chronic RfD derived in the draft PFOS document (U.S. EPA, 2021b) that was used to develop the lifetime iHA for PFOS.

Table 1. Draft Chronic RfD, Critical Effect, and Critical Study Used to Develop the Lifetime iHA for PFOS.

Source	For the Lifetime iHA for PFOS			
	RfD (mg/kg bw-day)	**PFOS Exposure in Critical Study**	**Critical Effect**	**Principal and Associated Studies (Study Type)**
Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water [Draft] (U.S. EPA, 2021b)	7.9×10^{-9}	PFOS measured in serum of 5-year-old children	Developmental immune health outcome (suppression of diphtheria vaccine response in 7-year-old children)	Grandjean et al., 2012; Budtz-Jorgensen and Grandjean, 2018 (epidemiological study)

Note: mg/kg bw-day = milligrams per kilogram body weight per day.

Decreased serum anti-diphtheria antibody concentration in children, which was associated with increased serum PFOS concentrations (Budtz-Jorgensen and Grandjean, 2018; Grandjean et al., 2012), was selected as the critical effect for draft chronic RfD derivation. As noted in the draft PFOS document (U.S. EPA, 2021b), selection of this draft critical effect is expected to be protective of all other adverse health effects in humans because this adverse effect of decreased immune response to vaccination was observed after exposure during a sensitive developmental life stage, and it yields the lowest point of departure (POD) human equivalent dose (POD_{HED}) among the candidate $PODs_{HED}$. Other candidate RfDs were derived based on other health effects (e.g., development/growth) observed in epidemiology studies; all of the candidate RfDs are associated with low daily oral exposure doses, ranging from $\sim 10^{-7}$ to 10^{-9} milligrams per kilogram body weight per day (mg/kg bw-day) (U.S. EPA, 2021b; Table 23).

The selected draft POD_{HED} for this critical effect was derived by performing BMD modeling (see Appendix B.1 of U.S. EPA, 2021b) on measured PFOS serum concentrations at age five reported in the critical study, which yielded an internal serum concentration POD in milligrams per liter (mg/L). This internal serum concentration POD was then converted to an external dose (POD_{HED}) in mg/kg bw-day using the updated physiologically-based pharmacokinetic (PBPK) model developed by Verner et al. (described in section 4.1.3.2 of U.S. EPA, 2021b). Specifically, the POD_{HED} was calculated as the external dose (*in utero* through age five) that results in the internal serum concentration measured at five years of age in the critical study. (Note that the model predicted slightly different values for male and female children; the lower POD_{HED} was selected to be more health protective). An intraspecies uncertainty factor (UF_H) of 10 was applied to the selected draft POD_{HED} to account for variability in the response within the human population in accordance with methods described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). EPA applied a value of 1 for the remaining four uncertainty factors (UFs): interspecies UF (UF_A), because the critical effect was observed in humans and there is no need to account for uncertainty associated with animal-to-human extrapolation; lowest-observed-adverse-effect level (LOAEL)-to-no-observed-adverse-

effect level (NOAEL) extrapolation UF (UF_L), because a benchmark lower dose confidence limit (BMDL) instead of a LOAEL was used as the basis for POD_{HED} derivation; subchronic-to-chronic exposure duration extrapolation UF (UF_S), because the critical effect on the developing immune system in children was observed after exposure during gestation and/or early childhood, a sensitive period that can lead to severe effects without lifetime exposure; and a database UF (UF_D), because the database of animal and human studies on the effects of PFOS is comprehensive (see the draft PFOS document [U.S. EPA, 2021b] for further details). Thus, the total or composite UF (UF_C) used to derive the PFOS RfD was 10.

2.2 Exposure Factors

To identify potentially sensitive populations, EPA considered the sensitive life stage of exposure associated with the critical effect on which the draft chronic RfD was based. The critical study that was selected for draft chronic RfD derivation (see Table 1) established an association in children between PFOS serum concentration (measured at age five, after three of four diphtheria vaccinations) and decreased anti-diphtheria antibody concentration (measured at age seven, approximately two years after all four diphtheria vaccinations) (Budtz-Jorgensen and Grandjean, 2018). Based on limited available data to inform the critical PFOS exposure window for this critical developmental immune effect, the serum PFOS concentrations measured in 5-year-old children in this study are assumed to represent PFOS exposure from birth to the time of measurement. EPA acknowledges that the DWI-BW varies between ages 0 and 5 years (U.S. EPA, 2019b); however, the available data do not permit a more precise identification of the most sensitive or critical PFOS exposure window for the developmental immune outcome because studies with different exposure intervals have not been performed.

EPA calculated and considered DWI-BWs for other potentially sensitive age ranges indicated by the critical study data (e.g., 0 to < 7 years, 1 to < 5 years, 1 to < 7 years; Table 2). The DWI-BW for children aged 0 to < 5 years was selected among the DWI-BWs (see Table 2) because it is the greatest value and therefore the most health-protective. EPA also considered the use of a DWI-BW for formula-fed infants (i.e., infants fed primarily or solely with water-reconstituted infant formula) because their DWI-BW is higher (U.S. EPA, 2019b) and the infant life stage occurs within the 0- to < 5-year age range. However, a greater RSC would be used for formula-fed infants than for 0- to < 5-year-olds, which would result in a less health-protective iHA value (see Section 2.3). Therefore, EPA selected the DWI-BW for 0- to < 5-year-olds.

Table 2. EPA Exposure Factors for Drinking Water Intake for Candidate Sensitive Populations Based on the Critical Effect and Study.

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Children aged 0 to < 5 yrs	**0.0701**	90th percentile direct and indirect consumption of community water, consumers-only population, two-day average[a]	*Exposure Factors Handbook*, Chapter 3 (U.S. EPA, 2019b), NHANES 2005–2010[b]
Children aged 0 to < 7 yrs	0.0553		
Children aged 1 to < 5 yrs	0.0447		
Children aged 1 to < 7 yrs	0.0426		

Notes: yrs = years; L/kg bw-day = liters of water consumed per kilogram body weight per day. The DWI-BW used to calculate the iHA is in bold.

[a] Community water = water from PWSs; consumers only population = quantity of water consumed per person in a population composed only of individuals who consumed water during a specified period.

[b] DWI-BWs are based on NHANES 2005−2010 data which is also reported in the EFH. DWI-BWs for the age ranges in this table were calculated using the FCID Commodity Consumption Calculator (available at https://fcid.foodrisk.org/percentiles).

2.3 Relative Source Contribution

When calculating HA values, EPA applies an RSC which represents the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee or tea, as well as from transfer to dietary items prepared with the local drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD is allocated to other potential exposure sources (U.S. EPA, 2000a); for PFOS, other potential exposure sources include food and food contact materials, consumer products (e.g., personal care products), ambient and indoor air, and indoor dust. The purpose of the RSC is to ensure that the level of a contaminant (e.g., the HA value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD (U.S. EPA, 2000a).

To determine the RSC, EPA follows the Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a). EPA conducted a broad literature search in 2019 to identify and evaluate information on sources of human PFAS (including PFOS) exposure to inform RSC determination, and subsequently updated the search through March 2021 (see U.S. EPA [2021b] for more details on the literature search methodologies and results). This literature search focused on real-world occurrences (measured concentrations) primarily in media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil). The initial search identified 3,622 peer-reviewed papers that matched search criteria (U.S. EPA, 2021b). Despite the U.S. phase-out of production, EPA has found widespread PFOS contamination in water, sediments, and soils. Exposure to PFOS can occur through food (including fish and shellfish), water, house dust, and contact with consumer products. The search did not identify adequate exposure information across potential exposure sources and specific to children aged 0 to < 5 years that could be used to quantify exposure and inform RSC derivation. The findings indicate that many other sources of PFOS exposure beyond drinking water ingestion exist (e.g., food, indoor dust), but that data are insufficient to allow for quantitative characterization of the different exposure sources.

EPA's Exposure Decision Tree approach states that when there is insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20% (U.S. EPA, 2000a). This means that 20% of the exposure equal to the RfD is allocated to drinking water, and the remaining 80% is attributed to all other potential exposure sources.

2.4 Derivation of Health Advisory Value: Interim Lifetime Noncancer HA

The lifetime iHA for PFOS is calculated as follows:

$$\textbf{Lifetime iHA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 1)}$$

$$\textbf{Lifetime iHA} = \left(\frac{0.0000000079 \ \frac{\text{mg}}{\text{kg bw-day}}}{0.0701 \frac{\text{L}}{\text{kg bw-day}}}\right) * 0.2$$

$$\textbf{Lifetime iHA} = 0.00000002 \ \frac{\text{mg}}{\text{L}}$$

$$= 0.00002 \ \frac{\mu\text{g}}{\text{L}}$$

$$= 0.02 \ \frac{\text{ng}}{\text{L}}$$

Based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, the lifetime iHA can be applied to short-term scenarios because the critical effect identified for PFOS is a developmental effect that can potentially result from short-term PFOS exposure during a critical period of development (U.S. EPA, 1991). EPA concludes that the lifetime iHA of 0.02 nanograms per liter (ng/L) (or 20 parts per quadrillion [ppq]) for PFOS can be applied to both short-term and chronic risk assessment scenarios.

3.0 Analytical Methods

EPA developed the following liquid chromatography/tandem mass spectrometry (LC/MS/MS) analytical methods to quantitatively monitor drinking water for targeted PFAS that include PFOS: EPA Method 533 (U.S. EPA, 2019a) and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020).

EPA Method 533 monitors for 25 select PFAS with published measurement accuracy and precision data for PFOS in reagent water, finished ground water, and finished surface water. For further details about the procedures for this analytical method, please see *Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry* (U.S. EPA, 2019a).

EPA Method 537.1 (an update to EPA Method 537 [U.S. EPA, 2009c]) monitors for 18 select PFAS with published measurement accuracy and precision data for PFOS in reagent water, finished ground water, and finished surface water. For further details about the procedures for this analytical method, please see *Method 537.1, Version 2.0, Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)* (U.S. EPA, 2020).

Drinking water analytical laboratories have different performance capabilities dependent upon their instrumentation (manufacturer, age, usage, routine maintenance, operating configuration, etc.) and analyst experience. Some laboratories will effectively generate accurate, precise, quantifiable results at lower concentrations than others. Organizations leading efforts that include the collection of data need to establish data quality objectives (DQOs) to meet the needs of their program. These DQOs should consider establishing reasonable quantitation limits that laboratories can routinely meet, without recurring quality control (QC) failures that will necessitate repeating sample analyses, increase costs, and potentially reduce laboratory capacity. Establishing a quantitation limit that is too high may result in important lower-concentration results being overlooked.

EPA's approach to establishing DQOs within the UCMR program serves as an example. EPA established MRLs for UCMR 5,[9] and requires laboratories approved to analyze UCMR samples to demonstrate that they can make quality measurements at or below the established MRLs. EPA calculated the UCMR 5 MRLs using quantitation-limit data from multiple laboratories participating in an MRL-setting study. The laboratories' quantitation limits represent their lowest concentration for which future recovery is expected, with 99% confidence, to be between 50 and 150%.

The UCMR 5-derived and promulgated MRL for PFOS is 0.004 µg/L (4 ng/L).

4.0 Treatment Technologies

This section summarizes the available drinking water treatment technologies that have been demonstrated to remove PFOS from drinking water, but it is not meant to provide specific operational guidance or design criteria. In terms of treatment efficacy, PFOS generally shares many characteristics with PFOA but in most circumstances will be removed more easily using the same technologies (Sörengård et al., 2020). Sorption-based treatment processes such as granular activated carbon (GAC), powdered activated carbon (PAC), and anion exchange (AIX), as well as high-pressure membrane processes such as nanofiltration (NF) and reverse osmosis (RO), have been shown to successfully remove PFOS from drinking water to below the 0.004 µg/L MRL for UCMR 5 (Hölzer et al., 2009). These treatment processes may have additional benefits on finished water quality by removing other contaminants and disinfection byproduct (DBP) precursors. Care should be taken when introducing one of these processes into a well-functioning treatment train, as there can be interactions with other treatment processes. Care should also be taken for system operators unfamiliar with proper operation and potential hazards. General information and published PFAS treatment data for these processes may be found in EPA's Drinking Water Treatability Database (U.S. EPA, 2022b).

[9] Information about UCMR 5 is available at https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule

Non-treatment PFOS management practices such as changing source waters, source water protection, or consolidation are also viable PFOS drinking water reduction options. One resource for protecting source water from PFAS, including PFOS, is the *PFAS − Source Water Protection Guide and Toolkit* (ASDWA, 2020), which shares effective strategies for addressing PFAS contamination risk in source waters. Source water protection is particularly important since PFOS can withstand biotic and abiotic degradation mechanisms except in unique situations that cannot be controlled *in situ* or result in complete defluorination (Huang and Jaffe, 2019; Rahman et al., 2014), indicating that PFOS is persistent and thus, natural attenuation is not a valid PFOS management strategy.

4.1 Sorption Technologies

Sorption technologies remove substances present in liquids by accumulation onto a solid phase (Crittenden et al., 2012). The two main sorption technologies that have been successfully used for full-scale PFOS removal are activated carbon and AIX. Activated carbon has been successfully applied in contactors as GAC or in powdered as well as slurry forms (PAC). Key considerations in choosing sorption technologies include influent water quality and desired effluent quality. Influent water quality can greatly impact the ability of sorption technologies to treat drinking water. Desired water quality can drive both operational and capital expenditures. When using a technology requiring a contactor, sizing the contactor is an important consideration that should include a pilot study. Pilot scale testing is highly recommended to ensure the treatment performance will be maximized for given source waters. EPA's *ICR Manual for Bench- and Pilot-Scale Treatment Studies* (U.S. EPA, 1996) contains guidance on conducting pilot studies for contactors which are used for GAC and AIX. Contactor efficacy can be compromised by particulate, organic, and inorganic constituents.

Both GAC and AIX can typically be regenerated when treatment performance reaches an unacceptable level. The choice between regeneration and replacement is a key planning decision. Regeneration can be on- or off-site. On-site regeneration typically requires a higher spatial footprint and capital outlay. Given water quality and other considerations, regenerated media can become totally exhausted or "poisoned" with other contaminants not removed during the regeneration process and must be replaced. However, most AIX resins in current use for PFOS technologies are single-use resins and not designed to be regenerated.

Two common interferences with sorption technologies relevant to PFAS are preloading (when a non-targeted compound is removed ahead of the targeted contaminant and prevents the targeted contaminant from accessing the sorption site) and competitive sorption (when one compound inhibits the removal of another by direct competition). The interferences can result in slowed sorption kinetics and reduced sorption capacities. It is also important to note that sorption technologies are largely reversible. PFAS in general, and PFOS specifically, can detach from sorbents and re-enter drinking water under certain conditions. In addition, direct competition with stronger sorbing constituents can lead to effluent PFOS concentrations temporarily exceeding influent concentration (known as chromatographic peaking). This has been documented in full-scale treatment plants (Appleman et al., 2013; Eschauzier et al., 2012; McCleaf et al., 2017; Takagi et al., 2011). Common PFOS competitors for binding sites on sorptive media include natural or dissolved organic matter (NOM/DOM) which lowers treatment efficacy (McNamara et al., 2018; Park et al., 2020; Pramanik et al., 2015; Yu et al., 2012). Preloading may be controlled in the design phase through pretreatment processes. For more

information about managing preloading, see AWWA (2018a). Competitive sorption may be controlled by changing or regeneration of the sorptive media at appropriate intervals.

4.1.1 Activated Carbon

Activated carbon is a highly porous media with high internal surface areas (U.S. EPA, 2017b). Activated carbon can be made from a variety of materials. Designs that work with carbon made from one source material activated in a specific way may not be optimized for other carbon types. There is some indication that of the common trace capacity tests, higher rose bengal numbers are most correlated with higher PFOS removal (Sörengård et al., 2020). Installing activated carbon as a treatment method may also have ancillary benefits on finished water quality, particularly regarding DBP control, other contaminants, as well as taste-and-odor compounds.

Activated carbon tends to remove non-polar, larger compounds more easily from water than smaller, more polar compounds. Adsorption of acids and bases on activated carbon is pH-dependent. Adsorption of neutral forms, as opposed to anionic forms, is generally stronger, so lowering the pH increases PFOS sorption. However, the calculated acid dissociation constant (pKa) of PFOS is about 3 (Larsen and Giovalle, 2015) and lowering the pH may not be practical operationally.

Before the addition of activated carbon to an existing treatment train, there are issues which should be considered. For instance, activated carbon may change system pH or release leachable metals (particularly arsenic and antimony) especially when new carbon media is first used without acid washing. These effects are typically mitigated through an acid wash or forward flushing. Activated carbon may also impact disinfection efficacy depending on process placement and requires consideration to mitigate its effects; for more information, please see the American Water Works Association (AWWA) GAC standard (American National Standards Institute (ANSI)/AWWA B604-18; AWWA, 2018a) or the AWWA published standard for PAC (ANSI/AWWA B600-16; AWWA, 2016). Activated carbon can also shift the bromide-to-total organic carbon ratio and increase brominated (Br)-DBP concentrations (Krasner et al., 2016); however, despite increased Br-DBP, studies have indicated a decreased overall DBP concentration and risk (Wang et al., 2019). DBPs may be mitigated through NOM (DBP precursor) removal; please see Zhang et al. (2015) for additional information.

4.1.1.1 Granular Activated Carbon

PFOS can be effectively removed from water by using GAC; contactors are normally placed as a post-filter step. Key design criteria include empty bed contact time (EBCT), superficial velocity, and carbon type. Typical EBCTs for PFOS removal are 10–20 minutes and superficial linear velocities are normally 5–15 meters per hour (m/hr). Normal height-to-diameter ratios are around 1.5 to 2.0; lower ratios can cause problems with too-shallow beds and require more space, and higher ratios can induce greater head drops. AWWA has published a GAC standard (ANSI/AWWA B604-18; AWWA, 2018a) and a standard for GAC reactivation (ANSI/AWWA B605-18; AWWA, 2018b).

4.1.1.2 Powdered Activated Carbon

PAC is the same material as GAC, but it has a smaller particle size and is applied differently. PAC is typically dosed intermittently although it can be employed continuously if there are spatial constraints restricting contactor use. PAC dosage and type, along with dosing location, contact time, and water quality, often influence process cost as well as treatment efficiency (Heidari et al., 2021). For more information on employing PAC, please see the Drinking Water Treatability Database (U.S. EPA, 2022b).

While relatively unstudied in PFAS, increasing PAC dose with other contaminants increases removal to a point, after which it starts to decrease. Jar testing is typically used to empirically determine the optimal PAC dosage; doses between 45 and 100 mg/L are generally suitable for PFOS (Dudley, 2012; Hopkins et al., 2018; Sun et al., 2016). Standardized jar testing procedures have been published (ASTM International, 2019; AWWA, 2011). The AWWA published standard for PAC is ANSI/AWWA B600-16 (AWWA, 2016).

PAC can pose additional safety considerations including depleting oxygen in confined or partially enclosed areas, fire hazards including spontaneous combustion when stored with hydrocarbons or oxidants, and inhalation hazards and must be managed accordingly. PAC is also a good electrical conductor and can create dangerous conditions when it accumulates (AWWA, 2016). These dangers can be effectively mitigated through various occupational safety programs such as confined space or fire safety programs. See AWWA (2016) for more information.

4.1.2 Ion Exchange

Ion exchange involves the exchange of an aqueous ion (e.g., contaminant) for an ion on an exchange resin. Once the resin has exchanged all its ions for contaminants, it can either be replaced (single use) or regenerated (i.e., restoring its ions for further use).

Different resin types preferentially bind certain ions over others; therefore, resin selection is an important consideration. As PFOS will predominantly exist in an anionic form in water and is a strong acid (U.S. EPA, 2021g), strongly basic AIX resins will be the most relevant for PFOS. Regenerating PFOS-saturated resins has been accomplished effectively with a brine of > 20% sodium chloride and ammonium chloride. Sodium hydroxide may be added to the sodium chloride solution to combat organic fouling; this is referred to as ‘brine squeeze’ and helps in solubilizing NOM and unplugging pores (Dixit et al., 2021). Regenerated media can be “poisoned,” meaning that a non-target ion not removed by the in-place regeneration procedures eventually crowds out available active sites. When this happens or if media is not regenerated, it must be disposed of appropriately. Once PFAS-contaminated spent brine is recovered, it must be treated or disposed of. Resin regeneration may not be practical for water utilities from safety and/or cost perspectives (Liu and Sun, 2021).

In some situations, AIX may outperform activated carbon for removing PFOS from drinking water (Liu and Sun, 2021). Key design parameters for GAC also apply to AIX, and they can be operated similarly. AIX typically uses 2-to-5-minute EBCTs, allowing for lower capital costs and a smaller footprint; compared to GAC, smaller height-to-diameter ratios are typically used in exchange columns. However, AIX resin is typically more costly compared to GAC which may increase overall operational costs. Columns used in pilot studies are scaled directly to full-scale if loading rates and EBCTs are kept constant (Crittenden et al., 2012).

Before the addition of AIX to an existing treatment train, there are effects which must be considered. For instance, AIX can increase water corrosivity and/or release amines and will increase concentrations of the counter-ion used (typically chloride). These effects may usually be mitigated through prior planning which may include corrosion control adjustments; for more information about corrosion control, see U.S. EPA (2016d). Additionally, PFOS-saturated resin regeneration creates an additional PFOS waste stream which will require appropriate handling. For more information about AIX, please see Crittenden et al. (2012), Dixit et al. (2021), Tanaka (2015), Tarleton (2014), and the EPA Drinking Water Treatability Database (U.S. EPA, 2022b).

4.2 High-Pressure Membranes

NF and RO are high-pressure processes where water is forced across a membrane. The water that transverses the membrane is known as permeate or produce, and has few solutes left in it; the remaining water is known as concentrate, brine, retentate, or reject water and forms a waste stream with concentrated solutes. NF has a less dense active layer than RO, which enables lower operating pressures but also makes it less effective at removing contaminants. Higher operating pressures and initial flux generally enhance removal. Temperature and pH are also significant parameters affecting performance. In general, organic NF membranes have lower operating costs and easier processing than inorganic membranes while maintaining appropriate robustness for PFOS treatment (Jin et al., 2021). NF and RO tend to take up less space than sorptive separation technologies; however, both NF and RO also tend to have higher operating expenses, use a significant amount of energy, and generate concentrate waste streams which require disposal. Generally, NF and RO require pre- and post-treatment processes. Higher expenses typically associated with NF and RO are only rarely competitive from an economic perspective for removing a specific contaminant; however, for waters requiring significant treatment and where concentrate disposal options are reasonably available, NF and RO may be the best option.

PFOS removal fluxes are generally 20–80 liters per square meter per hour (L/[m^2·hr]) at 0.2–1.2 megapascal (MPa) operating pressure (Mastropietro et al., 2021) with removal from 90% to > 99% (Jin et al., 2021). Temperature can dramatically impact flux; it is common to normalize flux to a specific reference temperature for operational purposes (U.S. EPA, 2005b). It is also common to normalize flux to pressure ratios to identify productivity changes attributable to fouling (U.S. EPA, 2005b). It is important to note that water may traverse the membranes from outside-in or inside-out; different system configurations operating at the same flux produce differing quantities of finished water. This means that membrane systems with differing configurations cannot be directly compared based on flux. Total flow per module and cost per module are more important decision support indicators for capital planning. Unlike low-pressure membranes, NF and RO systems are not manufactured as proprietary equipment and membranes from one manufacturer are typically interchangeable with those from others (U.S. EPA, 2005b).

High-pressure membranes may have effects when added onto a well-functioning treatment train. For instance, high-pressure membranes may remove beneficial minerals and increase corrosivity. Increased water corrosivity may need to be addressed through corrosion control treatment modifications and water may require remineralization. For more information, see AWWA (2007) or U.S. EPA (2016d).

4.3 Point-of-Use Devices for Individual Household PFOS Removal

Although the focus of this treatment technologies section is the different available options for removal of PFOS at drinking water treatment plants, centralized treatment technologies can also often be used in a decentralized fashion as point-of-entry (where the distribution system meets a service connection) or point-of-use (at a specific tap or application) treatment in cases where centralized treatment is impractical or individual consumers wish to further reduce their individual household risks. Many home drinking water treatment units are certified by independent third-party accreditation organizations using ANSI standards to verify contaminant removal claims. NSF International has developed protocols for NSF/ANSI Standards 53 (sorption) and 58 (RO) that establish minimum requirements for materials, design, and construction, and performance of point-of-use systems. Previously, NSF P473 was designed to certify PFOS reduction technologies below EPA's 2016 HA of 70 ppt for PFOS; in 2019, these standards were retired and folded into NSF/ANSI 53 and 58. PFOS removal by faucet filters has reportedly averaged 99%, whereas pitcher filters had an average of 71% removal, refrigerator filters 61%, single-stage under-sink filters > 99%, two-stage filters 99%, and RO filters 100%. Some filters can remove PFOS to below the 0.004 µg/L UCMR 5 reporting limit (Herkert et al., 2020). Boiling water is not an effective point-of-use PFOS treatment, as it will concentrate PFOS.

4.4 Treatment Technologies Summary

Non-treatment PFOS management options, such as changing source waters, source water protection, or consolidation are viable strategies for reducing PFOS concentrations in finished drinking water. Should treatment be necessary, GAC, PAC, AIX, NF, and RO are the best means for removing PFOS from drinking water and can be used in central treatment plants or in point-of-use applications. These treatment processes are separation technologies and produce waste streams with PFOS, and all processes may have unintended effects on the existing treatment trains. Some treatment processes have been shown to increase PFOS concentrations, most likely through precursor oxidation. PFOS treatment technologies often require pre- as well as post-treatment and may help remove other unwanted contaminants and DBP precursors. Boiling water will concentrate PFOS and should not be considered as an emergency action.

5.0 Consideration of Noncancer Health Risks from PFAS Mixtures

EPA recently released a *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (U.S. EPA, 2021h) that is currently undergoing SAB PFAS Panel review. That draft document describes a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on current, available EPA chemical mixtures approaches and methods (U.S. EPA, 2000b). Examples are presented for three approaches—Hazard Index (HI), Relative Potency Factor (RPF), and Mixture BMD—to demonstrate application to PFAS mixtures. To use these approaches, specific input values and information for each PFAS are needed or can be developed. These approaches may help to inform PFAS evaluation(s) by federal, state, and tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential noncancer human health hazards and risks associated with PFAS mixtures.

The HI approach, for example, could be used to assess the potential noncancer risk of a mixture of four component PFAS for which HAs, either final or interim, are available from EPA (PFOA, PFOS, GenX chemicals [hexafluoropropylene oxide dimer acid and its ammonium salt], and perfluorobutane sulfonic acid [PFBS]). In the HI approach described in the draft framework (U.S. EPA, 2021h), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (U.S. EPA, 1986). The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Eq. 2). In the context of PFAS in drinking water, a mixture PFAS HI can be calculated when health-based water concentrations (e.g., HAs, Maximum Contaminant Level Goals [MCLGs]) for a set of PFAS are available or can be calculated. In this example, HQs are calculated by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HA (e.g., expressed as ng/L) (Eqs. 3, 4). The component chemical HQs are then summed across the PFAS mixture to yield the mixture PFAS HIs based on interim and final HAs.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

(Eq. 2)

$$HI = HQ_{PFOA} + HQ_{PFOS} + HQ_{GenX} + HQ_{PFBS}$$

(Eq. 3)

$$HI = \left(\frac{[PFOA_{water}]}{[PFOA_{iHA}]}\right) + \left(\frac{[PFOS_{water}]}{[PFOS_{iHA}]}\right) + \left(\frac{[GenX_{water}]}{[GenX_{HA}]}\right) + \left(\frac{[PFBS_{water}]}{[PFBS_{HA}]}\right)$$

(Eq. 4)

Where:
HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS
RfV_i = human health-based toxicity value for component (i) PFAS
HQ_{PFAS} = hazard quotient for a given PFAS
$[PFAS_{water}]$ = concentration for a given PFAS in water
$[PFAS_{HA}]$ = HA value, interim or final, for a given PFAS

In cases when the mixture PFAS HI is greater than 1, this indicates an exceedance of the health protective level and indicates potential human health risk for noncancer effects from the PFAS mixture in water. When component health-based water concentrations (in this case, HAs) are below the analytical method detection limit, as is the case for PFOA and PFOS, such individual component HQs exceed 1, meaning that any detectable level of those component PFAS will result in an HI greater than 1 for the whole mixture. Further analysis could provide a refined assessment of the potential for health effects associated with the individual PFAS and their contributions to the potential joint toxicity associated with the mixture. For more details of the

approach and illustrative examples of the RPF approach and Mixture BMD approaches, please see U.S. EPA (2021h).

6.0 Interim Health Advisory Characterization

The purpose of developing the lifetime iHA for PFOS is to reflect the best available scientific information which indicates that PFOS can lead to adverse noncancer health effects at exposure levels that are much lower than previously understood (U.S. EPA, 2016c). The PFOS iHA of 0.02 ng/L is considered applicable to both short-term and chronic risk assessment scenarios because the critical effect identified for PFOS can result from developmental exposure and leads to long-term adverse health effects. Therefore, short-term PFOS exposure during a critical period of development may lead to adverse health effects across life stages.

In 2019, EPA initiated an updated literature search and analysis of health effects information for PFOS to better characterize the health hazards and risks of exposure using information published since EPA developed the 2016 HA for PFOS (draft PFOS document; U.S. EPA, 2021b). The draft PFOS document includes an updated draft chronic RfD and draft RSC. The draft PFOS document is currently undergoing review by the SAB PFAS Panel as part of EPA's process for developing a NPDWR for PFOS under SDWA. The draft report of the SAB PFAS Panel's review (U.S. EPA, 2022a) is supportive of the draft conclusions; however, the SAB PFAS Panel is recommending analyses that may impact the final RfD and RSC. Because the iHA is based on draft values, it is subject to change.

EPA expects to propose an MCLG and NPDWR for PFOS in the fall of 2022 and to promulgate a final MCLG and NPDWR by the fall of 2023 after considering public comment. EPA will complete its revisions to address the final SAB report's comments in the proposed PFOS MCLG and NPDWR. EPA may update or remove the iHA for PFOS at that time. Based, however, on the updated systematic review of the best available science on PFOS exposure and health effects and taking into consideration the work EPA is doing now to address SAB comments, the health-based drinking water values for PFOS (HA and MCLG) are anticipated to remain below the current UCMR 5 analytical MRL (0.004 µg/L or 4 ng/L).

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Attachment 7

United States Environmental Protection Agency

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Drinking Water Health Advisory: Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)

Drinking Water Health Advisory: Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)

Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Office of Science and Technology
Health and Ecological Criteria Division
Washington, DC 20460

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- Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics
- Office of Land and Emergency Management
- Office of Policy
- Office of Children's Health Protection
- Office of Research and Development

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Abbreviations and Acronyms

6:2 FTS	6:2 fluorotelomer sulfonic acid
ADAF	age-dependent adjustment factor
AFFF	aqueous film-forming foam
ANSI	American National Standards Institute
ASTSWMO	Association of State and Territorial Solid Waste Management Officials
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower limit
bw or BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CDPHE	Colorado Department of Public Health and Environment
CPHEA	Center for Public Health and Environmental Assessment
CSF	cancer slope factor
DF	detection frequency
DOH	Department of Health
DQO	data quality objective
dw	dry weight
DWI	drinking water intake
DWI-BW	body weight-adjusted drinking water intake
DWTP	drinking water treatment plant
ECHA	European Chemicals Agency
EEE	electronic equipment
EF	exposure factor
EFH	Exposure Factors Handbook
EFSA	European Food Safety Authority
EGLE	Michigan Department of Environment, Great Lakes, and Energy
EPA	United States Environmental Protection Agency
Eq	equation
EU	European Union
FCID	Food Commodity Intake Database
FCM	food contact material
FDA	United States Food and Drug Administration
fw	fresh weight
GCA	groundwater contamination area
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
HA	Health Advisory
HED	human equivalent dose
HI	hazard index
HIDOH	Hawai´i Department of Health
HQ	hazard quotient
IBWA	International Bottled Water Association
IDEM	Indiana Department of Environmental Management
iHA	Interim Health Advisory
Illinois EPA	Illinois Environmental Protection Agency
ITRC	Interstate Technology and Regulatory Council
K_{aw}	air-water partition coefficient
km^2	square kilometers
K^+PFBS	potassium perfluorobutane sulfonate
L	liters

L/kg bw-day	liters per kilogram body weight per day
$L/m^2 \cdot hr$	liters per square meter per hour
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LOAEL	lowest observed adverse effect level
LOQ	limit of quantification
Maine DEP	Maine Department of Environmental Protection
MCLG	Maximum Contaminant Level Goal
MDH	Minnesota Department of Health
mg/kg bw-day	milligrams per kilogram body weight per day
MPa	megapascal
MRL	minimum reporting level
MS/MS	tandem mass spectrometry
MW	molecular weight
NCHS	National Center for Health Statistics
NCOD	National Contaminant Occurrence Database
ND	non-detect
NDEP	Nevada Division of Environmental Protection
NF	nanofiltration
ng/g	nanograms per gram
ng/kg bw-day	nanograms per kilogram body weight per day
ng/L	nanograms per liter
ng/mL	nanograms per milliliter
NHANES	National Health and Nutrition Examination Survey
NHIS	Human Nutrition Information Service
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no observed adverse effect level
NRSA	National Rivers and Streams Assessment
ODH	Ohio Department of Health
OEHHA	Office of Environmental Health Hazard Assessment
Ohio EPA	Ohio Environmental Protection Agency
ORD	Office of Research and Development
OST	Office of Science and Technology
OW	Office of Water
PECO	populations, exposures, comparators, and outcomes
PFAA	perfluoroalkylated acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFC	perfluorinated chemical
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFDoS	perfluorododecane sulfonic acid
PFDS	perfluorodecane sulfonic acid
PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptane sulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFNS	perfluorononane sulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid

PFOSA	perfluorooctanesulfonamide
PFPA	perfluoropentanoic acid
PFPeA	perfluoropentanoic acid
PFPS	perfluoropentane sulfonic acid
PFSA	perfluoroalkane sulfonate
PFTrDA	perfluorotridecanoic acid
PFTrS	perfluorotridecane sulfonic acid
PFUnDA	perfluoroundecanoic acid
PFUnS	perfluoroundecane sulfonate
pg/cm^2	picograms per square centimeter
pg/m^3	picograms per cubic meter
PM	particulate matter
PMN	pre-manufacture notice
PND	post-natal day
POD	point of departure
ppm	parts per million
ppt	parts per trillion
POE	point-of-entry
POU	point-of-use
PWS	public water system
QC	quality control
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RfD	reference dose
RO	reverse osmosis
RPF	relative potency factor
RSC	relative source contribution
SDWA	Safe Drinking Water Act
T_4	thyroxine
TCEQ	Texas Commission on Environmental Quality
TSCA	Toxic Substances Control Act
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor(s)
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL extrapolation uncertainty factor
UF_S	subchronic to chronic exposure duration extrapolation uncertainty factor
WEEE	wastes of electrical and electronic equipment
Wisconsin DHS	Wisconsin Department of Health Services
WTP	water treatment plant
ww	wet weight
WWTP	wastewater treatment plant
µg/kg	micrograms per kilogram
$µg/m^2$	micrograms per square meter

Executive Summary

Perfluorobutane sulfonic acid (PFBS; CASRN 375-73-5) and its related compound potassium perfluorobutane sulfonate (K^+PFBS; CASRN 29420-49-3) are shorter-chain members of a group of substances known as per- and polyfluoroalkyl substances (PFAS). In water, K^+PFBS fully dissociates to the deprotonated anionic form of PFBS (PFBS–; CASRN 45187-15-3) and the K^+ cation at environmental pH levels (pH 4–9). Herein, these three PFBS chemical forms are referred to collectively as PFBS.

PFBS is a replacement chemical for the longer-chain perfluorooctane sulfonic acid (PFOS), a PFAS that was voluntarily phased out (with some exceptions) by its primary U.S. manufacturer (3M Company) between 2000 and 2002 (U.S. EPA, 2007; 3M, 2002). Prior to its use as a replacement for PFOS, PFBS was produced as a byproduct during production of perfluorooctane sulfonyl fluoride-based chemicals and was present in consumer products as an impurity (AECOM, 2019). PFBS is used in the manufacture of paints, cleaning agents, and water- and stain-repellent products and coatings (U.S. EPA, 2021a). PFBS has been detected in drinking water, groundwater, and surface water and has been found in dust, carpeting and carpet cleaners, floor wax, foods including seafood (fish and shellfish) and vegetables, food packaging, indoor and outdoor air, soil, biosolids, and some consumer products (ATSDR, 2021; U.S. EPA, 2021a; see Section 3.3.1). PFBS can enter the aquatic environment through releases from manufacturing sites, industrial uses, fire/crash training areas, and wastewater treatment facilities, as well as from land application of contaminated biosolids (ATSDR, 2021; U.S. EPA, 2021a). PFBS is water soluble (52.6 g/L at 22.5–24 °C for the potassium salt) and volatilization from water surfaces is not expected to be an important fate process (ATSDR, 2021; U.S. EPA, 2021a). PFBS has been detected in the serum of humans in the general population (U.S. EPA, 2021a).

The U.S. Environmental Protection Agency (EPA) is issuing a lifetime noncancer drinking water Health Advisory (HA) for PFBS of 2,000 nanograms per liter (ng/L) or 2,000 parts per trillion (ppt). This is the first HA for PFBS and its finalization fulfills a commitment described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021b). The final PFBS toxicity assessment titled *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)* (U.S. EPA, 2021a) serves as the basis of the toxicity information used to derive the lifetime noncancer HA for PFBS. The critical adverse effect is thyroid effects in mice (specifically, decreased serum levels of the thyroid hormone thyroxine [T_4]) observed at post-natal day (PND) 1, after 20-day gestational exposure to PFBS (Feng et al., 2017). Based on this critical effect, a chronic reference dose (RfD) of 3×10^{-4} milligrams per kilogram body weight per day (mg/kg bw-day) for PFBS was derived.

In accordance with EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), serum PFBS half-lives were used to scale a toxicologically equivalent dose of orally administered PFBS from animals to humans. Following EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b), benchmark dose (BMD) modeling of thyroid effects in offspring after gestational exposure to PFBS resulted in a benchmark dose lower confidence limit (BMDL) for 0.5 SD change from the control ($BMDL_{0.5SD}$) human equivalent dose (HED) of 0.095 mg/kg bw-day. This HED point of departure (POD) based on decreased levels of T_4 in newborn offspring was divided by a composite uncertainty factor (UF_C) of 300 to derive the chronic RfD.

Sensitive populations or life stages within the general population indicated by the critical study used to derive the chronic RfD for PFBS are the developing embryo and fetus. Therefore, drinking water exposure to pregnant women as well as women of childbearing age, who may be or become pregnant, were identified as two sensitive populations or life stages. EPA selected the body weight-adjusted drinking water intake (DWI-BW) exposure factor (EF) of 0.0354 liters per kilogram body weight per day (L/kg bw-day) for women of childbearing age because it is more health protective than the DWI-BW for pregnant women. However, PFBS HA values, when rounded to one significant figure, were the same when calculated using EFs for either women of childbearing age, pregnant women, or the general population (all ages).

The physical/chemical properties and available exposure information for PFBS suggest multiple potentially significant exposure sources (seafood, other foods, indoor air, and some consumer products) other than drinking water ingestion. However, information is not available to quantitatively characterize the relative exposure contributions from non-drinking water exposure sources. Therefore, following the Exposure Decision Tree approach within EPA's 2000 *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a), EPA recommends a relative source contribution (RSC) of 20 percent (0.20) for use in PFBS HA derivation.

There is insufficient toxicity information available to derive a one-day HA for PFBS. Derivation of a 10-day HA was considered because the subchronic and chronic RfDs are both based on a 20-day exposure study, which may be used to derive a 10-day HA. However, EPA did not derive a 10-day HA because the critical health effect on which the chronic RfD used to calculate the lifetime HA is based (i.e., decreased serum levels of T_4 in newborn mice) resulted from PFBS exposure during a developmental life stage (Feng et al., 2017). EPA's risk assessment guidelines for developmental toxicity indicate that adverse effects can result from even brief exposure during a critical period of development (U.S. EPA, 1991). The critical study observed persistent health effects into adulthood, suggesting the potential for long-term health consequences of gestational-only PFBS exposure and that gestation is at least one critical exposure window for PFBS. Therefore, the lifetime HA for PFBS of 2000 ng/L and the chronic RfD from which it is derived are considered applicable to short-term PFBS exposure (including during pregnancy) as well as lifetime exposure via drinking water. This lifetime HA applies to PFBS (CASRN 375-73-5), K^+PFBS (CASRN 29420-49-3), and PFBS– (CASRN 45187-15-3).

No studies evaluating the carcinogenicity of PFBS in humans or animals were identified (U.S. EPA, 2021a). In accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), EPA concluded that there is *"Inadequate Information to Assess Carcinogenic Potential"* for PFBS by any route of exposure (U.S. EPA, 2021a). Therefore, a 10^{-6} cancer risk concentration cannot be derived for PFBS at this time.

EPA developed two analytical methods to quantitatively assess drinking water for targeted PFAS that include PFBS: EPA Method 533 (U.S. EPA, 2019b), which has a quantitation limit of 3.5 ng/L for PFBS, and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020b), which has a quantitation limit of 6.3 ng/L for PFBS. These analytical methods can both effectively and accurately measure PFBS in drinking water at levels significantly lower than the lifetime HA of 2,000 ng/L. EPA finished drinking water sampling results have not identified PFBS levels that approached the lifetime HA of 2,000 ng/L. However, treatment technologies, including reverse osmosis (RO), nanofiltration (NF), and sorption-based processes such as activated carbon and

ion exchange are available and have been shown to remove PFBS in drinking water; however, sorption has less efficacy with PFBS than similar longer-chained PFAS.

1.0 Introduction and Background

The Safe Drinking Water Act (SDWA) (42 U.S.C. § § 300f - 300j-27) authorizes the U.S. Environmental Protection Agency (EPA) to develop drinking water Health Advisories (HAs).[1] HAs are national non-enforceable, non-regulatory drinking water concentration levels of a specific contaminant at or below which exposure for a specific duration is not anticipated to lead to adverse human health effects.[2] HAs are intended to provide information that tribal, state, and local government officials and managers of public water systems (PWSs) can use to determine whether actions are needed to address the presence of a contaminant in drinking water. HA documents reflect the best available science and include HA values as well as information on health effects, analytical methodologies for measuring contaminant levels, and treatment technologies for removing contaminants from drinking water. EPA's lifetime HAs identify levels to protect all Americans, including sensitive populations and life stages, from adverse health effects resulting from exposure throughout their lives to contaminants in drinking water.

In April 2021, EPA published a final toxicity assessment for two per- and polyfluoroalkyl substances (PFAS): perfluorobutane sulfonic acid (PFBS) and its related compound potassium perfluorobutane sulfonate (K^+PFBS) (U.S. EPA, 2021a). K^+PFBS differs from PFBS by being associated with a potassium ion. In water, K^+PFBS fully dissociates to the deprotonated anionic form of PFBS (PFBS–; CASRN 45187-15-3) and the K^+ cation at environmental pH levels (pH 4–9). Herein, these three PFBS chemical forms are referred to collectively as PFBS. Completing the toxicity assessment was an essential step to better understanding the potential human health effects of exposure to PFBS. The chronic noncancer reference dose (RfD) calculated in the toxicity assessment allows EPA to develop a final lifetime HA that will help communities make informed decisions to better protect human health. The final PFBS HA satisfies a commitment described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021b).

1.1 History under SDWA

PFBS is not currently regulated under SDWA. The 1996 amendments to SDWA require that EPA issue a new list of unregulated contaminants (once every five years) to be monitored by PWSs.[3] Under the Unregulated Contaminant Monitoring Rule (UCMR), EPA samples drinking water systems to collect data for contaminants that are known or suspected to be found in drinking water and do not have health-based standards under SDWA. The first four UCMRs required monitoring of all large public drinking water systems (>10,000 people), and a subset of smaller systems serving <10,000 people. PFBS was one of six PFAS monitored in drinking water under the third UCMR (UCMR 3) between 2013 and 2015 (U.S. EPA, 2012a). It is also one of 29 PFAS that will be monitored under the fifth UCMR (UCMR 5) between 2023 and 2025 (U.S. EPA, 2021c). The collection of drinking water occurrence data supports EPA's future

[1] SDWA § 1412(b)(1)(F) authorizes EPA to "publish health advisories (which are not regulations) or take other appropriate actions for contaminants not subject to any national primary drinking water regulation." www.epa.gov/sites/default/files/2020-05/documents/safe_drinking_water_act-title_xiv_of_public_health_service_act.pdf

[2] This document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community. This document is not enforceable against any person and does not have the force and effect of law. No part of this document, nor the document as a whole, constitutes final agency action that affects the rights and obligations of any person. EPA may change any aspects of this document in the future.

[3] SDWA § 1445(a)(1)(D)(2)(B) — "Not later than 3 years after the date of enactment of the Safe Drinking Water Act Amendments of 1996 and every 5 years thereafter, the Administrator shall issue a list pursuant to subparagraph (A) of not more than 30 unregulated contaminants to be monitored by public water systems and to be included in the national drinking water occurrence data base maintained pursuant to subsection (g)."

regulatory determinations and may support additional actions to protect public health (U.S. EPA, 2021c).

1.2 Current Advisories and Guidelines

Table 1 provides final drinking water guideline values for PFBS that have been developed by states. The state values range from 100 to 667,000 parts per trillion (ppt) or nanograms per liter (ng/L); this broad range of values may in part reflect differences in the type of value derived, state guidance/methodology for deriving values, or data included in the evaluation (see references for more details).

Table 1. State Guideline Values for PFBS

State [a,b]	PFBS Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
California	500	Notification level	Drinking water	California OEHHA (2021)
Colorado	400,000	Translation level	Groundwater; Surface water	CDPHE (2020b)
Hawai'i	600	Environmental action levels	Groundwater	HIDOH (2021)
Illinois	2,100	Health-based guidance level	Drinking water; Groundwater	Illinois EPA (2021a)
Indiana	> 2,100	Action level	Drinking water	IDEM (2022)
Maine	400,000	Remedial action guideline	Groundwater	Maine DEP (2018)
Michigan	420	Maximum contaminant level	Drinking water; Groundwater	EGLE (2020)
Minnesota	100	Health-based value	Drinking water; Groundwater	MDH (2022)
Nevada	667,000	Basic comparison level	Drinking water	NDEP (2020)
Ohio	2,100	Action level	Drinking water	Ohio EPA and ODH (2022)
Pennsylvania	10,000	Medium-specific concentration	Groundwater; Residential use	Environmental Quality Board (2021)
	29,000	Medium-specific concentration	Groundwater; Non-residential use	

State [a,b]	PFBS Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
Texas	34,000	Tier 1 protective concentration level	Groundwater	TCEQ (2021)
Washington	345	State action level	Drinking water	Washington DOH (2021)
Wisconsin	450,000	Recommended enforcement standard	Groundwater	Wisconsin DHS (2020)
	90,000	Recommended preventive action limit	Groundwater	

Notes:
[a] The information was compiled from two sources: 1) EPA regional office outreach by EPA's Office of Science and Technology (OST) in March 2022; and 2) information from the Interstate Technology and Regulatory Council's (ITRC) *Standards and guidance values for PFAS in groundwater, drinking water, and surface water/effluent (wastewater)* PFAS Water and Soil Values Table, last updated in April 2022 (available for download here: https://pfas-1.itrcweb.org/fact-sheets/).
[b] Only states with final guidelines are included; other states may be developing guidelines for PFBS.

In 2020, the European Chemicals Agency (ECHA) adopted an agreement that identified PFBS as a "Substance of Very High Concern" (ECHA, 2020) based on a "very high potential for irreversible" human and environmental health effects, and properties including moderate bioaccumulation in humans, high persistence and mobility in the environment, high potential for long-range transport, and difficulty of remediating and purifying water.

Table 2 provides drinking water guideline values for PFBS that were developed by international agencies. The international guideline values range from 90 to 15,000 ppt or ng/L.

Table 2. International Guideline Values for PFBS

Country [a,b]	PFBS Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
Canada	15,000	Screening value	Drinking water	Health Canada (2016)
European Union (EU)	100 ng/L[c,d]	Parametric value	Water intended for human consumption	EU (2020)
	500 ng/L[c,e]	Parametric value	Water intended for human consumption	
Denmark	100[f]	Health based	Groundwater	Danish EPA (2021)
Germany	6,000	Significance threshold	Groundwater	Von der Trenck et al. (2018)
Italy	3,000	Environmental quality standard	Drinking water	Valsecchi et al. (2017)

Country [a,b]	PFBS Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
Sweden	90[g]	Administrative	Drinking water	Concawe (2016)

Notes:

[a] The information was collected from the Interstate Technology and Regulatory Council's (ITRC) *Standards and guidance values for PFAS in groundwater, drinking water, and surface water/effluent (wastewater)* PFAS Water and Soil Values Table, last updated in April 2022 (available for download here: https://pfas-1.itrcweb.org/fact-sheets/).

[b] Only countries with guideline values provided in the ITRC table are included; other countries may be developing guidelines for PFBS.

[c] Parametric values from Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. By January 12, 2026, Member States shall take measures necessary to ensure that water intended for human consumption complies with the parametric values set out in Part B of Annex I in the EU Directive 2020/2184 (EU, 2020).

[d] Pertains to a sum of a subset of 20 individual PFAS that includes PFBS: PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFPS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnS, PFDoS, PFTrS.

[e] Total PFAS

[f] Applies to the individual results for PFOA, PFOS, PFNA, PFBA, PFBS, PFHxS, PFHxA, PFHpA, PFOSA, PFDA, 6:2 FTS, PFPS, PFHpS, PFNS, PFDS, PFUnS, PFDoS, PFTrS, PFPA, PFUnDA, PFDoDA, PFTrDA as well as the sum of concentrations of these 22 PFAS.

[g] This limit also applies to the sum of PFOS, PFHxS, PFBS, PFOA, PFHpA, PFHxA and PFPeA.

1.3 Uses and Sources of PFBS

PFBS is a replacement chemical for perfluorooctane sulfonic acid (PFOS), a chemical that was voluntarily phased out (with some exceptions) by its primary U.S. manufacturer, 3M Company, by 2002 (3M, 2002; U.S. EPA, 2007). PFBS and its potassium salt were listed on the original EPA Toxic Substances Control Act (TSCA) Chemical Substance Inventory[4] as existing chemicals that were already in commerce when TSCA was enacted in 1976 (15 U.S.C. § 2601 *et seq.*). Therefore, PFBS and its potassium salt were not subject to the pre-manufacture notice (PMN) reporting process. They are listed as "active" on the inventory but have not been reviewed under the TSCA New Chemicals program.[5] EPA also evaluates existing chemicals under amended TSCA;[6] however, to date, PFBS has not been designated as a high priority substance for risk evaluation. PFBS and its potassium salt are subject to Section 8 Chemical Data Reporting.[7] While there has not been recent reporting on PFBS, in 2020 there was a report on the potassium salt (K^+PFBS) for one industrial processing and use scenario[8] but not for consumer/commercial uses.

Prior to its use as a replacement chemical, PFBS had been produced solely as a byproduct and was present in consumer products as an impurity (AECOM, 2019). Concerns arising in the early 2000s about the environmental persistence, bioaccumulation potential, and long half-lives in humans of longer-chain PFAS resulted in the use of shorter-chain PFAS such as PFBS as

[4] TSCA Inventory. Available at https://www.epa.gov/tsca-inventory/how-access-tsca-inventory

[5] Mandated by section 5 of TSCA, EPA's New Chemicals program helps manage the potential risk to human health and the environment from chemicals new to the marketplace. Section 5 of TSCA is available at https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/15-usc-ch-53-toxic-substances-control-act

[6] On June 22, 2016, President Obama signed the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which updates TSCA. Available at https://www.congress.gov/114/plaws/publ182/PLAW-114publ182.pdf

[7] Basic information about Chemical Data Reporting available here https://www.epa.gov/chemical-data-reporting/basic-information-about-chemical-data-reporting

[8] Section 8 reporting: Processing—incorporation into formulation, mixture, or reaction product; Sector: Electrical Equipment, Appliance, and Component Manufacturing; Function Category: Flame retardant

replacements for longer-chain PFAS in consumer products and applications (U.S. EPA, 2021a). PFBS and other shorter-chain PFAS possess the desired chemical properties of longer-chain PFAS, but have shorter half-lives in humans (U.S. EPA, 2021a).

Environmental releases of PFBS may result directly from the production and use of PFBS itself, production and use of PFBS-related substances for various applications, and/or from the degradation of PFBS precursors (i.e., substances that may form PFBS during use, as a waste, or in the environment). PFBS is used in the manufacture of paints, cleaning agents, and water- and stain-repellent products and coatings (U.S. EPA, 2021a). PFBS has also been used as a mist suppressant for chrome electroplating and has been detected in association with the use of aqueous film-forming foam (AFFF) (U.S. EPA, 2021a). PFBS has been detected in dust, carpeting and carpet cleaners, floor wax, and food packaging (ATSDR, 2021; U.S. EPA, 2021a).

1.4 Environmental Fate, Occurrence in Water, and Exposure to Humans

1.4.1 Environmental Fate and Transport in the Environment

The ionic nature of PFAS, including PFBS, influences physicochemical properties such as water or lipid solubility and bioaccumulative potential, which impacts environmental fate and transport and potential human health and ecological effects after exposure (U.S. EPA, 2021a). ECHA reports that PFBS is stable to hydrolysis, oxidation, and photodegradation in the atmosphere, and there have been no reports of abiotic degradation under environmental conditions (ECHA, 2019). PFBS has a high solubility in water (52.6 g/L at 22.5–24 °C for the potassium salt) and high mobility in the environment (log K_{oc} 1.2 to 2.7) (ECHA, 2019).

The Norwegian Environment Agency conducted a literature review of physicochemical properties and environmental monitoring data for PFBS to assist an evaluation under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) (Arp and Slinde, 2018). No studies were identified that observed degradation of PFBS under environmental conditions, including atmospheric photolysis. The review determined that the air-water partition coefficient (K_{aw}) for PFBS is too low to measure and that volatilization from water is negligible, but that the presence of PFBS in ambient air can result from direct emissions or transport of droplets in contaminated water. ECHA (2019) modeled photodegradation of PFBS in air and concluded that PFBS has the potential for long-range transport.

1.4.2 Occurrence in Water

PFBS can enter the aquatic environment through releases from manufacturing sites, industrial uses, fire/crash training areas, and wastewater treatment facilities, as well as from the use of contaminated biosolids (ATSDR, 2021; U.S. EPA, 2021a). PFBS has been found in rain as well as in snow/ice in the Arctic and Antarctic (Arp and Slinde, 2018). EPA collected information about PFBS occurrence in water (described below and in Appendix B, Tables B-1 to B-3). To better understand PFBS sources and occurrence patterns in water, this section includes studies conducted within and outside the United States. Overall, studies that analyzed water from sites receiving inputs from or in proximity to known sources of PFAS (as reported by study authors) did not provide a consistent pattern of detection; increased PFBS detection frequencies (DFs) or concentrations were not only observed in studies of sites with known sources of PFAS contamination. Specifically, DFs of 0% were reported at some sites with known, suspected, or historic PFAS contamination, and DFs of 100% were reported at some sites with no known

sources of PFAS contamination. However, the maximum reported PFBS concentrations were measured at sites with known PFAS contamination from manufacturing facilities (drinking water) (Pitter et al., 2020) or AFFF usage (groundwater and surface water) (Anderson et al., 2016).

1.4.2.1 Drinking Water

EPA required the most nationally representative sampling for PFBS in drinking water to date under the UCMR 3. Sampling for the UCMR 3 was conducted between 2013 and 2015. PFBS was detected above the minimum reporting level (MRL)[9] of 90 ng/L in eight PWSs (across four U.S. states and one U.S. territory) out of a total of 4,920 PWSs with results (U.S. EPA, 2017). PFBS concentrations ranged from 90 (the MRL) to 370 ng/L. Results are available in EPA's National Contaminant Occurrence Database (NCOD).[10] EPA included PFBS among the analytes that will be monitored under the UCMR 5 and will use EPA analytical Method 533, which was demonstrated through multilab validation of the method to support a lower UCMR 5 defined MRL of 3 ng/L.

Some states have monitored for PFBS in drinking water since the UCMR 3 using improved EPA analytical methods 533 and 537.1 (see Section 5.0). PFBS has been detected in the finished drinking water from at least 17 U.S. states (ADEM, 2020; CADDW, 2021; CDPHE, 2020a; Illinois EPA, 2021b; KYDEP, 2019; MA EEA, 2020; Maine DEP, 2020; MDE, 2021; Michigan EGLE, 2021; NCDEQ, 2021; NHDES, 2021; NJDEP, 2021; NMED, 2021; Ohio DOH, 2021; PADEP, 2021; SCDHEC, 2020; VTDEC, 2021). State drinking water PFBS monitoring studies often focus on investigating areas known to be affected by PFBS. In states where samples were collected using random sampling site selection (AL, CO, IL, KY, MA, MI, NH, ND, NJ, OH, SC, and VT), PFBS concentrations ranged from non-detect (ND) to 310 ng/L (ppt). Where monitoring was targeted to areas known or suspected to have sources of PFBS (CA, ME, MD, NC, and PA), concentrations were higher and the percentage of samples with PFBS concentrations above the reporting limit often exceeded 20%. Based on the available finished drinking water sampling from states, no finished drinking water samples from any state had PFBS at concentrations exceeding 310 ng/L.

Peer-reviewed studies on PFBS occurrence in drinking water (including bottled water, tap water, and well water intended for consumption) reporting results from North America and/or Europe were reviewed (see literature search methods in Appendix A and study details in Appendix B, Table B-1).

Seven studies analyzed drinking water in areas of North America where study authors did not indicate whether sampling sites were associated with known or suspected sources of PFAS release (Appleman et al., 2014; Boone et al., 2014, 2019; Bradley et al., 2020; Dasu et al., 2017; Hu et al., 2019; Kaboré et al., 2018; Subedi et al., 2015). Three of these seven studies (Appleman et al., 2014; Boone et al., 2019; Bradley et al., 2020) evaluated finished or treated water from drinking water treatment plants (DWTPs). Appleman et al. (2014) detected PFBS in 100% of finished water samples taken from DWTPs that used surface water, groundwater, or blended

[9] The MRL refers to the quantitation level selected by EPA to ensure reliable and consistent results. It is the minimum quantitation level that can be achieved with 95 percent confidence by capable analysts at 75 percent or more of the laboratories using a specified analytical method (EPA, 2021g).
[10] EPA's NCOD is available at https://www.epa.gov/sdwa/national-contaminant-occurrence-database-ncod

water as source water, some of which were reportedly known to have been impacted by upstream wastewater effluent discharge. PFBS levels ranged from 0.43 – 37 ng/L across 11 sites with finished water samples. Boone et al. (2019) also reported that some sampling locations in their study had known or suspected sources of wastewater in the source water but did not identify which ones; PFBS levels in this study ranged from ND to 11.9 ng/L. Bradley et al. (2020) reported PFBS concentrations of ND–0.5 ng/L in treated pre-distribution tap water from four sites. Six studies analyzed tap water from homes (Boone et al., 2014; Bradley et al., 2020; Dasu et al., 2017; Hu et al., 2019; Kaboré et al., 2018; Subedi et al., 2015). Across these six studies, PFBS was detected in at least one sample per study (DFs 5–100%) at concentrations ranging from ND to 14.15 ng/L; in three of the six studies, the maximum PFBS concentration was < 1 ng/L. In Boone et al. (2014), tap water (for which Mississippi River water was the source) was tested at one private home during both low and high river stages, and PFBS concentrations were 14.15 ng/L and 2.12 ng/L, respectively. In Hu et al. (2019), the tested water samples were archived samples from 1989–1990 (PFBS concentrations in these samples ranged from ND–2.97 ng/L).

Three studies conducted in North America examined PFBS levels in drinking water from areas with known or suspected PFAS releases (Boone et al., 2014; Lindstrom et al., 2011; Scher et al., 2018) and two of the three studies detected PFBS. Boone et al. (2014) analyzed samples from three drinking-water wells at sites impacted by AFFF. PFBS was found in all three wells (mean PFBS concentrations 9.09–29 ng/L). Lindstrom et al. (2011) sampled six drinking-water wells in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer. PFBS was detected in four of the six wells, and concentrations were as high as 56.5 ng/L (mean PFBS concentration was 19.7 ng/L). Scher et al. (2018) found no PFBS in tap water from exterior taps of 23 homes near a former 3M PFAS production facility, 20 of which had been identified as being located within the groundwater contamination area (GCA).

Of the available studies conducted in Europe, 17 analyzed drinking water samples at sites for which authors did not indicate whether there were any known associations with PFAS sources or releases. Fourteen of these 17 studies analyzed tap water from private and/or public sources (cafes, homes, offices, public fountains); of these 14 studies, 12 detected PFBS in at least one sample. Across these 12 studies, mean PFBS concentrations ranged from 0.015 in Sweden (Filipovic and Berger, 2015) to 13.2 ng/L in the Netherlands (Ullah et al., 2011) and the maximum PFBS concentration was 69.43 ng/L (Barcelona; Ericson et al., 2009). Four of the 17 studies (Boiteux et al., 2012; Eriksson et al., 2013; Eschauzier et al., 2012, 2013) analyzed finished or treated water at DWTPs, and PFBS levels in these studies ranged from ND in the Faroe Islands (Eriksson et al., 2013) to 24 ng/L in the Netherlands (Eschauzier et al., 2012).

Nine European studies analyzed drinking water samples from areas near fluoropolymer manufacturing facilities, AFFF-contaminated military airfields, or fire training sites that may use AFFF. Six of the nine studies detected PFBS, with maximum concentrations ranging from 11 to 765 ng/L (Brandsma et al., 2019; Gebbink et al., 2017; Gyllenhammar et al., 2015; Li et al., 2018; Pitter et al., 2020; Weiss et al., 2012). The other three studies (all performed in France) found no detectable levels of PFBS in treated water from DWTPs located downstream of fluorochemical manufacturing facilities or a wastewater treatment plant (WWTP) that processes raw sewage from a fluorochemical manufacturing facility (Bach et al., 2017; Boiteux et al.,

2017; Dauchy et al., 2012). Among the six studies that detected PFBS, the highest measured PFBS concentration (765 ng/L) was detected in municipal water in Veneto, Italy, sampled from areas near a fluoropolymer manufacturing facility (Pitter et al., 2020). The study authors reported that the facility was the only likely source of PFAS and estimated a groundwater contamination plume with an area of 190 square kilometers (km^2) affecting public and private drinking water sources (Pitter et al., 2020). In the studies that analyzed water samples from areas near AFFF-contaminated military airfields or fire training sites (Gyllenhammar et al., 2015; Li et al., 2018; Weiss et al., 2012), PFBS DFs ranged from 0 to 100%, PFBS concentrations ranged from ND to 130 ng/L, and maximum PFBS concentrations ranged from 11 to 130 ng/L.

1.4.2.2 Bottled Water

The United States does not have standards for PFAS in bottled water. The Standard of Quality set by the International Bottled Water Association (IBWA) for PFAS in bottled water is 5 ng/L for one PFAS and 10 ng/L for more than one PFAS (IBWA, 2022). One available study analyzed bottled water in the United States (101 samples representing 66 brands) and reported a PFBS DF of 17% and PFBS concentrations ranging from ND to 1.44 ng/L (Chow et al., 2021). Of eight available studies that analyzed bottled water in Canada (one study) or Europe (seven studies), the study in Canada detected PFBS in 9% of samples at a maximum PFBS concentration of 0.23 ng/L (Kaboré et al., 2018). Four of seven studies that analyzed bottled water in different European countries detected PFBS at concentrations ranging from ND to 51 ng/L (DF 0–29%); however, most of the studies did not specify the origin of the bottled water (Gellrich et al., 2013; Harrad et al., 2019; Le Coadou et al., 2017; Ünlü Endirlik et al., 2019). The other three European studies did not detect PFBS in bottled water.

1.4.2.3 Groundwater

In addition to the studies described in Section 1.4.2.1 that reported groundwater PFBS concentrations in well water intended for direct consumption, several other studies evaluated the occurrence of PFBS in raw groundwater in the United States or Europe (see Table B-2). Most of the available studies sampled from groundwaters known or suspected to be contaminated with PFAS through various sources, as reported by the study authors. Importantly, some of these groundwaters are known to be used as input sources for PWSs.

Four U.S. studies assessed PFBS concentrations in groundwater at sites known to be contaminated with PFAS from the use of AFFF (Anderson et al., 2016; Eberle et al., 2017; Moody et al., 2003; Steele et al., 2018). Of the three studies that reported PFBS detections, two reported DFs of 78.26% and 100% (Anderson et al., 2016; Eberle et al., 2017); the third study did not report a PFBS DF across sample sites but indicated a range of PFBS concentrations (ND–48 ng/L) (Steele et al., 2018). The fourth study, which analyzed groundwater from the decommissioned Wurtsmith Air Force Base, did not detect PFBS at any of the ten sites sampled, though other PFAS were detected (Moody et al., 2003). However, a case study published by the Association of State and Territorial Solid Waste Management Officials (ASTSWMO) reported quantifiable levels of PFBS in four of seven samples tested from the Wurtsmith Air Force Base; one site sampled directly below the fire training area was reported to have a PFBS concentration of 4,100 ng/L (ASTSWMO, 2015).

Additionally, PFBS has been detected at concentrations ranging from 0.00211 ng/L to 0.0261 ng/L in groundwater wells (100% well DF) at a site near the 3M Cottage Grove perfluorochemical manufacturing facility in Minnesota (3M, 2007; ATSDR, 2021). Lee et al. (2015) evaluated urban shallow groundwater contaminated by wastewater effluent discharge and reported a DF of 20% (1 of 5 shallow sites) and a maximum PFBS level of 36.3 ng/L. In contrast, Procopio et al. (2017) collected groundwater from 17 sampling sites (53 total across all water types sampled), some of which were located downstream of an industrial facility that used materials containing PFOA. PFBS was not detected in groundwater collected from any of the sampling locations. Post et al. (2013) assessed raw water from PWS intakes in New Jersey; these intake locations were selected to represent New Jersey geographically and they were not necessarily associated with any known PFAS release. PFBS was detected pre-treatment in 1 of 18 systems at a concentration of 6 ng/L (MRL = 5 ng/L). Lindstrom et al. (2011) analyzed water from 13 wells intended for uses other than drinking water (e.g., livestock, watering gardens) in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer. PFBS was detected in three of the wells (mean concentration 10.3 ng/L; range: ND–76.6 ng/L).

Of the 10 identified studies conducted in Europe, seven studies evaluated groundwater samples from sites with known or suspected PFAS releases associated with AFFF use, fluorochemical manufacturing, or other potential emission sources including landfill/waste disposal sites, skiing areas, or areas of unspecific industries that use PFAS in manufacturing (e.g., metal plating) (Dauchy et al., 2012, 2017, 2019; Gobelius et al., 2018; Gyllenhammar et al., 2015; Høisæter et al., 2019; Wagner et al., 2013). All of these studies reported PFBS detections in at least one sample or site, though only two studies (both conducted in the vicinity of areas with known AFFF usage) reported PFBS concentrations ≥ 100 ng/L (Dauchy et al., 2019; Gyllenhammar et al., 2015). The remaining three studies of the 10 identified did not provide information on whether there were potential sources of PFAS at the sampling locations or were designed to be regionally, nationally, or internationally representative (Barreca et al., 2020; Boiteux et al., 2012; Loos et al., 2010). At these sites, PFBS was detected infrequently (DFs 4 to 18%) with a maximum concentration of 25 ng/L across the three studies.

1.4.2.4 Surface Water

Studies evaluating the occurrence of PFBS in surface water are available from North America, Europe, and across multiple continents (see Table B-3). Broadly, studies either targeted surface waters used as drinking water sources, surface waters known to be contaminated with PFAS (as reported by the study authors), or surface waters over a relatively large geographic area (i.e., statewide) with some or no known point sources of PFAS.

Zhang et al. (2016) identified major sources of surface water PFAS contamination by collecting samples from 37 rivers and estuaries in the northeastern United States (metropolitan New York area and Rhode Island). PFBS was detected at 82% of sites and the range of PFBS concentrations was ND to 6.2 ng/L. Appleman et al. (2014) collected samples of surface water that were impacted by wastewater effluent discharge in several states. PFBS was detected in 64% of samples from 11 sites with a range of PFBS concentrations from ND – 47 ng/L. Several other studies from North America (four from the United States and two from Canada) evaluated surface waters from sites for which authors did not indicate whether sites were associated with any specific, known PFAS releases (Nakayama et al., 2010; Pan et al., 2018; Subedi et al., 2015;

Veillette et al., 2012; Yeung et al., 2017). Nakayama et al. (2010) also collected samples across several states, but no specific source of PFAS was identified. The DF in the Nakayama et al. (2010) study was 43% with median and maximum PFBS levels of 0.71 and 84.1 ng/L, respectively. Pan et al. (2018) sampled surface water sites in the Delaware River and reported a 100% DF, though PFBS levels were relatively low (0.52 to 4.20 ng/L); Yeung et al. (2017) reported results for a creek (PFBS concentration of 0.02 ng/L) and a river (no PFBS detected) in Canada. Veillette et al. (2012) analyzed surface water from an Arctic lake and detected PFBS at concentrations ranging from 0.011 to 0.024 ng/L. Subedi et al. (2015) evaluated lake water potentially impacted by septic effluent from adjacent residential properties, and detected PFBS in only one sample at a concentration of 0.26 ng/L.

Additional available studies assessed surface water samples at U.S. sites contaminated with PFAS from nearby PFAS manufacturing facilities (ATSDR, 2021; Galloway et al., 2020; Newsted et al., 2017; Newton et al., 2017) or facilities that manufacture products containing PFAS (Lasier et al., 2011; Procopio et al., 2017; Zhang et al., 2016). A few of these studies identified potential point sources of PFAS contamination, including industrial facilities (e.g., textile mills, metal plating/coating facilities), airports, landfills, and WWTPs (Galloway et al., 2020; Zhang et al., 2016). Among these sites, DFs (0 to 100%) and PFBS levels (ND to 336 ng/L) varied. In general, DFs that ranged from 0 to 3% were associated with samples collected upstream of PFAS point sources, and higher DFs (up to 100%) and PFBS concentrations were associated with samples collected downstream of point sources. An additional study (Lindstrom et al., 2011) sampled pond and stream surface water in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer, and the maximum and mean PFBS concentrations were 208 and 26.3 ng/L, respectively.

Another group of studies from the United States evaluated sites known to be contaminated from military installations with known or presumed AFFF use (Anderson et al., 2016; Nakayama et al., 2007; Post et al., 2013). The highest PFBS levels reported among these available studies were from Anderson et al. (2016) who performed a national study of 40 AFFF-impacted sites across 10 military installations and reported a maximum PFBS concentration of 317,000 ng/L. Lescord et al. (2015) examined PFAS levels in Meretta Lake, a Canadian lake contaminated with runoff from an airport and military base, which are likely sources of PFAS from AFFF use. The authors reported a 70-fold higher mean PFBS concentration for the contaminated lake versus a control lake. In addition to AFFF, Nakayama et al. (2007) identified industrial sources, including metal-plating facilities and textile and paper production, as contributing to the total PFAS contamination in North Carolina's Cape Fear River Basin. Nakayama et al. (2007) reported a PFBS DF of 17% and PFBS concentrations ranging from ND to 9.41 ng/L at these sites.

Seven studies evaluated surface water samples from sites in Europe with known or suspected PFAS releases associated with AFFF use (Dauchy et al., 2017; Gobelius et al., 2018; Mussabek et al., 2019) or fluorochemical manufacturing (Bach et al., 2017; Boiteux et al., 2017; Gebbink et al., 2017; Valsecchi et al., 2015). PFBS levels were comparable at the AFFF-impacted sites (< 300 ng/L overall). Of the four study sites potentially contaminated based on proximity to fluorochemical manufacturing sites, two (from studies conducted in France) did not have PFBS detections (Bach et al., 2017; Boiteux et al., 2017). PFBS levels were low at most sampling locations of the remaining two studies (up to approximately 30 ng/L) except for the site in River

Brenta in Italy (maximum PFBS concentration of 1,666 ng/L) which is also impacted by nearby textile and tannery manufacturers (Valsecchi et al., 2015).

Eight studies in Europe evaluated areas close to urban areas, commercial activities, or industrial activities (e.g., textile manufacturing) (Boiteux et al., 2012; Eschauzier et al., 2012; Lorenzo et al., 2015; Rostkowski et al., 2009; Zhao et al., 2015) and/or wastewater effluent discharges (Labadie and Chevreuil, 2011; Lorenzo et al., 2015; Möller et al., 2010; Wilkinson et al., 2017). Among these sites, DFs varied (0 to 100%) and PFBS levels were < 250 ng/L overall.

Ten studies conducted in Europe evaluated sites with no known fluorochemical source of contamination (Ahrens et al., 2009a, 2009b; Barreca et al., 2020; Ericson et al., 2008b; Eriksson et al., 2013; Loos et al., 2017; Munoz et al., 2016; Pan et al., 2018; Shafique et al., 2017; Wagner et al., 2013). Pan et al. (2018) analyzed surface water from sites in the United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). None of the sites sampled were proximate to known sources of PFAS, but PFBS was detected in all three water bodies. Concentrations of PFBS ranged from 0.46 to 146 ng/L; the highest level (146 ng/L) was detected in the Rhine River and was more than 20 times greater than any maximum level found in the other water bodies. In the remaining nine studies, reported PFBS levels ranged from ND to 26 ng/L, except for one study in Italy that reported a PFBS DF of 39% and levels in the µg/L range at three out of 52 locations within the same river basin: Legnano (16,000 ng/L), Rho (15,000 ng/L), and Pero (3,400 ng/L) (Barreca et al., 2020).

1.4.3 Exposure in Humans

As described in EPA's final PFBS toxicity assessment, PFBS has been detected in the serum of humans in the general population (U.S. EPA, 2021a). In American Red Cross plasma samples collected in 2015, 8.4% of samples had a quantifiable serum PFBS concentration, ranging from the lower limit of quantitation (LOQ) to 4.2 nanograms per milliliter (ng/mL) (Olsen et al., 2017). Results for the majority of serum samples were below the lower LOQ for PFBS, and the 95th percentile concentration was 0.02 ng/mL (Olsen et al., 2017). Data from the 2013–2014 National Health and Nutrition Examination Survey (NHANES) reported a 95th percentile concentration for PFBS in serum that was at or below the level of detection (0.1 ng/mL) (Olsen et al., 2017). Another study studied temporal trends of PFBS in blood serum from primiparous nursing women in Sweden ~2000–2002 around the time of increased manufacturing of PFBS after it was introduced as a replacement for PFOS (Glynn et al., 2012). An increase in PFBS blood serum levels was observed between 1996 and 2010, and regression analysis suggested that PFBS levels doubled on average every six years (Glynn et al., 2012).

Studies in animals show that PFBS is well absorbed following oral administration and distributes to all tissues of the body (Bogdanska et al., 2014). Distribution is predominantly extracellular (Olsen et al., 2009) and based on its resistance to metabolic degradation, the majority of PFBS is eliminated unchanged in urine and feces. Two studies that measured PFBS half-life in humans found overlapping ranges of 21.6−87.2 days (Xu et al., 2020) and 13.1−45.7 days (Olsen et al., 2009). The relatively rapid rate of elimination (days to weeks) of PFBS, compared with longer-chain PFAS (years), could lead to a lack of detection in biomonitoring detects which should not be interpreted as a lack of occurrence or exposure potential (U.S. EPA, 2021a). For more information, see U.S. EPA (2021a).

2.0 Problem Formulation and Scope

2.1 Conceptual Model

A conceptual model provides useful information to characterize and communicate the potential health risks related to PFBS exposure from drinking water and to outline the scope of the HA. The sources of PFBS, the routes of exposure for biological receptors of concern (e.g., various human activities related to tap water ingestion such as drinking, food preparation, and consumption), the potential health effects, and exposed populations including sensitive populations and life stages are depicted in the conceptual diagram below (Figure 1).

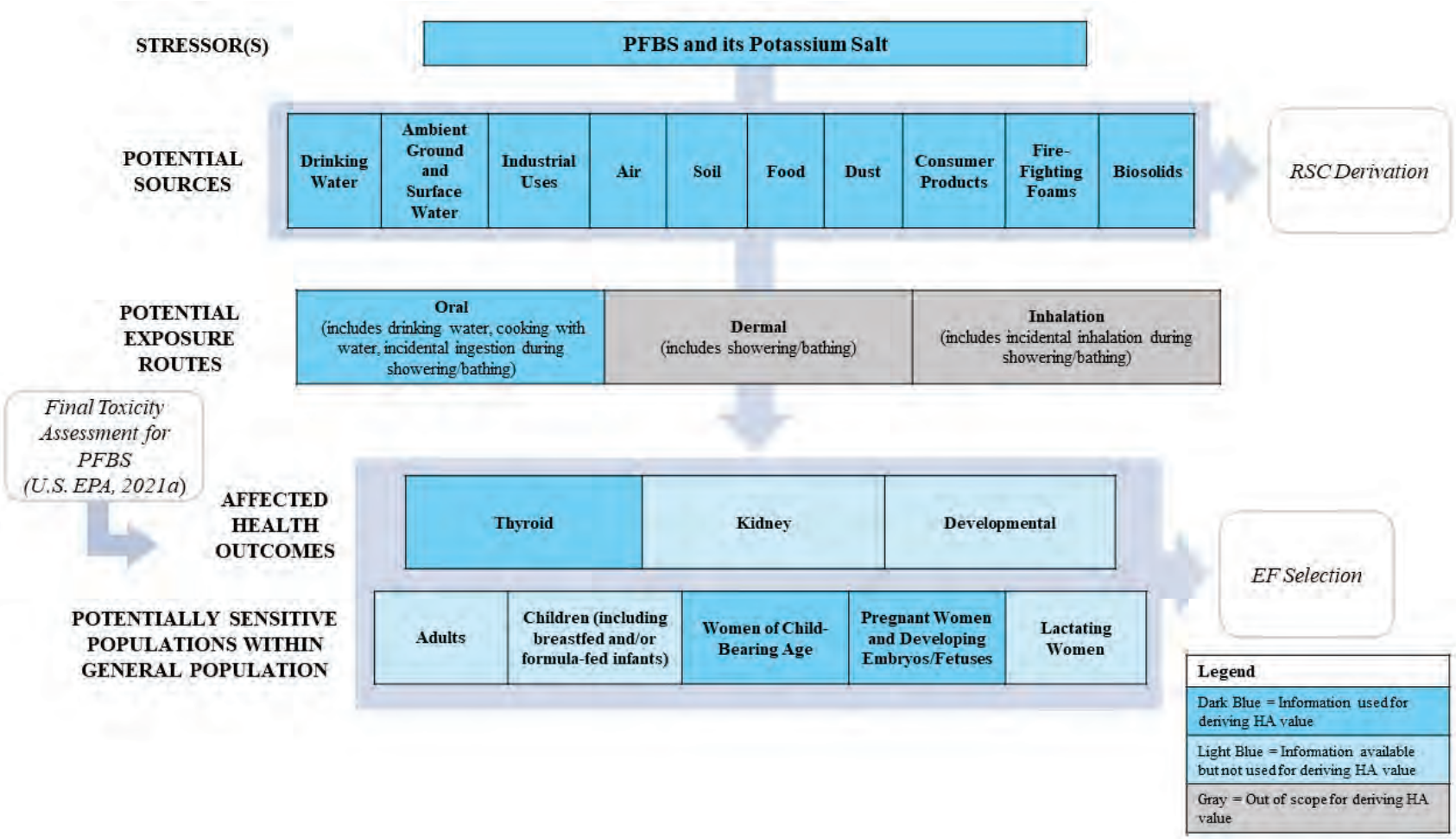

Figure 1. Conceptual Model for the Development of the Drinking Water Health Advisory for PFBS

The conceptual model is intended to explore potential links between exposure to a contaminant or stressor and the adverse health outcomes, and to outline the information sources used to identify or derive the input values used for the HA derivation, which are the RfD, relative source contribution (RSC), and exposure factor (EF). The conceptual model also illustrates the scope of the PFBS HA, which considers the following factors:

Stressors: The scope of this drinking water HA includes PFBS, its potassium salt (K^+PFBS), and PFBS– since K^+PFBS fully dissociates in water to the deprotonated anionic form of PFBS (PFBS–; CASRN 45187-15-3) and the K^+ cation at environmental pH levels (pH 4–9), consistent with the scope of the PFBS toxicity assessment (EPA, 2021a).

Potential Sources of Exposure: The scope of the HA derivation is limited to drinking water from public water facilities or private wells. Sources of PFBS exposure include both ground and surface waters used for drinking. To develop the RSC, information about non-drinking water sources was identified to determine the portion of the RfD attributable to drinking water. Potential non-drinking water sources of PFBS include but are not limited to foods, indoor dust, indoor and outdoor air, soil, biosolids, and consumer products (see Figure 1).

Potential Exposure Routes: Oral exposure to PFBS from contaminated drinking water sources (e.g., via drinking water, cooking with water, and incidental ingestion from showering) is the focus of the HA. The drinking water HA value does not apply to other exposure routes. However, information on other potential routes of exposure including dermal exposure (contact of exposed parts of the body with water containing PFBS during bathing or showering, dishwashing); and inhalation exposure (during bathing or showering or using a humidifier or vaporizer) was considered to develop the RSC.

Affected Health Outcomes: The PFBS final toxicity assessment (U.S. EPA, 2021a) considered all publicly available human, animal, and mechanistic studies of PFBS exposure and effects. The assessment identified associations between PFBS exposure and thyroid, developmental, and kidney effects. As part of the PFBS final toxicity assessment, human and animal studies of other health effects after PFBS exposure included the evaluation of effects on the reproductive system, liver, and lipid and lipoprotein homeostasis but the evidence did not support clear associations between exposure and effect. No cancer studies were identified for PFBS (U.S. EPA, 2021a).

Potentially Sensitive Populations or Life Stages: The receptors are humans in the general population who could be exposed to PFBS from oral exposure to tap water through ingestion at their homes, workplaces, schools, and daycare centers. Within all ages of the general population, there are potentially sensitive populations or life stages that may be more susceptible due to increased exposure and/or response. Potentially sensitive populations include the developing embryo and fetus (exposed to PFBS via the pregnant woman) and women of childbearing age who may be or become pregnant.

2.2 Analysis Plan

2.2.1 Health Advisory Guidelines

Assessment endpoints for HA guidelines or values can be developed, depending on the available data, for both short-term (one-day and ten-day) and lifetime exposure using information on the noncarcinogenic and carcinogenic toxicological endpoints of concern. Where data are available,

HAs can reflect sensitive populations or life stages that may be more susceptible and/or more highly exposed.

One-Day HA is protective of noncancer effects for up to 1 day of exposure and is typically based on an *in vivo* toxicity study with a duration of 7 days or less. It is typically calculated for an infant.

Ten-Day HA is protective of noncancer effects for up to 10 days of exposure and is typically based on an *in vivo* toxicity study with a duration of 7 to 30 days. It is typically calculated for an infant.

Lifetime HA is designed to be protective of noncancer effects over a lifetime of exposure and is typically based on a chronic *in vivo* experimental animal toxicity study and/or human epidemiological data.

10^{-6} Cancer Risk Concentration is the concentration of a carcinogen in water at which the population is expected to have a one in a million (10^{-6}) excess cancer risk above background after exposure to the contaminant over a lifetime. It is calculated for carcinogens classified as known or likely human carcinogens (U.S. EPA, 1986, 2005b). Cancer risk concentrations are not derived for substances for which there is suggestive evidence of carcinogenic potential unless the cancer risk has been quantified.

2.2.2 Sources of Toxicity Information for Health Advisory Development

The final toxicity assessment for PFBS, entitled *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)*, published in April 2021 by EPA's Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) (U.S. EPA, 2021a), serves as the basis of the toxicity information and chronic RfD used to derive the lifetime noncancer HA for PFBS. It also synthesizes and describes other information on PFBS including physicochemical properties and toxicokinetics. The PFBS toxicity assessment was published after rigorous scientific review, including internal and external review, and public comment.

To develop the final toxicity assessment for PFBS, EPA reviewed and analyzed the available toxicokinetics and toxicity data for PFBS. Briefly, online scientific databases (PubMed, Web of Science, TOXLINE, and TSCATS via TOXLINE) were searched using search terms focused on chemical name and synonyms with no limitations on publication type, evidence stream (i.e., human, animal, in vitro, and in silico), or health outcomes. The identified studies were screened using Populations, Exposures, Comparators, and Outcomes (PECO) criteria and relevant studies underwent study quality evaluation. Dose-response studies were identified for dose-response modeling and a point-of-departure (POD) and uncertainty factors (UFs) were selected for RfD derivation. For more information, please see Section 2.3 in U.S. EPA (2021a).

2.2.3 Approach and Scope for Health Advisory Derivation

2.2.3.1 Approach for Deriving Noncancer HAs

The following equations (Eqs. 1–3) are used to derive the HAs.[11] Lifetime HAs and 10^{-6} cancer risk concentrations are only derived for chemicals without an existing National Primary Drinking Water Regulation.

$$\textbf{One-Day HA} = \left(\frac{\text{POD}}{\text{UF}_\text{C} * \text{DWI-BW}}\right) \quad \text{(Eq. 1)}$$

POD is typically derived from a toxicity study of duration 7 days or less

$$\textbf{Ten-Day HA} = \left(\frac{\text{POD}}{\text{UF}_\text{C} * \text{DWI-BW}}\right) \quad \text{(Eq. 2)}$$

POD is typically derived from a toxicity study of duration 7–30 days

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \quad \text{(Eq. 3)}$$

RfD is typically derived from a chronic study

Where:

POD is the point of departure, typically a lowest observed adverse effect level (LOAEL), a no observed adverse effect level (NOAEL), or a BMDL from the critical study.

UF_C is the composite UF or total UF value after multiplying individual UFs. UFs are established in accordance with EPA best practices (U.S. EPA, 2002) and consider uncertainties related to the following: variation in sensitivity among the members of the human population (i.e., inter-individual variability), extrapolation from animal data to humans (i.e., interspecies uncertainty), extrapolation from data obtained in a study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure), extrapolation from a LOAEL rather than from a NOAEL, and extrapolation when the database is incomplete. For PFBS, the value of UF_C was determined in the final PFBS toxicity assessment (U.S. EPA, 2021a).

DWI-BW is the 90th percentile drinking water intake (DWI), adjusted for body weight (bw), for the selected population in units of liter per kilogram body weight per day (L/kg bw-day). The DWI-BW considers direct and indirect consumption of tap water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea and coffee). For PFBS, the value of this parameter is based on the critical study identified in the PFBS final toxicity assessment (U.S. EPA, 2021a), and is identified in Chapter 3 of EPA's *Exposure Factors Handbook* (U.S. EPA, 2019a).

RfD is the reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime. The value of this parameter

[11] https://www.epa.gov/system/files/documents/2022-01/dwtable2018.pdf

was derived in the final PFBS toxicity assessment and is based on the critical effect and study identified in that assessment (U.S. EPA, 2021a).

RSC is the relative source contribution—the percentage of the total oral exposure attributed to drinking water sources (U.S. EPA, 2000a) where the remainder of the exposure is allocated to other routes or sources. The RSC is calculated by examining other sources of exposure (e.g., air, food, soil) and pathways of exposure in addition to drinking water using the methodology described for calculation of an RSC described in U.S. EPA (2000a) and Section 3.3.

2.2.3.2 Scope of Noncancer Health Advisory Values

Adequate data are available to derive a lifetime HA for PFBS. Neither one-day nor ten-day HA values were derived for PFBS. U.S. EPA (2021a) derived subchronic and chronic RfDs but did not derive an RfD for exposure durations of 7 days or less on which to base a one-day HA for PFBS. Derivation of a 10-day HA was considered because the subchronic and chronic RfDs are both based on a 20-day exposure study, which may be used to derive a ten-day HA. However, the critical health effect on which the chronic RfD used to calculate the lifetime HA is based (i.e., decreased serum levels of the thyroid hormone thyroxine [T_4] in newborn mice) resulted from PFBS exposure during a developmental life stage. EPA's risk assessment guidelines for developmental toxicity indicate that adverse effects can result from even brief exposure during a critical period of development (U.S. EPA, 1991). The critical study for the subchronic and chronic RfDs for PFBS observed persistent health effects into adulthood suggesting the potential for long-term health consequences of gestational-only PFBS exposure and that gestation is at least one critical exposure window for PFBS. Therefore, the lifetime HA (calculated in Section 4.0) and the chronic RfD from which it is derived (see Table 4) are considered applicable to short-term PFBS exposure scenarios (including during pregnancy) via drinking water.

2.2.3.3 Approach and Scope for Deriving Cancer Risk Concentrations

The following equations (Eqs. 4-5) are used to derive cancer risk concentrations.

Calculated for non-mutagenic carcinogens[12] only:

$$\mathbf{10^{-6}\ Cancer\ Risk\ Concentration} = \frac{1\text{x}10^{-6}}{\text{CSF} * \text{DWI-BW}} \qquad \text{(Eq. 4)}$$

Calculated for mutagenic carcinogens only:

$$\mathbf{10^{-6}\ Cancer\ Risk\ Concentration} = \frac{1\text{x}10^{-6}}{\text{CSF}} * \sum_{i}\left(\frac{F_i * ADAF_i}{\text{DWI-BW}_i}\right) \qquad \text{(Eq. 5)}$$

Where:

CSF is the cancer slope factor—an upper bound, approximating a 95 percent confidence limit of the increased cancer risk from a lifetime of oral exposure to a stressor. The value for this parameter is derived in the final toxicity assessment when data are available.

[12] https://www.epa.gov/system/files/documents/2022-01/dwtable2018.pdf

$DWI\text{-}BW_i$ is the 90th percentile bw-adjusted DWI in units of L/kg bw-day for each age group (i), considered when calculating cancer risk concentrations for mutagenic carcinogens.

$ADAF_i$ is the age-dependent adjustment factor for each age group (i), used when calculating cancer risk concentrations for carcinogens that act via a mutagenic mode of action (U.S. EPA, 2005a,b).

F_i the fraction of life spent in each age group (i), used when calculating cancer risk concentrations for mutagens (U.S. EPA, 2005a).

2.2.3.4 Scope of Cancer Risk Concentration Derivation

As described in the toxicity assessment for PFBS, a CSF was not derived because no studies evaluating the carcinogenicity of PFBS in humans or animals had been identified (U.S. EPA, 2021a). In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), EPA concluded that there is *"Inadequate Information to Assess Carcinogenic Potential*" for PFBS by any route of exposure (U.S. EPA, 2021a). Therefore, a 10^{-6} cancer risk concentration cannot be derived for PFBS at this time.

2.2.4 Exposure Factors for Deriving Health Advisory

2.2.4.1 Exposure Factor Selection

An EF, such as body weight-adjusted drinking water intake (DWI-BW), is one of the input values for deriving a drinking water HA. EFs are factors related to human activity patterns, behavior, and characteristics that help determine an individual's exposure to a contaminant. EPA's *Exposure Factors Handbook* (EFH)[13] is a resource for conducting exposure assessments and provides EFs based on information from publicly available, peer-reviewed studies. Chapter 3 of the EFH presents EFs in the form of DWI and DWI-BW for various populations or life stages within the general population (U.S. EPA, 2019a). The use of EFs in HA calculations is intended to protect sensitive populations and life stages within the general population from adverse effects resulting from exposure to a contaminant.

When developing HAs, the goal is to protect all ages of the general population including potentially sensitive populations or life stages such as children. The approach to select the EF for the drinking water HA includes a step to identify potentially sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can prevent identification of the most sensitive population (e.g., not all windows of exposure or health outcomes have been assessed for PFBS), the critical effect and POD that form the basis for the RfD can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In those cases, EPA can select the corresponding DWI-BW for that sensitive population or life stage from the EFH (U.S. EPA, 2019a) for use in HA derivation. When multiple potentially sensitive populations or life stages are identified based on the critical effect or other health effects data (from animal or human studies), EPA selects the population or life stage with the greatest DWI-BW because it is

[13] EPA's EFH is available at https://www.epa.gov/expobox/about-exposure-factors-handbook

the most health protective. For deriving lifetime HAs, the RSC corresponding to the selected sensitive life stage is also determined when data are available (see Section 3.3). In the absence of information indicating a potentially sensitive population or life stage, the EF corresponding to all ages of the general population may be selected.

To derive chronic HAs, EPA typically uses DWI normalized to body weight (i.e., DWI-BW in liter [L] of water consumed/kg bw-day) for all ages of the general population or for a sensitive population or life stage, when identified. The Joint Institute for Food Safety and Applied Nutrition's Food Commodity Intake Database (FCID) Consumption Calculator Tool[14] includes the EFs from EPA's EFH and can also be used to estimate DWI-BW for specific populations, life stages, or age ranges. EPA uses the 90th percentile DWI-BW to ensure that the HA is protective of the general population as well as sensitive populations or life stages (U.S. EPA, 2000a, 2016a). In 2019, EPA updated its EFs for DWI-BW based on newly available science (EPA, 2019a).

Table 3 shows EPA EFs for some sensitive populations or life stages. Other populations or life stages may also be considered depending on the available information regarding sensitivity to health effects after exposure to a contaminant.

Table 3. EPA Exposure Factors for Drinking Water Intake

Populations or Life Stages	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
General population, all ages	0.0338	90th percentile direct and indirect consumption of community water, consumer-only two-day average, all ages.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)
Children	0.143	90th percentile direct and indirect consumption of community water, consumer-only two-day average, birth to < 1 year.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)
Formula-fed infants	0.249	90th percentile direct and indirect consumption of community water, formula-consumers only, 1 to < 3 months. Includes water used to reconstitute formula, plus all other community water ingested.	Kahn et al. (2013) Estimates of Water Ingestion in Formula by Infants and Children Based on CSFII 1994–1996 and 1998[a,b]

[14] Joint Institute for Food Safety and Applied Nutrition's FCID, Commodity Consumption Calculator is available at https://fcid.foodrisk.org/percentiles

Populations or Life Stages	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Lactating women	0.0469	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[c] (U.S. EPA, 2019a)

Notes: CSFII = continuing survey of food intake by individuals; L/kg bw-day = liter per kilogram body weight per day.
[a] The sample size does not meet the minimum reporting requirements as described in the Third Report on Nutrition Monitoring in the United States (LSRO, 1995).
[b] Chapter 3.2.3 in U.S. EPA (2019a) cites Kahn et al. (2013) as the source of drinking water ingestion rates for formula-fed infants. While U.S. EPA (2019a) provides the 95th percentile total direct and indirect water intake values, Office of Water/Office of Science and Technology (OW/OST) policy is to utilize the 90th percentile DWI-BW. OW/OST was able to identify the 90th percentile DWI-BW in Kahn et al. (2013) and report the value in this table.
[c] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports: Human Nutrition Information Service (HNIS)/National Center for Health Statistics (NCHS) Analytical Working Group Recommendations (NCHS, 1993).

2.2.4.2 Determining Proportion of RfD Attributable to Drinking Water

To account for aggregate risk from exposures and exposure pathways other than oral ingestion of drinking water, EPA applies an RSC when calculating HAs to ensure that total human exposure to a contaminant does not exceed the daily exposure associated with the RfD. The RSC represents the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee, tea, or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD is allocated to other potential exposure sources (U.S. EPA, 2000a). The purpose of the RSC is to ensure that the level of a contaminant (e.g., HA value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD (U.S. EPA, 2000a).

To determine the RSC, EPA follows the Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment in EPA's guidance, *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a). EPA considers whether there are significant known or potential uses/sources other than drinking water, the adequacy of data and strength of evidence available for each relevant exposure medium and pathway, and whether adequate information on each source is available to quantitatively characterize the exposure

profile. The RSC is developed to reflect the exposure to the general population or a sensitive population within the general population.

Per EPA's guidance, in the absence of adequate data to quantitatively characterize exposure to a contaminant, EPA typically recommends an RSC of 20%. When scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, thereby allocating the remaining 20% to other potential exposure sources (U.S. EPA, 2000a).

To inform the RSC determination, available information on all exposure sources and routes for PFBS was identified using the literature search and screening method described in Appendix A. To identify information on PFBS exposure routes and sources to inform RSC determination, EPA considered primary literature published between 2003–2020 and collected by EPA ORD as part of an effort to evaluate evidence for pathways of human exposure to eight PFAS, including PFBS. In order to consider more recently published information on PFBS exposure, EPA incorporated the results of a date-unlimited gray literature search that was conducted in February 2022 as well as an ad hoc process to identify relevant and more recently published peer-reviewed scientific literature. The literature resulting from the search and screening process included only final (not draft) documents and articles that were then reviewed to inform the PFBS RSC.

3.0 Health Advisory Input Values

3.1 Toxicity Assessment Values

Table 4 summarizes the peer-reviewed chronic noncancer toxicity values from EPA's *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)* (U.S. EPA, 2021a).

Table 4. Chronic Noncancer Toxicity Information for PFBS for Deriving the Lifetime HA

Health Assessment	PFBS Exposure in Critical Study	RfD (mg/kg bw-day)	Critical Effect	Principal Study
Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)	Days 1–20 of gestation	K^+PFBS: 3×10^{-4} PFBS: 3×10^{-4}	Decreased serum total T_4 in newborn (PND 1) mice	Oral gestational exposure study in mice (Feng et al., 2017)

Notes: mg/kg bw-day = milligram per kilogram body weight per day; PND = post-natal day.
Source: U.S. EPA, 2021a

As stated in U.S. EPA (2021a), the thyroid effect of decreased thyroid hormones, specifically serum total T_4, in newborn (PND1) mice exposed to K^+PFBS throughout gestation was selected as the critical effect (Feng et al., 2017). This critical effect and study were used to derive the chronic RfDs for K^+PFBS and PFBS of 3×10^{-4} milligrams per kilogram body weight per day (mg/kg bw-day).

Based on EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), serum half-lives were used to scale a toxicologically equivalent dose of orally administered K^+PFBS from animals to humans. Following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), benchmark dose (BMD) modeling of thyroid effects following gestational exposure to K^+PFBS resulted in a benchmark dose lower confidence limit for 0.5 standard deviation change from the control ($BMDL_{0.5SD}$) human equivalent dose (HED) of 0.095 mg/kg bw-day.

This POD (HED) served as the critical effect and was divided by a composite UF (UF_C) of 300. The UF_C is based on an animal-to-human UF (UF_A) of 3 to account for extrapolation from mice to humans; an intrahuman UF (UF_H) of 10 to account for interindividual differences in human susceptibility; and a database UF (UF_D) of 10 to account for deficiencies in the toxicity database. A value of 1 was applied for the extrapolation from subchronic to a chronic exposure duration UF (UF_S) because extrapolation from subchronic to chronic was not needed, and UF_L because a LOAEL to NOAEL approach was not used. Data for K^+PFBS were used to derive the chronic RfD for the free acid (PFBS), resulting in the same value (3×10^{-4} mg/kg bw-day), after adjusting for differences in molecular weight (MW) between K^+PFBS (338.19) and PFBS (300.10) (see Section 6.0 in U.S. EPA [2021a] for more details). This chronic RfD for PFBS was used to derive the lifetime HA.

No studies evaluating the carcinogenicity of PFBS in humans or animals were identified (U.S. EPA, 2021a). In accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), EPA concluded that there is *"Inadequate Information to Assess Carcinogenic Potential"* for PFBS by any route of exposure (U.S. EPA, 2021a) and did not derive a 10^{-6} cancer risk concentration.

3.2 Exposure Factors

To identify potentially sensitive populations or life stages, EPA considered the PFBS exposure interval used in the critical study selected for chronic RfD derivation in the final PFBS toxicity assessment (U.S. EPA, 2021a). In the critical study pregnant mice were orally exposed to K^+PFBS throughout all of gestation (days 1–20 of gestation) (Feng et al., 2017; U.S. EPA, 2021a), identifying the developing fetus (exposed via the pregnant mother) as a population that may be particularly susceptible to PFBS exposure. The critical study did not permit a more precise identification of the most sensitive or critical PFBS exposure window during prenatal development since exposure was throughout all of gestation. The critical effect of thyroid development in the developing mouse embryo and fetus is relevant to humans. Human thyroid development occurs in three phases during gestation, and while there are some timing differences in thyroid development between humans and rodents (see Section 6.1.1.3 in U.S. EPA, 2021a), two phases of thyroid development occur during gestation in both the mouse and human.

The gestational exposure in the critical study is relevant to two potentially sensitive populations or life stages—women of childbearing age (13 to < 50 years) who may be or become pregnant, and pregnant women and their developing embryo and fetus (Table 5). EPA selected women of childbearing age as the sensitive life stage for HA derivation because the DWI-BW is greater (0.0354 L/kg bw-day) than for pregnant women (0.0333 L/kg bw-day). EPA addresses exposure to the sensitive developing embryo and fetus because they are exposed to drinking water via the pregnant mother. Additional support for the women of childbearing age population including

pregnant women (and their developing embryo and fetus) includes the high rate of unintended pregnancies reported in the United States (30.6%) (United Health Foundation, 2021). To derive the HA value, EPA used the DWI-BW of 0.0354 L/kg bw-day representing the consumers-only two-day average of direct and indirect community water consumption at the 90th percentile for women of childbearing age (13 to < 50 years) (Table 5, in bold).

Table 5. EPA Exposure Factors for Drinking Water Intake for Different Candidate Sensitive Populations or Life Stages Based on the Critical Effect and Study

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Women of childbearing age	**0.0354**	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day. The DWI-BW used to calculate the PFBS lifetime HA is in bold.

3.3 Relative Source Contribution

As stated in the analysis plan, EPA collected and evaluated information about PFBS exposure routes and sources to inform RSC determination. Results from the literature search are described below.

3.3.1 Non-Drinking Water Sources and Routes

EPA presents information below from studies performed in the United States as well as studies published globally for this emerging contaminant to be as comprehensive as possible, given that the overall information is limited. While the studies from non-U.S. countries inform an understanding global exposure sources and trends, the RSC determination is based on the available data for the United States.

3.3.1.1 Dietary Sources

Food

PFBS was included in a suite of individual PFAS selected as part of PFAS-targeted reexaminations of samples collected for the U.S. Food and Drug Administration's (FDA's) Total Diet Study (U.S. FDA, 2020a,b, 2021a,b, 2022a,b); however, it was not detected in any of the food samples tested. It should be noted that FDA indicated that the sample sizes were limited and that the results should not be used to draw definitive conclusions about PFAS levels or presence in the general food supply (U.S. FDA, 2022c). PFBS was detected in cow milk samples collected from a farm with groundwater known to be contaminated with PFAS, as well as in produce

(collard greens) collected from an area near a PFAS production plant, in FDA studies of the potential exposure of the U.S. population to PFAS (U.S. FDA 2018, 2021c). Maximum residue levels for PFBS were not found in the Global MRL Database (Bryant Christie Inc., 2022).

In addition to efforts by FDA, 34 peer-reviewed studies conducted in North America (n = 7), Europe (n = 26), and across multiple continents (n = 1) analyzed PFBS in food items obtained from home, recreational, or commercial sources (see Table B-4). Food types evaluated include fruits and vegetables, grains, meat, seafood, dairy, and fats/other (e.g., eggs, spices, and oils), with seafood showing the highest levels of PFBS detected. PFBS was not detected in any of the eight studies that analyzed human milk for PFAS (not shown in Table B-4)—one in the United States (von Ehrenstein et al., 2009) and seven in Europe (Abdallah et al., 2020; Beser et al., 2019; Cariou et al., 2015; Kärrman et al., 2007, 2010; Lankova et al., 2013; Nyberg et al., 2018).

Of eight studies conducted in North America, four U.S. studies (Blaine et al., 2014; Byrne et al., 2017; Schecter et al., 2010; Scher et al., 2018) found PFBS in at least one food item. Locations and food sources varied in these studies. In Schecter et al. (2010), PFBS was detected in cod samples but not in any of the other foods collected from Texas grocery stores. Scher et al. (2018) detected PFBS in plant parts (leaf and stem samples) analyzed from garden produce collected at homes in Minnesota within a GCA impacted by a former 3M PFAS production facility (PFBS concentrations ranged from ND to 0.065 nanograms per gram [ng/g]). The authors suggested that the PFBS detections in plant parts were likely associated with PFAS present in irrigation water that had accumulated in produce. Blaine et al. (2014) found PFBS in radish, celery, tomato, and peas that were grown in soil amended with industrially impacted biosolids. They also found PFBS in these crops grown in soil that had received municipal biosolid applications over 20 years. In unamended control soil samples, PFBS was only detected in radish root with an average value of 22.36 ng/g (Blaine et al., 2014). In a similar study conducted by Blaine et al. (2013), PFBS was found in lettuce, tomato, and corn grown in industrially impacted biosolids-amended soils in greenhouses. Young et al. (2012) analyzed 61 raw and retail milk samples from 17 states for PFAS, but PFBS was not detected.

Based on the available data to date, seafood (including fish and shellfish) has been found to contain the highest concentrations of PFBS out of all food types examined. Several large-scale sampling efforts have been conducted by EPA and other agencies to determine PFAS levels in fish. In EPA's 2013–2014 National Rivers and Streams Assessment (NRSA), PFBS was detected at concentrations between the quantitation limit (1 ng/g) and the method detection limit (0.1 ng/g) at 0.571 ng/g in a largemouth bass fish fillet sample collected from Big Black River, Mississippi; 0.475 ng/g in a smallmouth bass fillet composite collected from Connecticut River, New Hampshire; and 0.148 ng/g in a walleye fillet composite collected from Chenango River, New York (U.S. EPA, 2020a). Notably, PFBS was not detected in any fish species sampled in the 2008–2009 NRSA (Stahl et al., 2014). PFBS was also detected at a concentration of 0.36 ng/g in a smallmouth bass fillet composite collected from Lake Erie, New York in EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study (U.S. EPA, 2021d). PFBS has been detected in Irish pompano, silver porgy, grey snapper, and eastern oyster from the St. Lucie Estuary in the National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). PFBS was not a target chemical in EPA's National Lake Fish Tissue Study (U.S. EPA, 2009a).

Several peer-reviewed publications that examined PFBS concentrations in fish and shellfish are also available. As mentioned previously, Schecter et al. (2010) detected PFBS in cod samples. Mean PFBS levels in cod from this study (0.12 ng/g wet weight [ww]) were much lower than maximum levels detected in Alaska blackfish obtained from the Suqi River, Alaska in remote locations upstream and downstream of a former (unnamed) defense site (59.2 ng/g ww) (Byrne et al., 2017). In this study, blackfish were considered sentinel species but are not among the traditional fish consumed in the area. The authors noted that the presence of PFAS in fish from remote sites is suggestive of atmospheric deposition. In two additional studies from North America, PFBS was not detected in samples of farmed and wild-caught seafood (Chiesa et al., 2019; Young et al., 2013).

The European Food Safety Authority (EFSA) reported the presence of PFBS in various food and drink items, including fruits, vegetables, cheese, and bottled water (EFSA, 2012). For average adult consumers, the estimated exposure ranges for PFBS were 0.03−1.89 nanograms per kilogram body weight per day (ng/kg bw-day) (minimum) to 0.10−3.72 ng/kg bw-day (maximum) (EFSA, 2012). Of 27 studies conducted in Europe, 12 found PFBS in at least one food type (Table B-4). Eight of the 12 studies included food samples obtained solely from markets where no particular source of PFAS contamination was identified (D'Hollander et al., 2015; Domingo et al., 2012; Eschauzier et al., 2013; Hlouskova et al., 2013; Pérez et al., 2014; Scordo et al., 2020; Surma et al., 2017; Sznajder-Katarzyńska et al., 2019). Across studies, PFBS detections were found in seafood; other animal products such as meat, dairy, and eggs; fruits and vegetables; tap water-based beverages such as coffee; sweets; and spices.

Papadopoulou et al. (2017) analyzed duplicate diet samples with PFBS detected in only one solid food sample (ND−0.001 ng/g; DF 2%; food category unspecified). Eriksson et al. (2013) evaluated foods that were farmed or freshly caught in the Faroe Islands, and only detected PFBS in cow milk (0.019 ng/g ww) and packaged dairy milk (0.017 ng/g ww) samples among the products analyzed. In eight of the European studies where PFBS was not detected, foods were primarily obtained from commercial sources, but wild-caught seafood was also included.

Two of the 12 European studies examined both market-bought and fresh-caught fish, and PFBS was detected in seafood from both sources (Vassiliadou et al., 2015; Yamada et al., 2014). Yamada et al. (2014) found higher PFBS in fresh-caught river fish samples (0.16 ng/g ww maximum) versus fresh or frozen market samples (0.03 ng/g ww maximum) in France. Vassiliadou et al. (2015) detected PFBS in raw shrimp (from Greek markets) but did not detect PFBS in either fried shrimp, raw hake (from Greek fishing sites), or fried hake.

In summary, in Europe and North America, PFBS has been detected in multiple food types, including fruits, vegetables, meats, seafoods, and other fats. Several large-scale fish tissue sampling efforts conducted by EPA and others indicate that fish consumption may be an important PFBS exposure source. Future large-scale sampling efforts by FDA and others may help to similarly elucidate PFBS concentrations in other food types. Although several U.S. studies have evaluated PFBS in meats, fats/oils, fruits, vegetables, and other non-seafood food types, many of these sampling efforts were localized to specific cities or markets and/or used relatively small sample sizes. Broader-scale sampling efforts will be helpful in determining the general levels of PFBS contamination in these food types, as well as the impact of known PFAS contamination sources on PFBS concentrations in foods.

Food Contact Materials

PFBS is not authorized for use in food packaging in the United States; however, PFBS has been detected in food packaging materials in the few available studies that investigate this potential route of exposure (ATSDR, 2021; U.S. EPA, 2021a). In one report from the United States, PFBS was detected in fast-food packaging (7/20 samples) although the concentrations detected were not reported (Schaider et al., 2017).

Five studies in Europe (conducted in Poland, Norway, Greece, Czech Republic, and Germany) analyzed the occurrence of PFBS in food packaging or food contact materials (FCMs), such as baking papers and fast-food boxes and wrappers. Surma et al. (2015) measured levels of 10 perfluorinated compounds in three different brands of common FCMs commercially available in Poland, including wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3). PFBS was detected in one brand of baking paper at 0.02 picograms per square centimeter (pg/cm^2), but PFBS was not detected at or below the LOQ in all other FCMs. Vestergren et al. (2015) analyzed paper plates (n = 2), paper cups (n = 1), baking covers (n = 1), and baking molds (n = 1) purchased from retail stores in Tromsø and Trondheim, Norway. PFBS was detected in one paper plate at 6.9 pg/cm^2.

The remaining three studies did not detect PFBS in FCMs. Zafeiraki et al. (2014) analyzed FCMs made of paper, paperboard, or aluminum foil collected from a Greek market. PFBS was not detected in any of the samples of beverage cups (n = 8), ice cream cups (n = 1), fast-food paper boxes (n = 8), fast-food wrappers (n = 6), paper materials for baking (n = 2), microwave bags (n = 3), and aluminum foil bags/wrappers (n = 14). The study concluded that the use of perfluorinated compound alternatives such as fluorophosphates and fluorinated polyethers in the local manufacturing process potentially explains the low levels of other PFAS (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluoroheptanoic acid [PFHpA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and perfluorododecanoic acid [PFDoDA]) detected in the sampled FCMs. Vavrous et al. (2016) analyzed 15 samples of paper FCMs acquired from a market in the Czech Republic. FCMs included paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220°C (n = 3), and paper filters for coffee preparation (n = 3). PFBS was not detected in any samples. Kotthoff et al. (2015) analyzed 82 samples for perfluoroalkane sulfonate (PFSA) and perfluoroalkyl carboxylic acid (PFCA) compounds in 10 consumer products including individual paper-based FCMs (n = 33) from local retailers in Germany in 2010. PFBS was not detected in paper-based FCMs.

Overall, the few available studies conducted in the United States and Europe indicate PFBS may be present in food packaging materials; however, further research is needed to understand which packaging materials generally contain PFBS at the highest concentrations and with the greatest frequency. There are also uncertainties related to data gaps on topics that may influence whether food packaging is a significant PFBS exposure source in humans, including differences in transfer efficiency from different packaging types directly to humans or indirectly through foodstuffs.

3.3.1.2 Consumer Products

Consumer products could also be a source of PFBS exposure as noted in Section 1.3. Several studies examined a range of consumer products and found multiple PFAS, including PFBS, at various levels (Bečanová et al., 2016; Favreau et al., 2016; Gremmel et al., 2016; Kotthoff et al., 2015; Liu et al., 2014; Schultes et al., 2018; van der Veen et al., 2020; Vestergren et al., 2015; Zheng et al., 2020). Two of the studies collected consumer products in the United States, five purchased consumer products in Europe, and two studies did not report the purchase location(s) of the consumer products that were tested. Additionally, two European studies analyzed commercially available AFFF products which have been formulated with PFAS and are associated with elevated levels of these chemicals in environmental media (Favreau et al., 2016; Høisæter et al., 2019).

Zheng et al. (2020) determined the occurrence of ionic and neutral PFAS in items collected from childcare environments in the United States Nap mats (n = 26; 20 polyurethane foam, 6 vinyl cover samples) were collected from seven Seattle childcare centers. PFBS was detected in 5% of nap mat samples at a maximum concentration of 0.04 ng/g. Liu et al. (2014) analyzed the occurrence of PFAS in commonly used consumer products (carpet, commercial carpet-care liquids, household carpet/fabric-care liquids, treated apparel, treated home textiles, treated non-woven medical garments, floor waxes, membranes for apparel, and thread-sealant tapes) purchased from retail outlets in the United States. PFBS was detected in 100% of commercial carpet/fabric-care liquids samples (n = 2) at concentrations of 45.8 and 89.6 ng/g, in 75% of household carpet/fabric-care liquids and foams samples (n = 4) at concentrations up to 911 ng/g, in one treated apparel samples (n = 2) at a concentration of 2 ng/g, in the single treated floor wax and stone/wood sealant sample (143 ng/g, n = 2), and in the single apparel membrane sample (30.7 ng/g, n = 2). PFBS was not detected in treated home textile and upholstery (n = 2) or thread-sealant tapes and pastes (n = 2).

van der Veen et al. (2020) examined the effects of weathering on PFAS content in durable water-repellent clothing collected from six suppliers in Sweden (1 pair of outdoor trousers, 7 jackets, 4 fabrics for outdoor clothes, 1 pair of outdoor overalls). Two pieces of each of the 13 fabrics were cut. One piece of each fabric was exposed to elevated ultraviolet radiation, humidity, and temperature in an aging device for 300 hours (assumed lifespan of outdoor clothing); the other was not aged. Both pieces of each fabric were analyzed for ionic PFAS (including PFBS) and volatile PFAS. In general, aging of outdoor clothing resulted in increased perfluoroalkylated acid (PFAA) levels of 5-fold or more. For 8 of 13 fabrics, PFBS was not detected before or after aging. For three fabrics, PFBS was detected before and after aging, increasing approximately 3- to 14-fold in the aged fabric (i.e., from 43 to 140 micrograms per square meter [$\mu g/m^2$], 45 to 350 $\mu g/m^2$, and 9.6 to 130 $\mu g/m^2$ respectively for the 3 fabrics). For the remaining two fabrics, PFBS was not detected prior to aging but was detected afterward at concentrations of 0.57 and 1.7 $\mu g/m^2$, respectively. The authors noted that possible explanations for this could be weathering of precursor compounds (e.g., fluorotelomer alcohols) to PFAAs such as PFBS or increased extractability due to weathering.

Kotthoff et al. (2015) analyzed 82 samples for PFSA and PFCA compounds in outdoor textiles (n = 3), gloves (n = 3), carpets (n = 6), cleaning agents (n = 6), impregnating sprays (n = 3), leather (n = 13), wood glue (n = 1), ski wax (n = 13), and awning cloth (n = 1). Individual samples were bought from local retailers or collected by coworkers of the involved institutes or

local clubs in Germany. The age of the samples ranged from a few years to decades. PFBS was detected in outdoor textiles (level not provided), carpet samples (up to 26.8 μg/m^2), ski wax samples (up to 3.1 micrograms per kilogram [μg/kg]), leather samples (up to 120 μg/kg), and gloves (up to 2 μg/kg). Favreau et al. (2016) analyzed the occurrence of 41 PFAS in a wide variety of liquid products (n = 132 consumer products, 194 total products), including impregnating agents, lubricants, cleansers, polishes, AFFFs, and other industrial products purchased from stores and supermarkets in Switzerland. PFBS was not detected in impregnation products (n = 60), cleansers (n = 24), or polishes (n = 18). PFBS was detected in 13% of a miscellaneous category of products (n = 23) that included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances, with mean and maximum concentrations of 998 and 2,992 parts per million (ppm), respectively (median = ND).

The remaining two European studies from Norway (Vestergren et al., 2015) and Sweden (Schultes et al., 2018) did not detect PFBS in the consumer products analyzed. Vestergren et al. (2015) analyzed furniture textile, carpet, and clothing samples (n = 40) purchased from retail stores in Tromsø and Trondheim, Norway, while Schultes et al. (2018) determined levels of 39 PFAS in 31 cosmetic products collected in Sweden. Both studies found measurable concentrations of at least one PFAS; however, PFBS was not detected in any of the samples.

Of the two studies for which purchase location(s) were not specified, Gremmel et al. (2016) determined levels of 23 PFAS in 16 new outdoor jackets since it has been shown that outdoor jackets emit PFAS to the air as well as into water during washing. The jackets were selected based on factors such as fabric and origin of production (primarily Asia, with some origins not specified). PFBS (concentration of 0.51 μg/m^2) was only detected in one large hardshell jacket made of 100% polyester that was polyurethane-coated and finished with Teflon® (production origin unknown). Bečanová et al. (2016) analyzed 126 samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment (EEE), and plastics); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, facade materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) for 15 target PFAS, including PFBS. The condition (new versus used) and production year of the samples varied; the production year ranged from 1981 to 2010. The origin(s) of production were not specified. PFBS was detected in 31/55, 9/54, 7/10, and 6/7 household equipment, building materials, car interior, and WEEE samples, respectively. The highest level was 11.4 μg/kg found in a used 1999 screen associated with WEEE.

PFBS was also evaluated in AFFFs in Switzerland (Favreau et al., 2016) and Norway (Høisæter et al., 2019). In currently commercially available AFFFs from Switzerland, PFBS was detected in 11% of samples (n = 35) with a maximum concentration of 0.1 ppm (Favreau et al., 2016). In AFFFs used at a firefighting training facility in Norway, PFAS concentrations in 1:100 diluted AFFF were predominately PFOS (88.7%). PFBS contributed to 1.2% of the concentration of the 23 total PFAS tested in the diluted foam, with a concentration of 1,400,000 ng/L (Høisæter et al., 2019).

In summary, in the few studies available from North America and Europe, PFBS was detected in a wide range of consumer products including clothing, household textiles and products, children's products, and commercial/industrial products. However, there is some uncertainty in these results as the number and types of products tested in each study were often limited in terms

of sample size. While there is evidence indicating PFBS exposure may occur through the use of or contact with consumer products, more research is needed to understand the DF and concentrations of PFBS that occur in specific products, as well as how the concentrations of PFBS change in these products with age or weathering.

3.3.1.3 Indoor Dust

Dust ingestion may be an important exposure source of PFAS including PFBS (ATSDR, 2021), though it should be noted that dust exposure may also occur via inhalation and dermal routes. Eighteen studies conducted in the United States, Canada, various countries in Europe, and across multiple continents analyzed PFBS in dust of indoor environments (primarily in homes, but also schools, childcare facilities, offices, and vehicles; see Table B-5). Most of the studies sampled dust from areas not associated with any known PFAS activity or release. PFBS concentrations in dust measured in these studies ranged from ND to 170 ng/g with three exceptions: two studies (Kato et al., 2009; Strynar and Lindstrom, 2008) reported maximum PFBS concentrations > 1,000 ng/g in dust from homes and daycare centers, and a third study (Huber et al., 2011) reported a PFBS concentration of 1,089 ng/g in dust from a storage room that had been used to store "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years."

Of the two available studies that measured PFBS in dust from vehicles, one (in the United States) detected no PFBS (Fraser et al., 2013) and the other (in Ireland) reported a DF of 75% and PFBS concentrations ranging from ND to 170 ng/g (Harrad et al., 2019).

One U.S. study, Scher et al. (2019) evaluated indoor dust from 19 homes in Minnesota within a GCA impacted by the former 3M PFAS production facility. House dust samples were collected from both interior living rooms and entryways to the yard. The DFs for PFBS were 16% and 11% for living rooms and entryways, respectively, and a maximum PFBS concentration of 58 ng/g was reported for both locations.

Haug et al. (2011) indicated that house dust concentrations are likely influenced by a number of factors related to the building (e.g., size, age, floor space, flooring type, ventilation); the residents or occupants (e.g., number of people, housekeeping practices, consumer habits such as buying new or used products); and the presence and use of certain products (e.g., carpeting, carpet or furniture stain-protective coatings, waterproofing sprays, cleaning agents, kitchen utensils, clothing, shoes, cosmetics, insecticides, electronic devices). In addition, the extent and use of the products affects the distribution patterns of PFAS in dust of these buildings.

At this time, there is uncertainty regarding the extent of human exposure to PFBS through indoor dust compared with other exposure pathways.

3.3.1.4 Air

PFAS have been released to air from WWTPs, waste incinerators, and landfills (U.S. EPA, 2016a). ATSDR (2021) noted that PFAS have been detected in particulates and in the vapor phase in air and can be transported long distances via the atmosphere; they have been detected at low concentrations in areas as remote as the Arctic and ocean waters. However, EPA's Toxic Release Inventory did not report release data for PFBS in 2020 (U.S. EPA, 2022a). In addition, PFBS is not listed as a hazardous air pollutant (U.S. EPA, 2022b).

Indoor Air

Three studies in Europe, conducted in Norway (Barber et al., 2007), Spain (Jogsten et al., 2012), and Ireland (Harrad et al., 2019), analyzed the occurrence of PFBS in indoor air samples.

In Norway, neutral and ionic PFAS were analyzed in four indoor air samples collected from homes in Tromsø (Barber et al., 2007). PFBS levels were below the limit of quantitation. The authors noted that measurable amounts of other ionic PFAS were found in indoor air samples, but levels were not significantly elevated above levels in outdoor air. In Spain, Jogsten et al. (2012) collected indoor air samples (n = 10) from selected homes in Catalonia and evaluated levels of 27 perfluorinated chemicals (PFCs). PFBS was not detected; PFOS and PFBA were the only detected PFCs in these indoor air samples.

In Ireland, Harrad et al. (2019) measured eight target PFAS in air from cars (n = 31), home living rooms (n = 34), offices (n = 34), and school classrooms (n = 28). PFBS was detected in all four indoor microenvironments, at DFs of 53%, 90%, 41%, and 54% in samples from homes, cars, offices, and classrooms, respectively. The mean (maximum) concentrations were 22 (270) picograms per cubic meter (pg/m^3) in homes, 54 (264) pg/m^3 in cars, 37 (313) pg/m^3 in offices, and 36 (202) pg/m^3 in classrooms.

There is some evidence from European studies indicating PFBS exposure via indoor air. However, further research is needed to understand the DF and concentrations of PFBS that occur in indoor environments in the United States.

Ambient Air

Four studies conducted across Europe (Barber et al., 2007; Beser et al., 2011; Harrad et al., 2020; Jogsten et al., 2012) and one study conducted in Canada (Ahrens et al., 2011) analyzed ambient air samples for PFBS. Two of the studies (Barber et al., 2007; Harrad et al., 2020) found detectable levels of PFBS in outdoor air. Barber et al. (2007) collected air samples from four field sites in Europe (one semirural site [Hazelrigg] and one urban site [Manchester] in the United Kingdom, one rural site from Ireland, and one rural site from Norway) for analysis of neutral and ionic PFAS. Authors did not indicate whether any of the sites had a history of PFAS impact. PFBS was detected in the particle phase of outdoor air samples during one of the two sampling events in Manchester at 2.2 pg/m^3 and one of the two sampling events in Hazelrigg at 2.6 pg/m^3. PFBS was not detected above the method quantification limit at the Ireland and Norway sites. Harrad et al. (2020) measured PFBS in air near 10 Irish municipal solid waste landfills located in non-industrial areas. Air samples were collected upwind and downwind of each landfill. PFBS was detected in more than 20% of the samples, with mean concentrations (ranges) at downwind and upwind locations of 0.50 (< 0.15–1.4) pg/m^3 and 0.34 (< 0.15–1.2) pg/m^3, respectively. Beser et al. (2011) and Jogsten et al. (2012) did not detect PFBS in ambient air samples in Spain. Beser et al. (2011) analyzed fine airborne particulate matter ($PM_{2.5}$) in air samples collected from five stations located in Alicante province, Spain (3 residential, 1 rural, 1 industrial) to determine levels of 12 ionic PFAS. PFBS was below the method quantification limit at all five locations. Jogsten et al. (2012) did not detect PFBS in ambient air samples collected outside homes in Catalonia, Spain.

In the one study identified from North America, Ahrens et al. (2011) determined levels of PFAS in air around a WWTP and two landfill sites in Canada. PFBS was not detected in any sample above the method detection limit.

PFBS has been detected in Artic air in one study, with a DF of 66% and mean concentration of 0.1 pg/m^3 (Arp and Slinde, 2018; Wong et al., 2018).

As with exposure to PFBS via indoor air, there is some evidence from European studies indicating PFBS is present in some ambient air samples. Further research is needed to understand the DF and concentrations of PFBS that occur in ambient environments in the United States.

3.3.1.5 Soil

PFBS can be released into soil from manufacturing facilities, industrial uses, fire/crash training sites, and biosolids containing PFBS (ATSDR, 2021, U.S. EPA, 2021a). EPA identified 16 studies that evaluated the occurrence of PFBS and other PFAS in soil, with studies conducted in the United States, Canada, and Europe (see Table B-6). Two U.S. studies and two Canadian studies (Blaine et al., 2013; Cabrerizo et al., 2018; Dreyer et al., 2012; Venkatesan and Halden, 2014) were conducted in areas not reported to be associated with any known PFAS release or were experimental studies conducted at research facilities. At these sites, PFBS levels were low (≤ 0.10 ng/g) or below detection limits in non-amended or control soils. Two U.S. studies by Scher et al. (2018, 2019) evaluated soils at homes in Minnesota within and outside of a GCA impacted by a former 3M PFAS production facility; for sites within the GCA, one of the studies reported a DF of 10% and a 90th percentile PFBS concentration of 0.02 ng/g, and the other reported a DF of 9% and a maximum PFBS concentration of 0.017 ng/g. For sites outside of the GCA, the DF was 17% and the maximum PFBS concentration was 0.031 ng/g. Three U.S. studies and one Canadian study analyzed soils potentially impacted by AFFF used to fight fires—one at U.S. Air Force installations with historic AFFF use (Anderson et al., 2016), two at former fire training sites (Eberle et al., 2017; Nickerson et al., 2020), and another at the site of a train derailment and fire in Canada (Mejia-Avendaño et al., 2017). In these four studies, DFs ranged from 35 to 100%. PFBS concentrations in the study of the U.S. Air Force installations ranged from ND–79 ng/g, and PFBS concentrations ranged from ND–58.44 ng/g at one fire training site (Nickerson et al., 2020). The study of the other fire training site measured PFBS pre-treatment (0.61–0.6.4 ng/g) and post-treatment (0.07–0.83 ng/g) (Eberle et al., 2017). The DFs and range of PFBS concentrations measured in soils at the site of the train derailment were 75% DF and ND–3.15 ng/g, respectively, for the AFFF run-off area (measured in 2013, the year of accident) and 36% DF and ND–1.25 ng/g, respectively, at the burn site and adjacent area (measured in 2015) (Mejia-Avendaño et al., 2017).

Of the six European studies, one study (Harrad et al., 2020) analyzed soil samples collected upwind and downwind of 10 municipal solid waste landfills in Ireland and found PFBS levels to be higher in soils from downwind locations. Based on the overall study findings, however, the authors concluded there was no discernible impact of the landfills on concentrations of PFAS in soil surrounding these facilities. Grønnestad et al. (2019) investigated soils from a skiing area in Norway to elucidate exposure routes of PFAS into the environment from ski products, such as ski waxes. The authors found no significant difference in mean total PFAS in soil samples from the Granåsen skiing area and the Jonsvatnet reference area but noted that the skiing area samples were dominated by long-chain PFAS (C8–C14; ≥ 70%) and the reference area samples were

dominated by short-chain PFAS (> 60%), which included PFBS. A study in Belgium (Groffen et al., 2019) evaluated soils collected at a 3M fluorochemical plant in Antwerp and at four sites located at increasing distances from the plant. PFBS levels were elevated at the plant site and decreased with increasing distance from the plant. The other three studies analyzed soil samples from areas near firefighting training sites in Norway and France, and reported PFBS concentrations varying from ND to 101 ng/g dry weight (Dauchy et al., 2019; Hale et al., 2017; Skaar et al., 2019).

A U.S. study of biosolid samples from 94 WWTPs across 32 states and the District of Columbia detected PFBS in 60% of samples at a mean concentration (range) of 3.4 (2.5–4.8) ng/g (Venkatesan and Halden, 2013). As mentioned, PFBS has been detected in drinking water wells, food types, and plant samples from soils or fields that have received biosolids applications that were industrially impacted (Blaine et al., 2013, 2014; Lindstrom et al., 2011).

In summary, results of some available studies suggest that proximity to a PFAS production facility or a site with historical AFFF use or firefighting is correlated with increased PFBS soil concentrations compared to soil from sites not known to be impacted by PFAS. However, few available studies examined PFBS concentrations in soils not known to have nearby sources of PFBS. Additional research is needed that quantifies ambient levels of PFBS in soils in the United States.

3.3.2 RSC Determination

In summary, based on the physical properties, detected levels, and available exposure information for PFBS, multiple non-drinking water sources (seafood [including fish and shellfish]) and other foods including vegetables, indoor air, and some consumer products) are potentially significant exposure sources. Following the Exposure Decision Tree within EPA's 2000 *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a), significant potential sources other than drinking water ingestion were identified (Box 8A in the Decision Tree). However, information is not available to quantitatively characterize the relative exposure contributions from the non-drinking water sources (Box 8B in the Decision Tree, U.S. EPA, 2000a).

EPA also considered the exposure information specifically for the identified sensitive population. The identified sensitive lifestage, based on the critical study and effect, is women of childbearing age (13 to <50 years) who may be or become pregnant. However, the literature search did not identify non-drinking water exposure information specific to women of childbearing age that could be used quantitatively to derive an RSC. Since neither the available data for the general population (all ages) nor the sensitive population enabled quantitative characterization of relative exposure sources and routes, EPA relied on an RSC of 20% (see Section 2.2.4.2 above; U.S. EPA, 2000a), which means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential exposure sources such as food, indoor air, and some consumer products.

4.0 Lifetime Noncancer Health Advisory Derivation

The lifetime noncancer HA for PFBS is calculated as follows:

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 3)}$$

$$\textbf{Lifetime HA} = \left(\frac{0.0003 \ \frac{\text{mg}}{\text{kg bw-day}}}{0.0354 \frac{\text{L}}{\text{kg bw-day}}}\right) * 0.2$$

$$\textbf{Lifetime HA} = 0.0017 \ \frac{\text{mg}}{\text{L}} \left(\text{rounded to } 0.002 \ \frac{\text{mg}}{\text{L}}\right)$$

$$= 2 \ \frac{\mu\text{g}}{\text{L}}$$

$$= 2{,}000 \ \frac{\text{ng}}{\text{L}}$$

EPA is issuing a lifetime noncancer drinking water HA for PFBS of 2,000 ng/L (ppt). The critical health effect on which the chronic RfD used to calculate the lifetime HA is based (i.e., decreased serum levels of the T_4 in newborn mice) resulted from PFBS exposure during a developmental life stage. In Feng et al. (2017), developmental effects occurred at PND 1 and were sustained through pubertal (PND 30) and adult periods (PND 60). This is consistent with the potential for long-term health consequences of gestational-only PFBS exposure and suggests that gestation is at least one critical window for PFBS. EPA's risk assessment guidelines for developmental toxicity indicate that adverse effects can result from even brief exposure during a critical period of development (U.S. EPA, 1991). Therefore, the lifetime HA for PFBS of 2000 ng/L and the chronic RfD from which it is derived are considered applicable to short-term PFBS exposure scenarios (including during pregnancy) as well as lifetime exposure scenarios via drinking water. This lifetime HA applies to PFBS (CASRN 375-73-5), K^+PFBS (CASRN 29420-49-3), and PFBS– (CASRN 45187-15-3).

5.0 Analytical Methods

EPA developed two liquid chromatography/tandem mass spectrometry (LC/MS/MS) analytical methods to quantitatively monitor drinking water for targeted PFAS that include PFBS: EPA Method 533 (U.S. EPA, 2019b) and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020b). The methods discussed below can be used to accurately and reasonably quantitate PFBS at ng/L levels that are three orders of magnitude below the PFBS lifetime HA of 2000 ng/L.

EPA Method 533 monitors for 25 select PFAS with published measurement accuracy and precision data for PFBS in reagent water, finished groundwater, and finished surface water and a single laboratory-derived MRL or approximate quantitation limit for PFBS at 3.5 ng/L (0.0035 µg/L). For further details about the procedures for this analytical method, please see *Method*

533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (U.S. EPA, 2019b).

EPA Method 537.1 (representing an update to EPA Method 537 [U.S. EPA, 2009b]) monitors for 18 select PFAS with published measurement accuracy and precision data for PFBS in reagent water, finished groundwater, and finished surface water and a single laboratory-derived MRL or approximate quantitation limit for PFBS at 6.3 ng/L (0.0063 µg/L). For further details about the procedures for this analytical method, please see *Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)* (U.S. EPA, 2020b).

Drinking water analytical laboratories have different performance capabilities dependent upon their instrumentation (manufacturer, age, usage, routine maintenance, operating configuration, etc.) and analyst experience. Some laboratories will effectively generate accurate, precise, quantifiable results at lower concentrations than others. Organizations leading efforts that include the collection of data need to establish data quality objectives (DQOs) to meet the needs of their program. These DQOs should consider establishing reasonable quantitation limits that laboratories can routinely meet, without recurring quality control (QC) failures that will necessitate repeating sample analyses, increase costs, and potentially reduce laboratory capacity. Establishing a quantitation limit that is too high may result in important lower-concentration results being overlooked.

EPA's approach to establishing DQOs within the UCMR program serves as an example. EPA established MRLs for UCMR 5,[15] and requires laboratories approved to analyze UCMR samples to demonstrate that they can make quality measurements at or below the established MRLs. EPA calculated the UCMR 5 MRLs using quantitation-limit data from multiple laboratories participating in an MRL-setting study. The laboratories' quantitation limits represent their lowest concentration for which future recovery is expected, with 99% confidence, to be between 50 and 150%. The UCMR 5-derived and promulgated MRL for PFBS is 0.003 µg/L (3 ng/L).

6.0 Treatment Technologies

This section summarizes available drinking water treatment technologies that have been demonstrated to remove PFBS from drinking water, but it is not meant to provide specific operational guidance or design criteria. High-pressure membrane processes such as nanofiltration (NF) and reverse osmosis (RO) are generally effective at removing organic solutes and dissolved ions and have been shown to successfully reduce or remove PFBS from drinking water (Appleman et al., 2014). NF generally removes 20–70% of PFBS (Jin et al., 2021), although 93% (Appleman et al., 2013) and 99.8% (U.S. EPA, 2021e) removal have been reported with NF. The amount of contaminant removed by membranes is referred to as a rejection rate; RO tends to have a higher rejection rate than NF. Direct filtration NF and RO membranes have been successful in removing PFBS at full-scale water treatment works to below the 3 ppt EPA UCMR 5 reporting limit (Appleman et al., 2014; Konradt et al., 2021; Liu et al., 2021; Quiñones and Snyder, 2009; Thompson et al., 2011). Absorption-based NF and RO membranes have had

[15] Information about UCMR 5 is available at https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule

success with PFBS treatment at laboratory scale (Zhang et al., 2019). Hybrid membrane processes, such as applying direct-current electrical fields or photocatalysts across lower pressure membranes, have had success with other short-chain sulfonates at laboratory scale (Tsai et al., 2010; Urtiaga, 2021). For more information about hybrid membrane processes, see Soriano et al. (2020) or (2017). Installing high-pressure membranes may have additional benefits on finished water quality by removing other contaminants and disinfection byproduct precursors. Sorption-based processes such as activated carbon and ion exchange have been shown to remove PFBS in drinking water to below the EPA method reporting limit of 3 ppt for UCMR 5; however, the media usage rate is higher than for other PFAS with longer carbon backbones (McCleaf et al., 2017; Murray et al., 2021). Information about PFBS treatment efficacy with sorption-based processes is still emerging; more information about the suitability of these technologies is expected to be available in the future. Most other treatment processes are viewed as not sufficiently effective or cost efficient to reduce PFBS concentrations in drinking water. For example, coagulation, flocculation, sedimentation, and biologically active carbon filtration are generally ineffective at removing PFBS (Quiñones and Snyder, 2009; Sun et al. 2016). Ozonation has increased concentrations of PFBS at full-scale water treatment plants (WTPs), possibly due to PFAS precursor compound oxidation (Sun et al., 2016). Boiling water will concentrate PFBS and should not be considered as an emergency action.

Non-treatment PFBS management practices such as changing source waters, source water protection, or consolidation are also viable options for reducing PFBS concentrations in finished drinking water. One resource for protecting source water from PFAS, including PFBS, is the *PFAS − Source water Protection Guide and Toolkit* (ASDWA, 2020), which shares effective strategies for addressing PFAS contamination risk in source waters. Source water protection is particularly important since natural attenuation is not a valid PFBS management strategy. PFBS will not degrade by abiotic reaction mechanisms such as hydrolysis and photolysis under environmental conditions (Lassen et al., 2013; NICNAS, 2005). Likewise, Quinete et al. (2010) studied biotic PFBS degradability using the manometric respirometry test (OECD, 1992b) and the closed-bottle test (OECD, 1992a) with River Rhine water as inoculum; PFBS did not show signs of biodegradation in either test.

NF and RO are high pressure processes where water is forced across a membrane. The water that transverses the membrane is known as permeate or produce, and has few solutes left in it; the remaining water is known as concentrate, brine, retentate, or reject water and forms a waste stream with concentrated solutes. The main PFBS removal mechanisms in NF and RO are steric exclusion, solution-diffusion, and electrostatic interaction (Jin et al., 2021). NF has a less dense active layer than RO, which enables lower operating pressures but also makes it less effective at removing contaminants. Higher operating pressures and initial flux generally enhance removal. Temperature and pH are also significant parameters affecting performance. In general, organic NF membranes have lower operating costs and easier processing than inorganic membranes while maintaining appropriate robustness for PFBS treatment (Jin et al., 2021). NF and RO tend to have high operating expenses, use significant amounts of energy, and generate concentrate waste streams which require disposal. Generally, NF and RO require pre- and posttreatment processes.

PFBS removal fluxes are generally around 40 liters per square meter per hour (L/[m^2·hr]) at about 0.7 megapascal (MPa) operating pressure (Wang et al., 2018). Temperature can

dramatically impact flux; it is common to normalize flux to a specific reference temperature for operational purposes (U.S. EPA, 2005c). It is also common to normalize flux to pressure ratios to identify productivity changes attributable to fouling (U.S. EPA, 2005c). It is important to note that water may traverse the membranes from outside-in or inside-out; different system configurations operating at the same flux produce differing quantities of finished water. This means that membrane systems with differing configurations cannot be directly compared based on flux. Total flow per module and cost per module are more important decision support indicators for capital planning.

High-pressure membranes may have effects when added onto a well-functioning treatment train. For instance, high-pressure membranes may remove beneficial minerals and increase corrosivity. Increased water corrosivity may need to be addressed through corrosion treatment modifications and water may require mineralization. For more information, see AWWA (2007).

6.1 Point-of-Use Devices for Individual Household PFBS Removal

Although the focus of this section is the different available options for removal of PFBS at DWTPs, centralized treatment technologies can also be used in a decentralized fashion as point-of-entry (POE) (where the distribution system meets a service connection) or point-of-use (POU) (at a specific tap or application) treatment in cases where centralized treatment is impractical or individual consumers wish to further reduce their individual household risks. Many home drinking water treatment units are certified by independent third-party accreditation organizations using American National Standards Institute (ANSI) standards to verify contaminant removal claims. NSF International has developed a protocol for NSF/ANSI Standard 58 (RO) that establishes minimum requirements for materials, design, construction, and performance of POU systems (NSF/ANSI, 2021). Currently, these standards provide certification procedures for PFOA and PFOS removal in drinking water to below EPA's 2016 PFOA and PFOS HA level of 70 ppt. When properly maintained, these systems may reduce other PFAS, including PFBS, although removal should not be automatically inferred for PFAS not specified within the protocol. PFBS removal by faucet filters has reportedly averaged 94%, whereas pitcher filters had an average of 65% removal, refrigerator filters 29%, single-stage under-sink filters 84%, two-stage filters > 92%, and RO filters 94% (Herkert et al., 2020). PFBS specific certification procedures may be developed in the future by voluntary consensus standards organizations. Individuals interested in POU or POE treatment should check with the manufacturers of these devices as to whether they have been independently certified for the reduction of PFBS levels in drinking water.

6.2 Treatment Technologies Summary

Non-treatment PFBS management options, such as changing source waters, source water protection, or consolidation, are viable strategies for reducing PFBS concentrations in finished drinking water. Should treatment be necessary, NF along with RO are the best means for removing PFBS from drinking water and can be used in central treatment plants or in POU/POE applications. Sorption processes such as activated carbon or ion exchange may successfully remove PFBS, but with lower efficacy than PFAS with a longer carbon backbone such as PFOS. PFBS treatment technologies often require pre- as well as post-treatment and may help remove other unwanted contaminants and disinfection byproduct precursors. These treatment processes are separation technologies and produce waste streams with PFBS on or in them.

7.0 Consideration of Noncancer Health Risks from PFAS Mixtures

EPA recently released a *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (U.S. EPA, 2021f) that is currently undergoing Science Advisory Board (SAB) review. That draft document describes a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on current, available EPA chemical mixtures approaches and methods (U.S. EPA, 2000b). Examples are presented for three approaches—Hazard Index (HI), Relative Potency Factor (RPF), and Mixture BMD—to demonstrate application to PFAS mixtures. To use these approaches, specific input values and information for each PFAS are needed or can be developed. These approaches may help to inform PFAS evaluation(s) by federal, state, and tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential noncancer human health hazards and risks associated with PFAS mixtures.

The HI approach, for example, could be used to assess the potential noncancer risk of a mixture of four component PFAS for which HAs, either final or interim (iHA), are available from EPA (PFOA, PFOS, GenX chemicals [hexafluoropropylene oxide dimer acid and its ammonium salt], and PFBS). In the HI approach described in the draft framework (U.S. EPA 2021f), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (U.S. EPA, 1986). The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Eq. 6). In the context of PFAS in drinking water, a mixture PFAS HI can be calculated when health-based water concentrations (e.g., HAs, Maximum Contaminant Level Goals [MCLGs]) for a set of PFAS are available or can be calculated. In this example, HQs are calculated by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HA (e.g., expressed as ng/L) (Eqs. 7, 8). The component chemical HQs are then summed across the PFAS mixture to yield the mixture PFAS HIs based on interim and final HAs.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

(Eq. 6)

$$HI = HQ_{PFOA} + HQ_{PFOS} + HQ_{GenX} + HQ_{PFBS}$$

(Eq. 7)

$$HI = \left(\frac{[PFOA_{water}]}{[PFOA_{iHA}]}\right) + \left(\frac{[PFOS_{water}]}{[PFOS_{iHA}]}\right) + \left(\frac{[GenX_{water}]}{[GenX_{HA}]}\right) + \left(\frac{[PFBS_{water}]}{[PFBS_{HA}]}\right)$$

(Eq. 8)

Where:
HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS

RfV = human health-based toxicity value for component (i) PFAS
HQ_{PFAS} = hazard quotient for a given PFAS
$[PFAS_{water}]$ = concentration of a given PFAS in water
$[PFAS_{HA}]$ = HA value, interim or final, for a given PFAS

In cases when the mixture PFAS HI is greater than 1, this indicates an exceedance of the health protective level and indicates potential human health risk for noncancer effects from the PFAS mixture in water. When component health-based water concentrations (in this case, HAs) are below the analytical method detection limit, as is the case for PFOA and PFOS, such individual component HQs exceed 1, meaning that any detectable level of those component PFAS will result in an HI greater than 1 for the whole mixture. Further analysis could provide a refined assessment of the potential for health effects associated with the individual PFAS and their contributions to the potential joint toxicity associated with the mixture. For more details of the approach and illustrative examples of the RPF approach and Mixture BMD approaches please see U.S. EPA (2021f).

8.0 Health Advisory Characterization

EPA is issuing a lifetime noncancer drinking water HA for PFBS of 2,000 ng/L or 2,000 ppt based on the best available science. This is the first HA for PFBS. The PFBS HA is considered applicable to both short-term and chronic risk assessment scenarios because the critical effect identified for PFBS can result from developmental exposure and leads to long-term adverse health effects (Feng et al., 2017). The input values for the HA include 1) the chronic RfD which was developed in the toxicity assessment for PFBS (U.S. EPA 2021a); 2) the RSC based on exposure information collected from a literature search and following EPA's Exposure Decision Tree (U.S. EPA, 2000a) and presented herein; and 3) the DWI-BW, described herein, selected for the sensitive population or lifestage. The PFBS toxicity assessment was published after rigorous scientific review, including internal and external review, and public comment.

Some of the uncertainties associated with the PFBS lifetime noncancer HA are due to data gaps. The PFBS toxicity assessment, which was the basis for the chronic RfD used to derive the HA, performed a systematic literature search and identified a limited number of studies examining health effects after PFBS exposure (U.S. EPA, 2021a). The toxicity assessment literature search did not identify available chronic studies or cancer studies for PFBS. Only a small number of human studies per health outcome category were identified. The identified animal studies of repeated-dose PFBS exposure used K^+PFBS as the tested substance and only examined noncancer effects. Further, since neurodevelopmental effects are of particular concern when perturbations in thyroid hormone occur during development, studies evaluating neurodevelopmental effects following PFBS exposure during development are needed (U.S. EPA, 2021a). Mechanistic studies were assessed as part of the systematic literature review but mechanism(s) of toxicity for PFBS for the various health outcomes have not been established.

Based on the data gaps and limitations described above, there is some uncertainty about whether the most sensitive population or life stage for PFBS exposure has been identified. Results of the literature search for information that could inform RSC determination for PFBS indicate that there is significant exposure from media other than drinking water, but the available data do not allow for quantitative characterization of the contributions of non-drinking water exposures. This

final HA is based on a recent toxicity assessment and recent literature searches of the publicly available scientific information regarding health effects, exposure, analytical methods, and treatment technologies for PFBS.

8.1 Comparative Analysis of Exposure Factors for Different Populations

The exposure duration in the critical study identified for PFBS in the toxicity assessment (U.S. EPA, 2021a) is throughout gestation which suggests that pregnant women and their developing embryo and fetus represent a sensitive life stage. In addition to drinking water exposure to pregnant women (and their developing embryo and fetus), the gestational exposure window is relevant to drinking water exposure to women of childbearing age (13 to < 50 years) who may be or become pregnant (Table 5).

EPA compared the impact of using the DWI-BW for the 90th percentile for the general population (all ages) with the DWI-BWs for the potentially sensitive populations identified, women of childbearing age and pregnant women on the HA value (Table 6). All three HA values are the same when rounded to one significant figure (i.e., all are 0.002 ppm). This indicates that the lifetime noncancer HA developed for PFBS based on the selected DWI-BW for women of childbearing age is protective of the 90th percentile of all ages of the general population.

Table 6. Comparison of HA Values Using EPA Exposure Factors for Drinking Water Intake for Different Candidate Populations

Population	DWI-BW (L/kg bw-day)	HA calculated/HA rounded to one significant figure	Description of Exposure Metric	Source
Pregnant women	0.0333	0.00180/ 0.002 ppm	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Women of childbearing age	**0.0354**	0.00169/ 0.002 ppm	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	
General population, all ages	0.0338	0.00177/ 0.002 ppm	90th percentile direct and indirect consumption of community water, consumer-only two-day average, all ages.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day. The DWI-BW used to calculate the PFBS lifetime HA is in bold.

9.0 References

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Appendix A: Relative Source Contribution – Literature Search and Screening Methodology

Information on all exposure sources and routes for perfluorobutane sulfonic acid (PFBS)was gathered through a literature search in a manner consistent with the Office of Science and Technology's (OST's) process the collection of information for relative source contribution (RSC) derivation. In this process, a literature search of both the peer reviewed and gray literature for the chemical of interest was conducted. All of the primary studies that were identified from the search are final documents or articles.

In 2020, U.S. Environmental Protection Agency's (EPA's) Office of Research and Development (ORD) conducted a broad literature search to evaluate evidence for pathways of human exposure to eight per- and polyfluoroalkyl substances (PFAS): perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA). This search was not date limited and spanned the information collected across the Web of Science, PubMed, and ToxNet/ToxLine (now ProQuest) databases. The results of the PFBS literature search of publicly available sources are available through EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2610.

The 654 literature search results for PFBS were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and non-human (i.e., those not identified as human) studies. Human studies were further categorized into seven major PFAS pathway categories (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) plus an additional category for Human Exposure Measures. Non-human studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill. Only studies published between 2003 and 2020 were considered. Application of the SWIFT-Review tags identified 343 peer-reviewed papers matching these criteria for PFBS.

After this 2020 literature search was conducted, the 343 articles were screened to identify studies reporting measured occurrence of PFBS in human matrices and media commonly related to human exposure (human blood/serum/urine, drinking water, food, food contact materials, consumer products, indoor dust, indoor and ambient air, and soil). For this synthesis, additional screening was conducted to identify studies relevant to surface water (freshwater only) and groundwater using a keyword[16] search for water terms.

Following the Populations, Exposures, Comparators, and Outcomes (PECO) inclusion criteria outlined in Table A-1, the title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree if a study should be included or excluded, a third reviewer made a final decision. The title and abstract screening of and of this synthesis resulted in 191 unique studies being tagged as relevant

[16] Keyword list: water, aquifer, direct water, freshwater, fresh water, groundwater, groundwater, indirect water, lake, meltwater, melt water, natural water, overland flow, recreation water, recreational water, river, riverine water, riverwater, river water, springwater, spring water, stream, surface water, total water, water supply

(i.e., having data on occurrence of PFBS in exposure media of interest) that were further screened with full-text review using the same inclusion criteria. After additional review of the evidence collected by ORD, 87 studies originally identified for other PFAS also contained information relevant to PFBS. Based on full-text review, 147 studies were identified as having relevant, extractable data for PFBS from the United States, Canada, or Europe for environmental media, not including studies with only human biomonitoring data. Of these 147 studies, 130 were identified from the ORD literature search, where primary data were extracted into a comprehensive evidence database. Parameters of interest included sampling dates and locations, numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics. Seventeen of the 147 studies were identified in this synthesis as containing primary data on only surface water and/or groundwater.

Table A-1. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults and/or children in the general and impacted populations from the United States, Canada, or Europe
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater[a], human blood/serum/urine, indoor air, landfill, sediment, soil, surface water[a] (freshwater), wastewater/biosolids/sludge
Comparator	Not applicable
Outcome	Measured concentrations of PFBS (or measured emissions from food packaging and consumer products only)

Note:
[a] Surface water and groundwater were not included as relevant media in ORD's literature search. Studies were re-screened for these two media in this synthesis.

The evidence database additionally identified 18 studies for which the main article was not available for review. As part of this synthesis, 17 of the 18 studies could be retrieved. An additional three references were identified through gray literature sources that were included to supplement the search results. The combined 20 studies underwent full-text screening using the inclusion criteria in Table A-1. Based on full-text review, four studies were identified as relevant.

Using the screening results from the evidence database and this synthesis, a total of 151 studies were identified as relevant and are summarized below.

To supplement the primary literature database, EPA also searched the following gray literature sources for information related to relative exposure of PFBS for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- U.S. EPA. 2021a. *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3).*
- ATSDR's *Toxicological Profiles*

- Centers for Disease Control's national reports on human exposures to environmental chemicals
- EPA's CompTox Chemicals Dashboard
- EPA's fish tissue studies
- EPA's Toxics Release Inventory
- EPA's Unregulated Contaminant Monitoring Rule (UCMR) data
- Relevant documents submitted under Toxic Substances Control Act (TSCA) and relevant reports from U.S. EPA's Office of Chemical Safety and Pollution Prevention
- FDA's *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada
- National Oceanic and Atmospheric Administration's National Centers for Coastal Ocean Science data collections
- National Science Foundation direct and indirect food and/or certified drinking water additives
- PubChem compound summaries
- Relevant sources identified in the RSC discussions (section 5) of EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA)/Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water*
- Additional sources, as needed

EPA has included available information from these gray literature sources for PFBS relevant to its uses, chemical and physical properties, and for occurrence in drinking water (directly or indirectly in beverages like coffee, tea, commercial beverages, or soup), ambient air, foods (including fish and shellfish), incidental soil/dust ingestion, and consumer products. EPA has also included available information specific to PFBS on any regulations that may restrict PFBS levels in media (e.g., water quality standards, air quality standards, food tolerance levels).

Appendix B: Compilation of Data on PFBS Occurrence in Environmental Media Collected from Primary Literature

This appendix includes tables resulting from the efforts to identify and screen primary literature (i.e., peer-reviewed journal articles), described in Appendix A, as well as extract data that may be relevant to informing the RSC derivation for PFBS.

Table B-1. Compilation of Studies Describing PFBS Occurrence in Drinking Water

Study	Location	Site Details	Results
North America			
Bradley et al. (2020)	United States (Chicago, Illinois; East Chicago, Indiana)	Residential tap water (45 sites); treated, pre-distribution tap water from water filtration plants (4 sites)	Residential tap water: DF[a] 47%, range = ND–0.8 ng/L Pre-distribution tap water = DF[a] 75%, range = ND–0.5 ng/L
Hu et al. (2019)	United States (national)	Archived tap water samples (collected 1989–1990) from 225 homes of Nurses' Health Study participants (across 22 states)	DF 5%, median (range) = 0.20 (ND–2.97) ng/L
Boone et al. (2019)	United States (national)	Treated water from 25 DWTPs; some locations reportedly had known or suspected sources of wastewater in the source water, but the study did not identify which	DF 96%, median (range) = 1.17 (ND–11.9) ng/L
Dasu et al. (2017)	United States (Ohio, Kentucky)	Tap water collected in 2003–2006 from 25 homes of Health Outcomes and Measures of the Environment study participants	DF 16%, range = ND–11.7 ng/L
Subedi et al. (2015)	United States (New York)	Tap water (from outdoor taps; 27 samples) from 4 homes around Skaneateles Lake that use an enhanced treatment unit for onsite wastewater treatment	DF 7%, mean (range) = 0.44 (ND–0.48) ng/L

Study	Location	Site Details	Results
Appleman et al. (2014)	United States (Wisconsin, Oklahoma, Alaska, California, Alabama, Colorado, Ohio, Nevada, Minnesota, New Jersey)	Finished water from DWTPs where source waters were impacted by upstream wastewater effluent discharge	DF 100% (n=19), mean[a] (range) = 4.27 (0.43 - 37) ng/L
Scher et al. (2018)	United States (Twin Cities metropolitan region, Minnesota)	Tap water from exterior taps of homes near former 3M PFAS production facility; 20 homes within and 3 homes outside of the GCA (GCA defined by well monitoring conducted by Minnesota Department of Health and the Minnesota Pollution Control Agency)	Within GCA: DF 0% Outside GCA: DF 0%
Boone et al. (2014)	United States (New Orleans, Louisiana)	Tap water from one home when the river source water was at a low stage (2.95 ft) or a high stage (8.32 ft); well water samples from wells on a firefighting training site that used AFFF (3 wells sampled before carbon adsorption treatment and 1 well sampled after; number of samples collected per well not reported)	Tap water (low river stage): DF 100%, mean of primary and duplicate = 14.15 ng/L Tap water (high river stage): DF 100%, mean of 4 replicates = 2.12 ng/L Well 1: DF NR, mean = 11.9 ng/L Well 1 (after carbon adsorption treatment): DF NR, mean = 9.09 ng/L Well 2: DF NR, mean = 9.265 ng/L Well 3: DF NR, mean = 29 ng/L
Lindstrom et al. (2011)	United States (Alabama)	Samples from 6 wells used for drinking water located in areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 66%, mean (range) = 19.7 (ND–56.5) ng/L

Study	Location	Site Details	Results
Chow et al. (2021)	United States (Baltimore, Maryland metropolitan area)	101 different non-carbonated bottled water products representing 66 brands, purchased from 19 different retail food and beverage chains	DF 17%, median (range) = 0.25 (ND–1.44) ng/L
Europe			
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick counties)	Bottled water (31) from Galway city shops; tap water (private supply) from 25 homes with private water supplies; tap water (main public supply) from 34 homes and 32 offices (combined)	Bottled water: DF 29%, mean (range) = 3.7 (ND–51) ng/L Tap water (private supply): DF 0% Tap water (main public supply): DF 8%, mean (range) = 0.52 (ND–15.06) ng/L
Ünlü Endirlik et al. (2019)	Turkey (33 provinces)	Bottled water (26 samples representing 18 different brands, both plastic- and glass-bottled); municipal tap water (94 samples)	Bottled water: DF 8%, mean (range) = 0.20 (ND–0.21) ng/L Tap water: DF 87%, mean (range) = 0.29 (ND–0.85) ng/L
Ciofi et al. (2018)	Italy (Tuscany)	8 drinking water samples from various rural, urban, and industrial districts of Tuscany (origins not further described, but latitudinal and longitudinal coordinates for sampling locations were provided)	DF 0%
Le Coadou et al. (2017)	France (national)	Bottled water (25 samples of natural mineral water and 15 samples of spring water)	DF 2.5% (only one detection); single detection value (range) = 1.4 (ND–1.4) ng/L
Shafique et al. (2017)	Germany (Leipzig)	Tap water (2 samples) from one location (authors' research institute)	DF NR, mean = 1.3 ng/L

Study	Location	Site Details	Results
Filipovic and Berger (2015)	Sweden (Bollebygd, Bromma, Umeå)	Tap water from four WWTPs (4 or 5 samples from each)	Bollebygd: DF 75%, mean = 0.015 ng/L Norrvatten, Bromma: DF 100%, mean = 1.33 ng/L Stockholm Vatten, Bromma: DF 100%, mean = 1.55 ng/L Umeå: DF 100%, mean = 0.035 ng/L
Zafeiraki et al. (2015)	Greece, the Netherlands	Bottled water (5 samples each from Greece and the Netherlands); tap water samples (37 samples from the Netherlands and 43 samples from Greece)	Tap water: Greece: DF 2.3% (only one detection); single detection value (range) = 0.7 (ND–0.7) ng/L The Netherlands: DF 35%, median (range) = 7.6 (ND–13.7) ng/L Bottled water: Greece: DF 0% The Netherlands: DF 0%
Eschauzier et al. (2013)	The Netherlands (Amsterdam)	Hot water and tap water from two different locations (A and B), where A and B originated from different DWTPs; additional tap water samples (n=4) from cafes, universities, and supermarkets	Hot water A: point = 3.3 ng/L Tap water A: point = 3.2 ng/L Hot water B: point = 19 ng/L Tap water B = 16 ng/L Tap water (n=4): DF NR, mean (range) = 16 (14–17) ng/L
Gellrich et al. (2013)	Germany (Hesse, Saxony Anhalt); Switzerland; Czech Republic	Bottled water; spring water; tap water from homes	Bottled mineral water: DF 16%, median (range) = 2.6 (ND–13.3) ng/L Spring water: DF 6%, median (range) = 3.2 (ND–3.2) ng/L Tap water: DF 42%, median (range) = 2.7 (ND–5.8) ng/L
Eriksson et al. (2013)	Denmark (Faroe Islands)	Treated water from DWTPs (source water from Havnardal Lake or Kornvatn Lake)	Havnardal Lake: DF 0% Kornvatn Lake: DF 0%

Study	Location	Site Details	Results
Boiteux et al. (2012)	France (national)	Treated water from DWTPs across two sampling campaigns (41 samples in first campaign, 69 samples in second campaign)	First campaign (treated water originating from surface water): DF 46%, median = < 1 ng/L, maximum = 3 ng/L First campaign (treated water originating from groundwater): DF 40%, median = < 1 ng/L, maximum = 3 ng/L Second campaign (treated water originating from surface water): DF NR, range = ND–< 10 ng/L Second campaign (treated water originating from groundwater): DF NR, range = ND–13 ng/L
Eschauzier et al. (2012)	The Netherlands (Amsterdam)	Finished water from DWTP (n=5); tap water from 1 home	Finished water from DWTP: DF NR, mean (range) = 20 (17–24) ng/L Tap water: point = 19 ng/L
Llorca et al. (2012)	Germany, Spain	Mineral bottled water (2 samples from Germany, 4 samples from Spain); tap water (84 samples from Spain, 5 samples from Germany); well water (2 samples from Spain, 0 samples from Germany)	Bottled water (both Germany and Spain): DF 0% Tap water: Germany: DF 0% Spain: DF 35%, mean (range) = 8.3 (ND–36 ng/L) Well water (Spain): DF 0%
Ullah et al. (2011)	Belgium (Antwerp); Germany (Schmallenberg); Italy (Ispra); the Netherlands (Amsterdam); Norway (Tromsø); Sweden (Stockholm)	Tap water from seven research institutes in six European countries	Belgium: point = 2.94 ng/L Germany: point = 0.092 ng/L Italy: point = 0.502 ng/L The Netherlands: DF[a] 100%, mean[a] (range) = 13.2 (7.61–18.8) ng/L Sweden: point = 0.955 ng/L Norway: point = ND

Study	Location	Site Details	Results
Hölzer et al. (2011)	Germany (Sauerland)	Tap water (56 samples) treated from Lake Möhne, which became contaminated by perfluorocompounds through application of polluted soil conditioner to agricultural fields	DF 43%, mean (range) = 11 (ND–36) ng/L
Ericson et al. (2009)	Spain (5 regions of Catalonia)	Tap water from 40 locations identified as important supply areas	Overall: DF 73%, mean (range) = 4.52 (ND–69.43) ng/L Barcelona: DF 86%, mean (range) = 11.99 (ND–69.43) ng/L Girona: DF 57%, mean (range) = 1.13 (ND–4.91) ng/L Lleida: DF 43%, mean (range) = 0.07 (ND–0.16) ng/L Tarragona: DF 86%, mean (range) = 0.32 (ND–0.55) ng/L Terres de l'Ebre: DF 80%, mean (range) = 0.45 (ND–1.28) ng/L
Ericson et al. (2008b)	Spain (Tarragona Province)	Bottled water; municipal tap water from public fountains of most populated towns in the province	Bottled water: DF 0% Tap water: DF 0%
Pitter et al. (2020)	Italy (Veneto region)	Treated water from DWTP where its source water was contaminated by PFAS manufacturing plant	DF 89.5%, median (range) = 91.5 (ND–765.0) ng/L
Brandsma et al. (2019)	The Netherlands (Dordrecht)	Tap water from homes within 50 km of fluorochemical manufacturing plant	DF[a] 100%, range = 2.5–11 ng/L
Li et al. (2018)	Sweden (Ronneby)	Finished water from Brantafors DWTP, near AFFF-contaminated military airfield; finished water from Kärragården DWTP	Brantafors: point = 130 ng/L Kärragården: DF 0%

Study	Location	Site Details	Results
Boiteux et al. (2017)	France (northern)	Treated water from DWTPs located 15–39 km downstream of industrial WWTP that processes raw sewage from fluorochemical manufacturing facility	DF 0%
Bach et al. (2017)	France (southern region)	Treated water from two DWTPs downstream of a fluoropolymer manufacturing facility	DF 0%
Gebbink et al. (2017)	The Netherlands (Zwijndrecht, Dordrecht, Papendrecht, Sliedrecht, Utrecht, Wageningen)	Drinking water collected from city halls in municipalities close to PFAS production plant (D1−D4), at residential home in Utrecht (D5), and at the RIKILT institute in Wageningen (D6)	D1: point = 3.4 ng/L D2: point = 3.4 ng/L D3: point = 19 ng/L D4: point = 2.3 ng/L D5: point = 1.0 ng/L D6: point = 0.54 ng/L
Gyllenhammar et al. (2015)	Sweden (Uppsala)	Finished water from DWTPs; private well (Klastorp) downstream of a military airport using AFFF	Bäcklösa: DF[a] 9%, range = ND–11 ng/L Gränby: DF 0% Private well: DF 0%
Dauchy et al. (2012)	France (unspecified)	Treated water from DWTPs located 15 km downstream of fluorochemical manufacturing facility	DF 0%
Weiss et al. (2012)	Germany (Cologne)	Private well water 950 m (Well A) and 2,000 m (Well B) downstream of a fire training area; Well A is inside the contamination plume.	Well A: DF 100%, mean[a] (range) = 50 (20–100) ng/L Well B: DF[a] 86%, range = ND–20 ng/L
Multiple Continents			
Kaboré et al. (2018)	Canada (Great Lakes, St. Lawrence River)	Tap water from homes (8 sites)	DF 100%, mean (range) = 0.5 (0.3–0.8) ng/L
	Canada (rest of Canada)	Tap water from homes (11 sites); bottled water (11 brands)	Tap water: DF 73%, mean (range) = 0.1 (ND–0.5) ng/L Bottled water: DF[a] 9%, range = ND–0.23 ng/L

Study	Location	Site Details	Results
	United States (Illinois, California)	Tap water from homes (2 sites)	DF[a] 50%; ND and 0.28 ng/L
	Norway (Oslo)	Tap water from a home (1 site)	Point = 0.72 ng/L
	France (Le Mans, Paris, Guadeloupe in French West Indies)	Tap water from homes (3 sites)	DF[a] 67%, range = ND–0.32 ng/L

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; DWTP = drinking water treatment plant; ft = feet; GCA = groundwater contamination area; km = kilometer; m = meter; ND = not detected; ng/L = nanogram per liter; NR = not reported; PFAS = per- and polyfluoroalkyl substances; WWTP = wastewater treatment plant; µg/L = microgram per liter.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

Table B-2. Compilation of Studies Describing PFBS Occurrence in Groundwater

Study	Location	Site Details	Results
North America			
Lee et al. (2015)	United States (California)	Samples from 5 urban shallow groundwater wells with wastewater contamination	DF[a] 20%, range = ND–36.3 ng/L
Appleman et al. (2014)	United States (New Jersey)	Samples from 5 New Jersey groundwater source waters for PWSs impacted by upstream wastewater effluent discharge	DF[a] 100%, mean[a] (range) = 2.4 (0.43–3.7) ng/L
Post et al. (2013)	United States (New Jersey)	Raw water from 18 public drinking water system groundwater intakes	DF 6%, range = ND–6 ng/L
Steele et al. (2018)	United States (Alaska)	Military base contaminated with PFAS from AFFF use (4 wells sampled once per month for 8 months)	DF[a] NR, range = ND–48 ng/L
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, VA)	Former fire training site, site characterization and pretreatment groundwater samples	Site characterization: DF 100%, mean[a] (range) = 3,700 (1,100–13,000) ng/L (10 wells) Pretreatment: DF 100%, mean[a] (range) = 3,400 (1,200–5,000) ng/L (5 wells, 2 laboratory samples/well)

Study	Location	Site Details	Results
Anderson et al. (2016)	United States (national)	Ten active U.S. Air Force installations with historic AFFF release	DF 78.26%, median of detects (range) = 200 (ND–110,000) ng/L
Moody et al. (2003)	United States (Oscoda, MI)	Groundwater plume at former Wurtsmith Air Force Base; firefighting training area active from 1952 to 1993	DF 0%
Procopio et al. (2017)	United States (New Jersey)	Samples collected from temporary wells in a small area of an industrial/business park located within the Metedeconk River Watershed	DF 0%
Lindstrom et al. (2011)	United States (Alabama)	Samples from 13 wells used for purposes aside from drinking water (e.g., livestock, watering gardens, washing), located in areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 23%, mean (range) = 10.3 (ND–76.6) ng/L
Europe			
Barreca et al. (2020)	Italy (Lombardia region)	Groundwater sampling stations representative of region	DF 18%[a], concentrations NR
Boiteux et al. (2012)	France (national)	Raw water from 2 sampling campaigns of DWTPs, some sites possibly affected by industrial or commercial releases	DF 4%, range = ND–9 ng/L
Loos et al. (2010)	23 European countries	Monitoring stations were not necessarily representative of surrounding area or contaminated	DF 15.2%, range = ND–25 ng/L
Gobelius et al. (2018)	Sweden (national)	Sampling locations selected based on potential vicinity of PFAS hot spots and importance as a drinking water source area	DF 26%[a] (triplicate samples removed), range = ND–22 ng/L

Study	Location	Site Details	Results
Dauchy et al. (2012)	France (unspecified)	Raw water from 2 DWTPs supplied by alluvial wells; DWTPs located 15 km downstream of fluorochemical manufacturing facility	DF[a] 40%, range = ND–4 ng/L
Høisæter et al. (2019)	Norway (unspecified)	Samples from 19 sampling campaigns of 5 pumping wells placed to intercept a groundwater contamination plume originating from a firefighting training facility that ceased usage of PFAS- and fluorotelomer-based AFFF 15 years prior	Detections reported but DF and concentrations not provided
Dauchy et al. (2019)	France (unspecified)	Samples collected over 2 campaigns from 6 areas (13 monitoring wells) of a firefighter training site	DF[a] 77%, range = ND–750 ng/L
Dauchy et al. (2017)	France (unspecified)	Samples collected near 3 sites (A, C, D) impacted by the use of AFFF. Site A results describe 1 sampling location with 2 sampling events. Site C results describe a single sampling location and event. Site D results describe 5 sampling locations, each with a single sampling event	Site A: DF[a] 100% mean[a] = 8 ng/L Site C: point = 6 ng/L Site D: DF[a] 20%, range = ND–59 ng/L

Study	Location	Site Details	Results
Gyllenhammar et al. (2015)	Sweden (Uppsala)	Samples from local aquifers extracted by 21 production wells, 6 observation wells or 1 private well located in the vicinity of a potential AFFF point source (military airport). Results for all well sites were not provided.	Site 1 (production well): DF 0% (n = NR) Site 3 (observation wells): DF 100%, median = 100 ng/L (n = 3) Site 5 (observation well): DF 0% (n = NR) Site 6 (production well): DF 0% (n = NR) Site 7 (observation well): DF 100%, median = 35 ng/L (n = 3) Site 8 (production well): DF[a] 91%, median = 13 ng/L (n = 103) Site 10 (production well): DF[a] 2%, median = ND (n = 50)
Wagner et al. (2013)	Germany (unspecified)	Samples (n = 3) taken downstream from a site contaminated by AFFF from firefighting activities	DF[a] 100%, concentrations NR

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; DWTP = drinking water treatment plant; km = kilometer; ND = not detected; ng/L = nanogram per liter; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; NR = not reported; WWTP = wastewater treatment plant.

[a] The DF and/or mean was calculated using point data. Means were calculated only when DF = 100%.

Table B-3. Compilation of Studies Describing PFBS Occurrence in Surface Water

Study	Location	Site Details	PFBS Results
North America			
Yeung et al. (2017)	Canada (Ontario; Mimico Creek, Rouge River)	Two water samples at each of the sites	Mimico Creek: point = 0.020 ng/L Rouge River: DF 0%
Subedi et al. (2015)	United States (New York; Skaneateles Lake)	Lake water along the shoreline of residences that use an enhanced treatment unit for onsite wastewater treatment	DF[a] 4% (n=28); single detection value = 0.26 ng/L

Study	Location	Site Details	PFBS Results
Appleman et al. (2014)	United States (Wisconsin, Oklahoma, Alaska, California, Alabama, Colorado, Ohio, Nevada, Minnesota, New Jersey)	Raw surface waters from 11 sites, some impacted by upstream wastewater effluent discharge	DF[a] 64% (n=25); range = ND - 47 ng/L (MRL = 0.3)
Veillette et al. (2012)	Canada (Ellesmere Island, Nunavut)	A lake near the northwest coast with no known sources of PFAS	DF[a] 100%, mean (range) = 0.016 (0.011–0.024) ng/L
Nakayama et al. (2010)	United States (Illinois, Iowa, Minnesota, Missouri, Wisconsin; Upper Mississippi River Basin and Missouri River Basin)	88 sampling sites from tributaries and streams	DF 43%, median (range) = 0.71 (ND–84.1) ng/L
Galloway et al. (2020)	United States (Ohio and West Virginia; Ohio River Basin)	Rivers and tributaries 58 km upstream to 130 km downwind of a fluoropolymer production facility, some sample locations potentially impacted by local landfills	DF NR, range[a] = ND–28.0 ng/L
Newsted et al. (2017)	United States (Minnesota; Upper Mississippi River Pool 2)	Upstream and downstream of 3M Cottage Grove facility outfall, which is a source of PFAS	Upstream: DF[a] 3%, point = 4.2 ng/L Downstream: DF[a] 67%, range = ND–336.0 ng/L
Procopio et al. (2017)	United States (New Jersey; Metedeconk River Watershed)	Downstream of suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs	DF[a] 5%, range = ND–100 ng/L
Newton et al. (2017)	United States (Decatur, Alabama; Tennessee River)	6 sites upstream and 3 sites downstream of fluorochemical manufacturing facilities	Upstream: DF 0% Downstream: DF[a] 100%, mean[a] (range) = 69 (10–160) ng/L

Study	Location	Site Details	PFBS Results
Zhang et al. (2016)	United States (Rhode Island, New York Metropolitan Region)	Rivers and creeks, some sampling locations downstream from industrial activities, airport, textile mills, and WWTP. PFAS are used for water resistant coating in textiles.	DF[a] 85%, range = ND–6.181 ng/L
Lescord et al. (2015)	Canada (Resolute Bay, Nunavut)	One lake (Meretta) contaminated with runoff from an airport, which is a known source of PFAS; one control lake (9 Mile)	Meretta: DF NR, mean = 4.9 ng/L 9 Mile: DF NR, mean = 0.07 ng/L
Lasier et al. (2011)	United States (Georgia; Coosa River watershed)	Upstream (sites 1 and 2) and downstream (sites 3–8) of a land-application site where effluents from carpet manufacturers (suspected of producing wastewaters containing perfluorinated chemicals) are processed at a WWTP and the treated WWTP effluent is sprayed onto the site. Site 4 was downstream of a manufacturing facility for latex and polyurethane backing material.	Upstream Sites 1 and 2: DF 0% Downstream Site 3: DF NR, mean = 205 ng/L Site 4: DF NR, mean = 260 ng/L Site 5: DF NR, mean = 125 ng/L Site 6: DF NR, mean = 134 ng/L Site 7: DF NR, mean = 122 ng/L Site 8: DF NR, mean = 105 ng/L
Anderson et al. (2016)	United States (national)	Ten U.S. Air Force installations with historic AFFF release	DF 80.00%, median (range) = 106 (ND–317,000) ng/L
Post et al. (2013)	United States (New Jersey)	6 rivers and 6 reservoirs from public drinking water system intakes, some sites may include nearby small industrial park and civil-military airport	DF 17%, range = ND–6 ng/L

Study	Location	Site Details	PFBS Results
Nakayama et al. (2007)	United States (North Carolina; Cape Fear River Basin)	80 sampling sites in river basin; some sites near industrial areas and Fort Bragg and Pope Air Force Base with suspected use of AFFF at the Air Force Base	DF 62%, mean (range) = 2.58 (ND–9.41) ng/L
Lindstrom et al. (2011)	United States (Alabama)	32 surface water samples (ponds and streams) from areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 63%, range = ND–208 ng/L
Bradley et al. (2020)	United States (Lake Michigan)	Untreated Lake Michigan water from treatment plant intake (4 sites)	DF 29%, range = ND–0.5 ng/L
Europe			
Barreca et al. (2020)	Italy (Lombardia Region)	Rivers and streams with no known fluorochemical sources	DF[a] 39%, range = ND–16,000 ng/L
Loos et al. (2017)	Austria, Bulgaria, Croatia, Moldova, Romania, Serbia, Slovakia (Danube River and tributaries)	Some sampling locations downstream of major cities	DF 94%, mean (range) = 1.6 (ND–3.7) ng/L
Wilkinson et al. (2017)	England (Greater London and southern England; Hogsmill River, Chertsey Bourne River, Blackwater River)	50 m upstream and 250 m and 1,000 m downstream from WWTP effluent outfalls	Upstream: DF NR, mean = 20.4 ng/L Downstream 250 m: DF NR, mean = 40.3 ng/L Downstream 1,000 m: DF NR, mean = 41.1 ng/L
Shafique et al. (2017)	Germany (Leipzig, Pleiße-Elster River, Saale River, and Elbe River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Pleiße-Elster: DF NR, mean = 1.2 ng/L Saale: DF NR, mean = 7.5 ng/L Elbe: DF NR, mean = 4.3 ng/L
Munoz et al. (2016)	France (Seine River)	Two sites downstream of Greater Paris and one site unaffected by the Greater Paris region	DF 70%, range = ND–3.1 ng/L

Study	Location	Site Details	PFBS Results
Lorenzo et al. (2015)	Spain (Guadalquivir River Basin, Ebro River Basin)	Guadalquivir sampling locations included downstream of WWTPs, near industrial areas, near a military camp, or through major cities; Ebro sampling locations included nearby ski resorts and downstream of WWTP and industrial areas	Guadalquivir: DF 8%, mean (range) = 10.1 (ND–228.3) ng/L Ebro: DF 0%
Zhao et al. (2015)	Germany (Elbe River and lower Weser River)	Some sampling sites near Hamburg city and industrial plants	Elbe: DF 100%, mean (range) = 7.4 (0.24–238) ng/L Weser: DF 100%, mean (range) = 1.41 (0.75–1.85) ng/L
Eriksson et al. (2013)	Denmark (Faroe Islands)	Lakes Leitisvatn, Havnardal, Kornvatn, and Á Mýranar with no known point sources of any fluorochemical facilities	Leitisvatn: DF 0% Havnardal Lake: DF 0% Kornvatn Lake: DF 0% Á Mýranar: DF 0%
Wagner et al. (2013)	Germany (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean[b] (range[b]) = 18 (9–26) ng/L
Boiteux et al. (2012)	France (national)	Rivers; some locations may have upstream industrial sources	DF 1%, range = ND–5 ng/L
Eschauzier et al. (2012)	The Netherlands (Amsterdam; Lek Canal, tributary of Rhine River)	Downstream of an industrial point source in the German part of the Lower Rhine	DF[a] 100%, mean (range) = 35 (31–42) ng/L
Labadie and Chevreuil (2011)	France (Paris; River Seine)	Urban stretch of the River Seine during a flood cycle, sampling location under the influence of two urban WWTPs and two major combined sewer overflow outfalls	DF 100%, mean (range) = 1.3 (0.6–2.6) ng/L

Study	Location	Site Details	PFBS Results
Möller et al. (2010)	Germany (Rhine River watershed)	Upstream and downstream of Leverkusen, where effluent of a WWTP treating industrial wastewater was discharged; other major rivers and tributaries	Rhine upstream Leverkusen: DF 100%, mean (range) = 3.19 (0.59–6.58) ng/L Rhine downstream Leverkusen: DF 100%, mean (range) = 45.4 (15.0–118) ng/L River Ruhr: DF 100%, mean (range) = 7.08 (2.87–11.4) ng/L River Moehne: point = 31.1 ng/L Other tributaries: DF 100%, mean (range) = 2.84 (0.22–6.82) ng/L
Ahrens et al. (2009b)	Germany (Elbe River)	Sampling sites in Hamburg city (sites 16–18) and from Laurenburg to Hamburg (sites 19–24)	Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.6 (1.1–2.5) ng/L Laurenburg to Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.1 (0.53–1.5) ng/L
Ahrens et al. (2009a)	Germany (Elbe River)	Sampling locations 53 to 122 km (sites 1 to 9)[c] upstream of estuary mouth of Elbe River	DF NR; range of mean (for different locations) = 1.8–3.4 ng/L
Rostkowski et al. (2009)	Poland (national)	Rivers, lakes, and streams in northern and southern Poland, some southern locations near chemical industrial activities	North: DF[a] 60%, range = ND–10 ng/L South: DF[a] 73%, range = ND–16.0 ng/L
Ericson et al. (2008b)	Spain (Tarragona Province; Ebro River, Francolí River, Cortiella River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Ebro site 1: DF 0% Ebro site 2: DF 0% Francolí: DF 0% Cortiella: DF 0%
Bach et al. (2017)	France (southern)	Upstream and downstream from discharge point that receives wastewater from an industrial site with two fluoropolymer manufacturing facilities	Upstream: DF 0% Downstream: DF 0%

Study	Location	Site Details	PFBS Results
Boiteux et al. (2017)	France (northern)	River samples from upstream and downstream of an industrial WWTP that processes raw sewage from fluorochemical manufacturing facility	Upstream: DF 0% Downstream: DF 0%
Gebbink et al. (2017)	The Netherlands (Dordrecht)	Upstream and downstream of Dordrecht fluorochemical production plant; two control sites	Control sites: DF[a] 100%, mean[a] (range) = 17 (12–22) ng/L Upstream: DF[a] 100%, mean[a] (range) = 19.7 (18–21) ng/L Downstream: DF[a] 100%, mean[a] (range) = 21 (16–27) ng/L
Valsecchi et al. (2015)	Italy (Po River Basin, Brenta River Basin, Adige River Basin, Tevere River Basin, and Arno River Basin)	Two river basins (Po and Brenta) which receive discharges from two chemical plants that produce fluorinated polymers and intermediates; three river basins (Adige, Tevere, Arno) with no known point sources of any fluorochemical facilities	Po: DF[a] 56%, range = ND–30.4 ng/L Brenta: DF[a] 100%, mean[a] (range) = 707 (23.1–1,666) ng/L Adige: DF[a] 20%, range = ND–4.3 ng/L Tevere: DF 0% Arno: DF[a] 58%, range = ND–31.4 ng/L
Mussabek et al. (2019)	Sweden (Luleå)	Samples from lake and pond near a firefighting training facility at the Norrbotten Air Force Wing known to use PFAS-containing AFFF	Lake: DF NR, mean = 200 ng/L Pond: DF NR, mean = 150 ng/L
Gobelius et al. (2018)	Sweden (national)	Sampling locations selected based on potential vicinity of PFAS hot spots and importance as a drinking water source area, some sites include firefighting training sites at airfields and military areas	DF[a] 29%, range = ND–299 ng/L

Study	Location	Site Details	PFBS Results
Dauchy et al. (2017)	France (unspecified)	Samples collected near 3 sites (B, C, D) impacted by the use of firefighting foams	Site B: DF 0% Site C: DF 0% Site D: DF[a] 30%, range = ND–138 ng/L
Multiple Continents			
Pan et al. (2018)	United States (Delaware River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 2.19 (0.52–4.20) ng/L
	United Kingdom (Thames River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 5.06 (3.26–6.75) ng/L
	Germany and the Netherlands (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 21.9 (0.46–146) ng/L
	Sweden (Mälaren Lake)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 1.43 (0.75–1.92) ng/L

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; km = kilometer; m = meter; ND = not detected; ng/L = nanogram per liter; NR = not reported; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; WWTP = wastewater treatment plant; µg/L = microgram per liter.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] For Wagner et al. (2013), PFBS concentrations were calculated using the fluorine concentrations reported in Table 4 from the study.

[c] Freshwater locations determined as sites with conductivity < 1.5 mS/cm.

Table B-4. Compilation of Studies Describing PFBS Occurrence in Food

Study	Location and Source	Food Types	Results
North America			
Schecter et al. (2010)	United States (Texas) Grocery stores	Dairy, fruits and vegetables, grain, meat, seafood, fats/other	**Cod: DF NR, mean = 0.12 ng/g ww** ND in salmon, canned sardines, canned tuna, fresh catfish fillet, frozen fish sticks, tilapia, cheeses (American, mozzarella, Colby, cheddar, Swiss, provolone, and Monterey jack), butter, cream cheese, frozen yogurt, ice cream, whole milk, whole milk yogurt, potatoes, apples, cereals, bacon, canned chili, ham, hamburger, roast beef, sausages, sliced chicken breast, sliced turkey, canola oil, margarine, olive oil, peanut butter, eggs
Byrne et al. (2017)	United States (Alaska) Upstream/downstream of former defense site (Suqi River)	Seafood	**Blackfish: DF 48%, range = ND–59.2 ng/g ww** Highest concentration was upstream
Scher et al. (2018)	United States (Minnesota) Home gardens Near former 3M PFAS production facility, homes within and outside a GCA	Fruits and vegetables	Within GCA: **Leaf: DF 6%, max = 0.061 ng/g** **Stem: DF 4%, max = 0.065 ng/g** ND in floret, fruit, root, seed Outside GCA: ND
Blaine et al. (2014)	United States (Midwestern) Greenhouse study, unamended controls	Fruits and vegetables	**Radish root: DF NR, mean = 22.36 ng/g** ND in celery shoot, pea fruit
Blaine et al. (2013)	United States (Midwestern) Greenhouse and field studies, unamended controls	Fruits and vegetables, grain	ND in corn, lettuce, tomato in unamended soil.
Young et al. (2013)	United States (Maryland, Mississippi, Tennessee, Florida, New York, Texas, Washington, D.C.) Retail markets	Seafood	ND in crab, shrimp, striped bass, farm raised catfish, farm raised salmon
Young et al. (2012)	United States (17 states) Retail markets	Dairy	ND in retail cow's milk

Study	Location and Source	Food Types	Results
Europe			
Domingo et al. (2012)	Spain (Catalonia) Local markets, small stores, supermarkets, big grocery stores	12 food categories	**Vegetables: DF NR, mean = 0.013 ng/g fw** **Fish and seafood: DF NR, mean = 0.054 ng/g fw** ND in meat and meat products, tubers, fruits, eggs, milk, dairy products, cereals, pulses, industrial bakery, oils
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Various supermarkets and retail stores	8 food categories	Categories included cereals, pulses and starchy roots, tree-nuts, oil crops and vegetable oils, vegetables and fruits, meat and meat products, milk, animal fats, dairy products, and eggs, fish and seafood, and others such as candies or coffee **Spain: DF 3.2%, range = ND–13 ng/g (primarily fish, oils)** **Serbia: DF 5.2%, range = ND–0.460 ng/g (primarily meat and meat products, cereals)**
D'Hollander et al. (2015)	Belgium, Czech Republic, Italy, Norway PERFOOD study; items from 3 national retail stores of different brands and countries of origin	Fruit, cereals, sweets, salt	**Sweets: DF[a] 25%, range = ND–0.0016 ng/g** **Fruit: DF[a] 19%, range = ND–0.067 ng/g** ND in cereals, salt
Hlouskova et al. (2013)	Belgium, Czech Republic, Italy, Norway Several national supermarkets	Pooled milk/dairy products, meat, fish, hen eggs	**DF 5%, mean (range) = 0.00975 (0.006–0.012) ng/g**
Eriksson et al. (2013)	Denmark Farm, dairy farm, fish from Faroe Shelf area	Dairy, fruits and vegetables, seafood	**Milk:** **Farmer (Havnardal): point = 0.019 ng/g ww** **Diary (Faroe Island): point = 0.017 ng/g ww;** ND or NQ in 4 samples ND in yogurt, creme fraiche, potatoes, farmed salmon, wild-caught cod, wild-caught saithe

Study	Location and Source	Food Types	Results
Sznajder-Katarzyńska et al. (2019)	Poland Markets	Dairy	**All dairy: sum PFBS = 0.04 ng/g** **Butter: range = 0.01–0.02 ng/g** ND in camembert-type cheese, cottage cheese, milk, natural yogurt, sour cream, kefir (bonny clabber)
Yamada et al. (2014)	France Freshwater fish from 6 major French rivers; fresh and frozen fish from markets	Seafood	**Freshwater fish: DF NR, range = 0.06–0.16 ng/g ww** **Fresh or frozen fish: DF NR, range = 0.02–0.03 ng/g ww**
Vassiliadou et al. (2015)	Greece Local fish markets, mariculture farm, fishing sites	Seafood	**Hake: raw mean = 0.45 ng/g ww, fried mean = 0.83 ng/g ww** **Shrimp: raw mean = 1.37 ng/g ww** ND in raw, fried, and grilled anchovy, bogue, picarel, sand smelt, sardine, squid, striped mullet, raw and fried mussel, fried shrimp, and grilled hake
Eschauzier et al. (2013)	The Netherlands (Amsterdam) Cafés, universities, supermarkets	Fats/other	**Brewed coffee (manual): mean (range) = 1.6 (1.3–2.0) ng/L** **Brewed coffee (machine): mean (range) = 2.9 (ND–9.8) ng/L** **Cola: mean (range) = 7.9 (ND–12) ng/L**
Surma et al. (2017)	Spain, Slovakia Source NR	Fats/other	**Spices: ND–1.01 ng/g** Spain: Detected in anise, star anise, fennel, coriander, cinnamon, peppermint, parsley, thyme, laurel, cumin, and oregano ND in white pepper, cardamon, clove, nutmeg, allspice, vanilla, ginger, garlic, black paper, and hot pepper (mild and hot) Slovakia: ND in anise, star anise, white pepper, fennel, cardamom, clove, coriander, nutmeg, allspice, cinnamon, vanilla, and ginger

Study	Location and Source	Food Types	Results
Papadopoulou et al. (2017)	Norway A-TEAM project: food and drinks collected by participants as duplicate diet samples	Solid foods (11 food categories), liquid foods (5 drinks)	**Solid foods (unspecific food category): DF 2%, range = ND–0.001 ng/g** ND in liquid foods (coffee, tea and cocoa, milk, water, alcoholic beverages and soft drinks)
Scordo et al. (2020)	Italy Supermarkets	Fruits	**Olives: DF[a] 100%, mean[a] (range) = 0.294 (0.185–0.403) ng/g dw** ND in strawberries
Ericson et al. (2008a)	Spain Local markets, large supermarkets, grocery stores	18 food categories	ND in all categories: veal, pork, chicken, lamb, white fish, seafood, tinned fish, blue fish, whole milk, semi-skimmed milk, dairy products, vegetables, pulses, cereals, fruits, oil, margarine, and eggs
Noorlander et al. (2011)	The Netherlands Several Dutch retail store chains with nationwide coverage	15 food categories	ND in all categories: flour, fatty fish, lean fish, pork, eggs, crustaceans, bakery products, vegetables/fruit, cheese, beef, chicken/poultry, butter, milk, vegetable oil, and industrial oil
Jogsten et al. (2009)	Spain (Catalonia) Local markets, large supermarkets, grocery stores	Fruits and vegetables, meat, seafood, fats/other	ND in lettuce, raw, cooked, and fried meat (veal, pork, and chicken), fried chicken nuggets, black pudding, lamb liver, pate of pork liver, foie gras of duck, “Frankfurt” sausages, home-made marinated salmon, and common salt
Sznajder-Katarzyńska et al. (2018)	Poland Markets	Fruits and vegetables	ND in apples, bananas, cherries, lemons, oranges, strawberries, beetroots, carrots, tomatoes, potatoes, and white cabbage
Falandysz et al. (2006)	Poland Gulf of Gdañsk, Baltic Sea south coast	Meat, seafood	ND in eider duck, cod
Barbosa et al. (2018)	Belgium, France, the Netherlands, Portugal Various markets	Seafood	ND in raw and steamed fish (*P. platessa, M. australis, M. capenis, K. pelamis*, and *M. edulis*)

Study	Location and Source	Food Types	Results
Hölzer et al. (2011)	Germany Fish from Lake Möhne and river Möhne, contaminated with PFCs from use of polluted soil conditioner on agricultural lands; retail trade, wholesale trade, supermarkets, and producers	Seafood	Lake Möhne /River Möhne: ND in cisco, eel, perch, pike, and roach Trade/markets: ND in eel, pike/perch, and trout
Jörundsdóttir et al. (2014)	Iceland Collected during biannual scientific surveys, commercially-produced	Seafood	ND in anglerfish, Atlantic cod, blue whiting, lemon sole, ling, lumpfish, plaice, and pollock
Rivière et al. (2019)	France Based on results of national consumption survey	Seafood, fats/other	ND in infant food, vegetables, non-alcoholic beverages, dairy-based desserts, milk, mixed dishes, fish, ultra-fresh dairy products, meat, poultry and game
Lankova et al. (2013)	Czech Republic Retail market	Fats/other	ND in infant formula
Zafeiraki et al. (2016a)	Greece, the Netherlands Home and commercially-produced	Fats/other	ND in chicken eggs
Gebbink et al. (2015)	Sweden Major grocery chain stores, market basket samples	12 food categories	ND in all categories: dairy products, meat products, fats, pastries, fish products, egg, cereal products, vegetables, fruit, potatoes, sugar and sweets, soft drinks
Herzke et al. (2013)	Belgium, Czech Republic, Italy, Norway PERFOOD study: items from 3 national retail stores of different brands per location	Vegetables	ND for all vegetables
Zafeiraki et al. (2016b)	The Netherlands Local markets and slaughterhouses	Meat	ND for horse, sheep, cow, pig, and chicken liver

Study	Location and Source	Food Types	Results
Multiple Continents			
Chiesa et al. (2019)	United States (Pacific Ocean) Wholesale fish market	Seafood	ND in wild-caught salmon
	Canada Wholesale fish market	Seafood	ND in wild-caught salmon
	Norway Wholesale fish market	Seafood	ND in farm salmon
	Scotland Wholesale fish market	Seafood	ND in wild-caught and farm salmon

Notes: DF = detection frequency; dw = dry weight; fw = fresh weight; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; ng/L = nanogram per liter; NR = not reported; PFAS = per- and polyfluoroalkyl substances; NQ = not quantified; µg/L = microgram per liter; ww = wet weight.
Bold indicates detected levels of PFBS in food.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

Table B-5. Compilation of Studies Describing PFBS Occurrence in Indoor Dust

Study	Location	Site Details	Results
North America			
Zheng et al. (2020)	United States (Seattle, Washington and West Lafayette, Indiana)	Childcare facilities (20 samples from 7 in Seattle and 1 in West Lafayette)	DF 90%, mean (range) = 0.34 (ND–0.86) ng/g
Byrne et al. (2017)	United States (St. Lawrence Island, Alaska)	Homes (49)	DF 16%, median = ND; 95th percentile = 1.76 ng/g
Fraser et al. (2013)	United States (Boston, Massachusetts)	Homes (30); offices (31); vehicles (13)	Homes: DF 3% (single detection), range = ND–4.98 ng/g Offices: DF 10%, range = ND–12.0 ng/g Vehicles: DF 0%
Knobeloch et al. (2012)	United States (Great Lakes Basin, Wisconsin)	Homes (39)	DF 59%, median (range) = 1.8 (ND–31) ng/g

Study	Location	Site Details	Results
Strynar and Lindstrom (2008)	United States (Cities in North Carolina and Ohio)	Homes (102) and daycare centers (10); samples had been collected in 2000–2001 during EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study	DF 33%, mean (range) = 41.7 (ND–1,150) ng/g
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility; 19 homes within the GCA	Entryway: DF 11%, median (range) = ND (ND–58 ng/g) Living room: DF 16%, median (range) = ND (ND–58 ng/g)
Kubwabo et al. (2005)	Canada (Ottawa)	Homes (67)	DF 0%
Europe			
de la Torre et al. (2019)	Spain (unspecified), Belgium (unspecified), Italy (unspecified)	Homes (65)	Spain: DF 52%, median (range) = 0.70 (ND–12.0) ng/g Belgium: DF 27%, median (range) = 0.40 (ND–56.7) ng/g Italy: DF 18%, median (range) = 0.40 (ND–11.6) ng/g
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick counties)	Homes (32); offices (33); cars (31); classrooms (32)	Homes: DF 81%, mean (range) = 17 (ND–110) ng/g Offices: DF 88%, mean (range) = 19 (ND–98) ng/g Cars: DF 75%, mean (range) = 12 (ND–170) ng/g Classrooms: DF 97%, mean (range) = 17 (ND–49) ng/g
Giovanoulis et al. (2019)	Sweden (Stockholm)	Preschools (20)	DF 0%
Winkens et al. (2018)	Finland (Kuopio)	Homes (63 children's bedrooms)	DF 12.7%, median (range) = ND (ND–13.5) ng/g
Padilla-Sánchez and Haug (2016)	Norway (Oslo)	Homes (7)	DF 14% (single detection), range = ND–3 ng/g

Study	Location	Site Details	Results
Jogsten et al. (2012)	Spain (Catalonia)	Homes (10)	DF 60%, range = ND–6.5 ng/g
Haug et al. (2011)	Norway (Oslo)	Homes (41)	DF 22%, mean (range) = 1.3 (0.17–9.8) ng/g
Huber et al. (2011)	Norway (Tromsø)	Homes (7; carpet, bedroom, sofa); one office; one storage room that had been used for storage of "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years"	All homes: DF NR, median = 1.1 ng/g Living room: DF[a] 57%, range = ND–10.6 ng/g Carpet, bedroom, sofa: DF 0% Office: point = 3.8 ng/g Storage room: point = 1,089 ng/g
D'Hollander et al. (2010)	Belgium (Flanders)	Homes (45); offices (10)	Homes: DF 47%, median = 0 ng/g dw Offices: DF NR, median = 0.2 ng/g dw
Multiple Continents			
Kato et al. (2009)	United States (Atlanta, Georgia), Germany (unspecified), United Kingdom (unspecified), Australia (unspecified)	Homes (39)	DF 92.3%, median (range) = 359 (ND–7,718) ng/g
Karásková et al. (2016)	United States (unspecified)	Homes (14)	DF 60%, mean (range) = 1.4 (ND–2.6) ng/g
	Canada (unspecified)	Homes (15)	DF 55%, mean (range) = 1.6 (ND–5.8) ng/g
	Czech Republic (unspecified)	Homes (12)	DF 37.5%, mean (range) = 3.6 (ND–14.4) ng/g

Notes: DF = detection frequency; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; NR = not reported; dw = dry weight

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

Table B-6. Compilation of Studies Describing PFBS Occurrence in Soil

Study	Location	Site Details	Results
North America			
Venkatesan and Halden (2014)	United States (Baltimore, Maryland)	Control (nonamended) soil from Beltsville Agricultural Research Center	DF 0%
Blaine et al. (2013)	United States (Midwestern)	Urban and rural full-scale field study control (nonamended) soil	Urban control: DF NR, mean = 0.10 ng/g Rural control: DF NR, mean = ND
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within a GCA	DF 10%, median (p90) = ND (0.02) ng/g
Scher et al. (2018)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within and outside a GCA	Within GCA: DF 9%, median (range) = ND (ND–0.17 ng/g) Outside GCA: DF 17%, median (range) = ND (ND–0.031 ng/g)
Anderson et al. (2016)	United States (unspecified)	Ten U.S. Air Force installations with historic AFFF release, surface and subsurface soils	Surface soil: DF 35%, median (range) = 0.775 (ND–52.0) ng/g Subsurface soil: DF 35%, median (range) = 1.30 (ND–79.0) ng/g
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Firefighting training site, pre- and posttreatment	Pretreatment: DF 60%, range = 0.61–6.4 ng/g Posttreatment: DF 100%, range = 0.07–0.83 ng/g
Nickerson et al. (2020)	United States (unspecified)	Two AFFF-impacted soil cores from former fire-training areas	Core E: DF[a] 91%, range = ND–27.37 ng/g dw Core F: DF 100%, range = 0.13–58.44 ng/g dw
Cabrerizo et al. (2018)	Canada (Melville and Cornwallis Islands)	Catchment areas of lakes	DF 100%, mean[a] (range) = 0.0024 (0.0004–0.0083) ng/g dw
Dreyer et al. (2012)	Canada (Ottawa, Ontario)	Mer Bleue Bog Peat samples (core samples)	Detected once at 0.071 ng/g in 1973 sample and not considered for further evaluation

Study	Location	Site Details	Results
Mejia-Avendaño et al. (2017)	Canada (Lac-Mégantic, Quebec)	Site of 2013 Lac-Mégantic train accident (oil and AFFF runoff area [sampled 2013], burn site and adjacent area [sampled 2015])	Background: DF NR, mean = 0.035 ng/g dw 2013: DF 75%, mean range = ND–3.15 ng/g dw 2015: DF 36%, mean range = ND–1.25 ng/g dw
Europe			
Harrad et al. (2020)	Ireland (multiple cities)	10 landfills, samples collected upwind and downwind	Downwind: DF NR, mean (range) = 0.0059 (ND–0.044) ng/g dw Upwind: DF NR, mean (range) = 0.0011 (ND–0.0029) ng/g dw
Grønnestad et al. (2019)	Norway (Granåsen, Jonsvatnet)	Granåsen (skiing area); Jonsvatnet (reference site)	Skiing area: DF 0%[b] Reference area: DF 70%, mean (range) = 0.0093 (ND–0.0385 ng/g dw)
Groffen et al. (2019)	Belgium (Antwerp)	3M perfluorochemical plant and 4 sites with increasing distance from plant	Plant: DF 92%, mean (range) = 7.84 (ND–33) ng/g dw Vlietbos (1 km from plant): DF 90%, mean (range) = 2.79 (ND–7.04) ng/g dw 2.3 km, 3 km, 11 km from plant: DF 0%
Dauchy et al. (2019)	France (unspecified)	Firefighting training site, samples collected in 6 areas collected up to 15-m depth; in areas 2 and 6, foams used more intensely and/or before concrete slab was built	Areas 1, 3, 4, and 5 combined: DF[a] 0–10%, range = ND–7 ng/g dw, across all depths Area 2: DF[a] 35%, range = ND–82 ng/g dw, across all depths Area 6: DF[a] 55%, range = ND–101 ng/g dw, across all depths
Skaar et al. (2019)	Norway (Ny-Ålesund)	Research facility near firefighting training site	Background: DF 0% Contaminated: DF 100%, mean[a] (range) = 4.9 (2.64–7.13) ng/g dw
Hale et al. (2017)	Norway (Gardermoen)	Firefighting training site	DF 0%

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; dw = dry weight; GCA = groundwater contamination area; km = kilometer; ND = not detected; ng/g = nanogram per gram; NR = not reported; PFAS = per- and polyfluoroalkyl substances; p90 = 90th percentile

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] Grønnestad et al. (2019) reported a DF = 10% but a range, mean, and standard deviation of < LOQ.

Attachment 8

United States Environmental Protection Agency

Office of Water
OST
EPA
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Drinking Water Health Advisory: Hexafluoropropylene Oxide (HFPO) Dimer Acid (CASRN 13252-13-6) and HFPO Dimer Acid Ammonium Salt (CASRN 62037-80-3), Also Known as "GenX Chemicals"

Drinking Water Health Advisory: Hexafluoropropylene Oxide (HFPO) Dimer Acid (CASRN 13252-13-6) and HFPO Dimer Acid Ammonium Salt (CASRN 62037-80-3), Also Known as "GenX Chemicals"

Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Office of Science and Technology
Health and Ecological Criteria Division
Washington, DC 20460

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- Office of Water
- Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics
- Office of Land and Emergency Management
- Office of Policy
- Office of Children's Health Protection
- Office of Research and Development

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Abbreviations and Acronyms

ADAF	age-dependent adjustment factor
AIX	anion exchange
AF	amorphous fluoropolymer
AFFF	aqueous film-forming foam
ANSI	American National Standards Institute
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower limit
bw or BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CDC	Centers for Disease Control and Prevention
CCL	Contaminant Candidate List
CCL 5	Fifth Safe Drinking Water Act Contaminant Candidate List
cm^3	cubic centimeters
CSF	cancer slope factor
DBP	disinfection byproduct
DF	detection frequency
DHS	Department of Health Services
DOM	dissolved organic matter
DQO	data quality objective
dw	dry weight
DWI	drinking water intake
DWI-BW	body weight-adjusted drinking water intake
DWTP	drinking water treatment plant
E	human exposure
EBCT	empty bed contact time
EF	exposure factor
EFH	Exposure Factors Handbook
EGLE	Michigan Department of Environment, Great Lakes, and Energy
EPA	United States Environmental Protection Agency
Eq.	equation
FDA	United States Food and Drug Administration
FEP	fluorinated ethylene propylene
g/L	grams per liter
GAC	granular activated carbon
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
H_3O^+	hydronium
HA	Health Advisory
HECD	Health and Ecological Criteria Division
HED	human equivalent dose
HFPO	hexafluoropropylene oxide
HFPO dimer acid	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid
HFPO-TA	hexafluoropropylene oxide trimer acid
HFPO-TeA	hexafluoropropylene oxide tetramer acid
HI	hazard index
HIDOH	Hawai´i State Department of Health
HNIS	Human Nutrition Information Service
HQ	hazard quotient
i	mixture component chemical

IDEM	Indiana Department of Environmental Management
iHA	interim Health Advisory
Illinois EPA	Illinois Environmental Protection Agency
ITRC	Interstate Technology and Regulatory Council
km	kilometers
L/kg bw-day	liters per kilogram body weight per day
$L/(m^2 \cdot hr)$	liters per square meter per hour
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LOAEL	lowest observed adverse effect level
LOQ	limit of quantification
m/hr	meters per hour
MCLG	Maximum Contaminant Level Goal
mg/kg bw-day	milligrams per kilogram body weight per day
MQL	method quantification limit
MRL	minimum reporting limit
NCDEQ	North Carolina Department of Environmental Quality
NCDHHS	North Carolina Department of Health and Human Services
ND	not detected
NH_4^+	ammonium cation
NF	nanofiltration
ng/g	nanograms per gram
ng/kg	nanograms per kilogram
ng/L	nanograms per liter
NHANES	National Health and Nutrition Examination Survey
NOAEL	no observed adverse effect level
NOM	natural organic matter
NR	not reported
NSF	National Science Foundation
OCHP	Office of Children's Health Protection
ODH	Ohio Department of Health
OGWDW	Office of Ground Water and Drinking Water
Ohio EPA	Ohio Environmental Protection Agency
OST	Office of Science and Technology
OW	Office of Water
PAC	powdered activated carbon
PECO	populations, exposures, comparators, and outcomes
PFA	perfluoroalkoxy
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutane sulfonic acid
PFCA	perfluoroalkyl carboxylic acid
PFECA	perfluoroalkyl ether carboxylic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
pg/g	picograms per gram
pg/L	picograms per liter
PHG	provisional health goal
pK_a	acid dissociation constant
POD	point of departure
POD_{HED}	point of departure human equivalent dose
POE	point-of-entry
POU	point-of-use

PPARα	peroxisome proliferator-activated receptor alpha
ppt	parts per trillion
PTFE	polytetrafluoroethylene
PWS	public water system
RfD	reference dose
RO	reverse osmosis
RPF	relative potency factor
RSC	relative source contribution
SAB	Science Advisory Board
SDWA	Safe Drinking Water Act
$t_{1/2}$	half-life
TSCATS	Toxic Substances Control Act Test Submissions
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor(s)
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	extrapolation from subchronic to chronic exposure duration uncertainty factor
µg/L	micrograms per liter
µg/kg bw-day	micrograms per kilogram body weight per day
Wisconsin DHS	Wisconsin Department of Health Services
WOS	Web of Science

Executive Summary

Hexafluoropropylene oxide (HFPO) dimer acid (2,3,3,3-tetrafluoro-2- [heptafluoropropoxy] propanoic acid) (Chemical Abstracts Service Registry Number [CASRN] 13252-13-6) and HFPO dimer acid ammonium salt (ammonium 2,3,3,3- tetrafluoro-2-[heptafluoropropoxy]propanoate) (CASRN 62037-80-3) are shorter-chain members of a group of substances known as per- and polyfluoroalkyl substances (PFAS). HFPO dimer acid and its ammonium salt are referred to as "GenX chemicals" because they are two of the main chemicals associated with the GenX processing aid technology that DuPont developed to make high-performance fluoropolymers without using perfluorooctanoic acid (PFOA) (U.S. EPA, 2021a). In water, both HFPO dimer acid and its ammonium salt dissociate to form the HFPO dimer acid anion (HFPO-) as a common analyte.

GenX chemicals are replacements for the longer-chain PFOA, which was phased out in the United States by 2015 as part of an agreement between manufacturers and the U.S. Environmental Protection Agency (EPA) under the PFOA Stewardship Program, established in 2006. GenX chemicals are used to manufacture fluoropolymers which have many industrial applications including in medical, automotive, electronics, aerospace, energy, and semiconductor industries. The Chemours Company uses GenX chemicals to produce four trademarked fluoropolymers: Teflon™ polytetrafluoroethylene (PTFE), Teflon™ perfluoroalkoxy (PFA), Teflon™ fluorinated ethylene propylene (FEP), and Teflon™ amorphous fluoropolymer (AF) (Chemours, 2022). Since GenX chemicals are substitutes for PFOA, products (e.g., some nonstick coatings) that were previously made using PFOA may now rely on GenX chemicals.

GenX chemicals have been detected around the globe in surface water, groundwater, finished drinking water, rainwater, and air emissions (U.S. EPA, 2021a). Potential sources of GenX in the environment include industrial facilities that use GenX technology for polymer production, facilities that produce fluoromonomers (as a byproduct), and contaminated water, air, soil, and biosolids. GenX chemicals may also be generated as a byproduct of other manufacturing processes including fluoromonomer production. For example, GenX chemicals have been discharged into the Cape Fear River for several decades as a byproduct of manufacturing (NCDEQ, 2017). GenX chemicals can enter the aquatic environment through industrial discharges, runoff into surface water, and leaching into groundwater from soil and landfills (U.S. EPA, 2021a). GenX chemicals are water-soluble, with solubilities of greater than 751 grams per liter (g/L) and greater than 739 g/L for HFPO dimer acid and its ammonium salt, respectively, at 20°C (U.S. EPA, 2021a). Volatilization from water surfaces is expected to be an important fate process for both HFPO dimer acid and its ammonium salt (U.S. EPA, 2021a). The limited data on human serum have detected GenX chemicals in studies of workers.

EPA is issuing a lifetime noncancer drinking water Health Advisory (HA) for GenX chemicals of 10 nanograms per liter (ng/L) or 10 parts per trillion (ppt). This is the first HA for GenX chemicals and its finalization fulfills a commitment described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021b). The final toxicity assessment for GenX chemicals titled *Final Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"* (U.S. EPA, 2021a) serves as the basis of the toxicity information used to derive the lifetime noncancer HA for GenX chemicals. This final toxicity assessment was published after a rigorous process including draft assessment development, agency and interagency review, public

comment, two independent peer reviews, and an independent review of data from two studies by the National Toxicology Program. The input values for deriving the HA include 1) the final chronic reference dose (RfD) for GenX of 0.000003 milligrams per kilogram body weight per day (mg/kg bw-day) (U.S. EPA, 2021a); 2) a 20% relative source contribution (RSC) based on EPA's Exposure Decision Tree approach in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a); and 3) the drinking water intake rate of 0.0469 L/kg bw-day for lactating women, which is the sensitive population identified based on the critical study selected for the final RfD (U.S. EPA, 2021a).

The final toxicity assessment for GenX chemicals (U.S. EPA, 2021a) derived both subchronic and chronic RfDs based on the critical adverse effect of a constellation of liver lesions (i.e., cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) observed in female mice in an oral reproductive/developmental toxicity study (DuPont-18405-1037, 2010; NTP, 2019). Using EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), EPA modeled the dose-response relationship in the range of observed data. Additionally, EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011) was used to allometrically scale a toxicologically equivalent dose from adult laboratory animals to adult humans. From benchmark dose modeling (BMD) of the DuPont-18045-1037 (2010) study, the resulting POD human equivalent dose (HED) is 0.01 mg/kg bw-day. The HED was divided by a composite UF (UFc) of 3,000 to obtain the chronic RfD of 0.000003 mg/kg bw-day or 0.003 micrograms per kilogram body weight per day (µg/kg bw-day) for GenX chemicals (U.S. EPA, 2021a).

There is insufficient toxicity information available to derive a one-day HA for GenX chemicals because U.S. EPA (2021a) does not have a final RfD for acute exposure (i.e., relevant to a 7 day or less exposure period). There is also insufficient toxicity information available to derive a ten-day HA because U.S. EPA (2021a) did not derive a final short-term exposure RfD for a 7-to-30-day exposure on which to base a ten-day HA for GenX chemicals.

For cancer toxicity, one chronic 2-year study in rats evaluating the carcinogenicity of GenX chemicals was identified (U.S. EPA, 2021a). In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), EPA concluded that there is *Suggestive Evidence of Carcinogenic Potential* following oral exposure in humans for GenX chemicals based on female hepatocellular adenomas and carcinomas and male combined pancreatic acinar adenomas and carcinomas observed in the chronic 2-year study in rats (U.S. EPA, 2021a). A cancer slope factor (CSF) was not derived for GenX chemicals in the toxicity assessment. This is consistent with EPA's guidelines which state that when the available evidence is suggestive for carcinogenicity, a quantitative risk estimate is generally not derived unless there exists a well-conducted study that could facilitate an understanding of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a). Therefore, EPA did not derive a 10^{-6} cancer risk concentration in the HA for GenX chemicals.

EPA developed two analytical methods to quantitatively monitor drinking water for targeted PFAS that include HFPO dimer acid: EPA Method 533 (U.S. EPA, 2019b), which has a quantitation limit of 3.7 ng/L for HFPO dimer acid, and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020b), which has a quantitation limit for HFPO dimer acid at 4.3 ng/L. These analytical methods can both effectively and accurately monitor drinking water for HFPO dimer acid at levels below the lifetime HA of 10 ng/L. Treatment technologies, including sorption-based

processes such as activated carbon and ion exchange, along with high pressure membrane processes such as reverse osmosis (RO), and nanofiltration (NF), are available and have been shown to remove HFPO dimer acid in drinking water.

1.0 Introduction and Background

The Safe Drinking Water Act (SDWA) (42 U.S.C. § § 300f - 300j-27) authorizes the U.S. Environmental Protection Agency (EPA) to develop drinking water Health Advisories (HAs).[1] HAs are national non-enforceable, non-regulatory drinking water concentration levels of a specific contaminant at or below which exposure for a specific duration is not anticipated to lead to adverse human health effects.[2] HAs are intended to provide information that tribal, state, and local government officials and managers of public water systems (PWSs) can use to determine whether actions are needed to address the presence of a contaminant in drinking water. HA documents reflect the best available science and include HA values as well as information on health effects, analytical methodologies for measuring contaminant levels, and treatment technologies for removing contaminants from drinking water. EPA's lifetime HAs identify levels to protect all Americans, including sensitive populations and life stages, from adverse health effects resulting from exposure throughout their lives to contaminants in drinking water.

In October 2021, EPA published a final toxicity assessment for two per- and polyfluoroalkyl substances (PFAS), hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt, collectively known as "GenX chemicals" (U.S. EPA, 2021a). EPA's final *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"* was an essential step to better understanding the potential human health effects of exposure to these two main GenX chemicals. The human health chronic reference dose (RfD) calculated in the toxicity assessment allows EPA to develop a lifetime HA that will help communities make informed decisions about GenX chemicals to better protect human health. The final HA for GenX chemicals satisfies a commitment described in EPA's PFAS Strategic Roadmap (U.S. EPA, 2021b).

1.1 History under SDWA

HFPO dimer acid and its ammonium salt are not currently regulated under SDWA. GenX is a trade name for a technology that is used to make high-performance fluoropolymers without the use of perfluorooctanoic acid (PFOA). In 2008, DuPont de Nemours, Inc. (hereinafter DuPont) submitted premanufacture notices to EPA under the Toxic Substances Control Act (Title 15 of the United States Code § 2601 et seq.) for two chemicals:

- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid (CASRN13252-13-6) or HFPO dimer acid
- ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate (CASRN 62037-80-3) or HFPO dimer acid ammonium salt

Both HPFO dimer acid and its ammonium salt are components of the GenX processing aid technology that DuPont developed to make high-performance fluoropolymers without using

[1] SDWA § 1412(b)(1)(F) authorizes EPA to "publish health advisories (which are not regulations) or take other appropriate actions for contaminants not subject to any national primary drinking water regulation." www.epa.gov/sites/default/files/2020-05/documents/safe_drinking_water_act-title_xiv_of_public_health_service_act.pdf

[2] This document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community. This document is not enforceable against any person and does not have the force and effect of law. No part of this document, nor the document as a whole, constitutes final agency action that affects the rights and obligations of any person. EPA may change any aspects of this document in the future.

PFOA (U.S. EPA, 2021a). These compounds fall into the perfluoroalkyl ether carboxylic acids (PFECAs) PFAS class or subgroup. Although not the only GenX chemicals, HFPO dimer acid and its ammonium salt are the major chemicals associated with the GenX processing aid technology (ECHA, 2015; U.S. EPA, 2021a). The lifetime HA for GenX chemicals derived in this document pertains only to the two major GenX chemicals, HFPO dimer acid and its ammonium salt, because this was the scope of the toxicity assessment for GenX chemicals (U.S. EPA, 2021a).

HFPO dimer acid and its ammonium salt were listed on the draft fifth SDWA Contaminant Candidate List (CCL 5) not as individual chemicals but as part of the PFAS group inclusive of any PFAS except for PFOA and perfluorooctanesulfonic acid (PFOS) (U.S. EPA, 2021c). The Contaminant Candidate List (CCL) is a list of contaminants that are not subject to any proposed or promulgated National Primary Drinking Water Regulations, are known or anticipated to occur in PWSs and may require regulation under SDWA.[3] EPA is currently evaluating public comments and additional information to inform the Final CCL 5 and any future regulatory actions for these chemicals under SDWA.

The 1996 amendments to SDWA require that EPA issue a new list of unregulated contaminants (once every five years) to be monitored by PWSs.[4] Under the Unregulated Contaminant Monitoring Rule (UCMR), EPA collects occurrence data for contaminants that may be present in drinking water but do not have health-based standards set under SDWA. HFPO dimer acid is one of 29 PFAS included for monitoring under the fifth Unregulated Contaminant Rule (UCMR 5) between 2023 and 2025 (U.S. EPA, 2021d). The collection of drinking water occurrence data supports EPA's future regulatory determinations and may support additional actions to protect public health (U.S. EPA, 2021d).

1.2 Current Advisories and Guidelines

Table 1 provides drinking water guideline values for GenX chemicals that have been developed by states. The state values range from 21 to > 700 parts per trillion (ppt) or nanograms per liter (ng/L). This broad range of values may in part reflect differences in the level type derived, state guidance, or use of different methods (see references for more details).

Table 1. State Guideline Values for GenX Chemicals

State[a,b]	GenX Chemical Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
Hawaii	160	Environmental Action Levels	Groundwater	HIDOH (2020)
Illinois	21	Health-Based Guidance Level	Drinking water; Groundwater	Illinois EPA (2022)

[3] https://www.epa.gov/ccl/basic-information-ccl-and-regulatory-determination

[4] SDWA § 1445 (a)(1)(D)(2)(B) — "Not later than 3 years after the date of enactment of the Safe Drinking Water Act Amendments of 1996 and every 5 years thereafter, the Administrator shall issue a list pursuant to subparagraph (A) of not more than 30 unregulated contaminants to be monitored by public water systems and to be included in the national drinking water occurrence data base maintained pursuant to subsection (g)."

State[a,b]	GenX Chemical	Standard/Guidance	Type of Medium	Reference
Indiana	> 700	Action Level	Drinking water	IDEM (2022)
Michigan	370	Drinking Water Maximum Contaminant Level	Drinking water; Groundwater	EGLE (2020)
North Carolina	140	Health Goal	Drinking water	NCDHHS (2017)
Ohio	21	Action Level	Drinking water	Ohio EPA and ODH (2022)
Wisconsin	300	Recommended Enforcement Standard	Groundwater	Wisconsin DHS (2020)
	30	Recommended Preventive Action Limit	Groundwater	

Notes:

[a] The information was collected via EPA regional office outreach by EPA's Office of Science and Technology (OST) in March 2022; and from the Interstate Technology and Regulatory Council's (ITRC) *Standards and guidance values for PFAS in groundwater, drinking water, and surface water/effluent (wastewater)* PFAS Water and Soil Values Table, last updated in April 2022 (available for download here: https://pfas-1.itrcweb.org/fact-sheets/).

[b] Only states with final guidelines are included in the table. Note: EPA regions report that New Jersey and New York are developing guidelines for GenX chemicals.

Table 2 provides drinking water guideline values for GenX chemicals that have been developed by international agencies; the Interstate Technology and Regulatory Council (ITRC) only reported guideline values for GenX chemicals for the Netherlands (ITRC, 2022). The guidelines presented are indicative levels for severe pollution in drinking water (660 ppt or ng/L) and groundwater (140,000 ppt or ng/L). Other countries may be developing guidelines for GenX chemicals.

Table 2. International Guideline Values for GenX Chemicals

Country[a,b]	GenX Chemical Level (ppt [ng/L])	Standard/Guidance	Type of Medium	Reference
The Netherlands	660	Indicative Level for Severe Pollution	Drinking water	ITRC (2022)
	140,000	Indicative Level for Severe Pollution	Groundwater	

Notes:

[a] The information was collected from ITRC *Standards and guidance values for PFAS in groundwater, drinking water, and surface water/effluent (wastewater)* PFAS Water and Soil Values Table, last updated in April 2022 (available for download here: https://pfas-1.itrcweb.org/fact-sheets/).

[b] Only countries with guideline values provided in the ITRC table are included; other countries may be developing guidelines for GenX chemicals.

1.3 Uses and Sources of GenX Chemicals

GenX chemicals are used to manufacture fluoropolymers. Since GenX chemicals are substitutes for PFOA, products (e.g., some nonstick coatings, aqueous film-forming foam [AFFF]) that were previously made using PFOA may now rely on GenX chemicals. PFOA was phased out between 2006 and 2015 in the United States under an agreement between EPA and eight major PFAS companies under the PFOA Stewardship Program[5] established in 2006. According to the Chemours Company,[6] fluoropolymers have "countless" industrial applications, including in the medical, automotive, electronics, aerospace, energy, and semiconductor industries.[7] The Chemours Company uses GenX chemicals to produce four trademarked fluoropolymers: Teflon™ polytetrafluoroethylene (PTFE), Teflon™ perfluoroalkoxy (PFA), Teflon™ fluorinated ethylene propylene (FEP), and Teflon™ amorphous fluoropolymer (AF) (Chemours, 2022). GenX chemicals may also be generated as a byproduct of fluoromonomer production. There is a paucity of publicly available information on specific end-use products made with GenX chemicals.

Potential sources of GenX chemicals in the environment include industrial facilities that use GenX technology for fluoropolymer or fluoromonomer production, and contaminated water, air, soil, and biosolids. GenX chemicals have been detected around the globe, in surface water, groundwater, finished drinking water, rainwater, air, soil, and sediment as further described below and in U.S. EPA (2021a).

1.4 Environmental Fate, Occurrence in Water, and Exposure to Humans

1.4.1 Environmental Fate and Transport in the Environment

As noted in U.S. EPA (2021a), HFPO dimer acid and its ammonium salt are stable to photolysis, hydrolysis, and biodegradation. The degradation data suggest that they will be persistent (i.e., have a half-life [$t_{1/2}$] longer than six months) in air, water, soil, and sediments. Measured physical-chemical and sorption data indicate that GenX chemicals are expected to run off into surface water and to leach to groundwater from soil and landfills. Based on chemicals with similar properties (e.g., PFOA), HFPO dimer acid and its ammonium salt might undergo long-range atmospheric transport in the vapor phase and associate with particulates. They are not expected to be removed during conventional wastewater treatment or conventional drinking water treatment processes such as coagulation, flocculation, or sedimentation.

When released to the freshwater environment, HFPO dimer acid will dissociate to the HFPO carboxylate anion and hydronium cation (H_3O^+). The ammonium salt will dissolve to the HFPO carboxylate anion and the ammonium cation (NH_4^+). Both HFPO dimer acid and its ammonium salt are highly water-soluble and are expected to remain in water with low sorption to sediment or soil. Based on its high vapor pressure, the HFPO dimer acid can partition to air. The ammonium salt can also be transported in air, although the mechanism of vapor phase transport is not well understood (DuPont CCAS, 2009). In the vapor phase, the HFPO dimer acid and its

[5] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program
[6] The GenX processing technology and associated chemicals are products of The Chemours Company, a spin-off of DuPont de Nemours, Inc. (Chemours, 2015).
[7] https://www.epa.gov/system/files/documents/2022-03/3.18.22-request-for-correction-letter-and-exhibits_0.pdf

ammonium salt are expected to be stable to direct photolysis and will undergo hydroxyl radical-catalyzed indirect photolysis very slowly (U.S. EPA, 2021a).

1.4.2 Occurrence in Water

GenX chemicals can enter the aquatic environment through industrial discharges, runoff into surface water, and leaching into groundwater from soil and landfills (U.S. EPA, 2021a). GenX chemicals are water-soluble, with solubilities of greater than 751 grams per liter (g/L) and greater than 739 g/L for HFPO dimer acid and its ammonium salt, respectively, at 20°C (U.S. EPA, 2021a). Volatilization from water surfaces is expected to be an important fate process for both HFPO dimer acid and its ammonium salt (U.S. EPA, 2021a). Due to the limited number of U.S. occurrence studies on GenX chemicals, this section includes studies conducted outside as well as inside the U.S. to better understand sources and occurrence patterns in water.

1.4.2.1 Drinking Water

GenX chemicals were not included in the suite of PFAS analyzed in EPA's Third Unregulated Contaminant Monitoring Rule (UCMR 3) monitoring; thus, national GenX chemicals occurrence data from drinking water facilities are not available at this time (U.S. EPA, 2017a). However, occurrence data for GenX chemicals in drinking water are available, collected using EPA methods 533 and 537.1, from studies investigating areas known to be affected by GenX chemicals in a subset of U.S. states. GenX chemicals have been detected in the finished drinking water of at least nine states (ADEM, 2020; CDPHE, 2020; KYDEP, 2019; Michigan EGLE, 2021; NCDEQ, 2021, NHDES, 2021; Ohio DOH, 2021; SCDHEC, 2020; VTDEC, 2021). In states where sampling locations were selected randomly, the percentage of total samples that had concentrations of GenX chemicals above the reporting limit is generally well below 1%. Where targeted sampling has been performed, some states have found GenX chemicals at relatively higher concentrations, whereas in other states, the total number of samples with GenX chemicals is low or there are no detections. Further, EPA is aware of four states in which state-level monitoring efforts have found GenX chemicals in at least one finished water sample at a concentration above 0.010 micrograms per liter (µg/L) (10 ng/L). For example, the Kentucky Department for Environmental Protection (KYDEP, 2019) detected HFPO dimer acid in 11 post-treatment samples from statewide drinking water treatment plants (DWTPs) (median concentration of < 1.32 ng/L and maximum concentration of 29.7 ng/L). There were 10 detections of HFPO dimer acid at DWTPs that use surface water and one detection at a DWTP that uses groundwater; all detections occurred at DWTPs that use the Ohio River and Ohio River Alluvium as sources. Many of the DWTPs tested did not utilize treatment technologies that remove PFAS at that time.

In addition to those data collected by some states, GenX chemicals have been detected in three on-site production wells and one on-site drinking water well at the Chemours Washington Works facility outside of Parkersburg, West Virginia (U.S. EPA, 2021a). EPA subsequently requested that Chemours test for GenX chemicals in both raw and finished water at four PWSs and 10 private drinking water wells in Ohio and West Virginia near the Washington Works facility. Chemours completed the additional testing in February 2018 and reported HFPO dimer acid concentrations of < 0.010–0.081 µg/L in the PWS samples before treatment and < 0.010–0.052 µg/L in the private drinking water wells before treatment (U.S. EPA, 2018). Results for all samples collected after treatment were below the reporting limit of 0.010 µg/L (10 ng/L)

achievable at that time (U.S. EPA, 2018). Additionally, a study by Galloway et al. (2020) analyzed eight drinking water samples from public buildings (e.g., schools and libraries) and private wells located more than 27 kilometers (km) northeast of the Washington Works facility. HFPO dimer acid was detected in only one sample, and at a concentration below the limit of quantification (LOQ).

Three published studies evaluated the occurrence of GenX chemicals in drinking water near Cape Fear River in North Carolina (McCord et al., 2018; Pritchett et al., 2019; Sun et al., 2016). In finished drinking water collected from a DWTP downstream of a fluorochemical manufacturer, McCord et al. (2018) reported an HFPO dimer acid concentration of approximately 500 ng/L. After this sampling, the fluorochemical manufacturer diverted waste stream emissions from one of its manufacturing lines, and subsequent measured concentrations at this location were close to or below the North Carolina Department of Health and Human Services (NCDHHS) provisional health goal (PHG) of 140 ng/L. Pritchett et al. (2019) reported that according to the North Carolina Department of Environmental Quality (NCDEQ), as of April 2018, 207 out of 837 private wells (25%) within a 5-mile radius of a PFAS manufacturing facility in the Cape Fear River basin had levels of GenX chemicals exceeding the NCDHHS PHG of 140 ng/L, with a maximum measured concentration of 4,000 ng/L. Sun et al. (2016) analyzed finished drinking water from a DWTP downstream of a PFAS manufacturing site and reported HFPO dimer acid concentrations of ~475 ng/L.

Three European studies on GenX chemicals occurrence in drinking water were identified: two studies that analyzed drinking water samples from the vicinity of the same fluorochemical plant in the Netherlands (Brandsma et al., 2019; Gebbink et al., 2017), and a third that analyzed drinking water from areas of Belgium and the Netherlands, some of which were in the vicinity of known PFAS point sources (Vughs et al., 2019). Gebbink et al. (2017) detected HFPO dimer acid in drinking water samples from three of four sites in the vicinity of the fluorochemical plant, at concentrations of 0.25, 0.48, and 11 ng/L, respectively. All three sites at which HFPO dimer acid was detected were downstream of the plant; the high concentration of 11 ng/L was measured at the downstream site closest to the plant. HFPO dimer acid was not detected in samples from two control sites nor in a sample from a site upstream of the plant. Brandsma et al. (2019) analyzed drinking water at residential homes from six different municipalities within 50 km of the same fluorochemical plant featured in the study by Gebbink et al. (2017). The measured levels of HFPO dimer acid ranged from 1.4 to 8.1 ng/L; the highest concentration (8.1 ng/L) was measured at the sampling site that was closest to and downstream of the plant. Vughs et al. (2019) analyzed drinking water from 11 water suppliers at sites in Belgium and the Netherlands, some of which were in the vicinity of a fluoropolymer manufacturing plant. HFPO dimer acid was detected in 46% of samples, with a mean concentration of 2.9 ng/L and maximum concentration of 28 ng/L. The study reported that concentrations above 4 ng/L were measured in drinking water from suppliers that sourced surface water in the vicinity of the fluoropolymer manufacturing plant in the Netherlands. However, the study did not map the distribution of reported concentrations by geographic location or with respect to distance from the fluoropolymer manufacturing plant.

1.4.2.2 Groundwater

Petre et al. (2021) quantified the mass transfer of PFAS, including GenX chemicals, from contaminated groundwater to five tributaries of the Cape Fear River. All sampling sites were

located within 5 km of a manufacturing plant known known to be a major source of PFAS contamination. HFPO dimer acid and another fluoroether (perfluoro-2-[perfluoromethoxy] propanoic acid) together accounted for 61% of the total quantified PFAS. The study authors calculated that approximately 32 kg/year of PFAS is discharged from contaminated groundwater to the five tributaries. These data indicate that the discharge of contaminated groundwater has led to long-term contamination from GenX chemicals in surface water and could lead to subsequent impacts on downstream drinking water (Petre et al., 2021).

In a European study, Vughs et al. (2019) reported that HFPO dimer acid was not detected in any of five samples of groundwater obtained from water suppliers in the Netherlands and Belgium. Some sampling locations were in the vicinity of a fluoropolymer manufacturing plant, but the study did not identify the locations of sites relative to the plant.

1.4.2.3 Surface Water

Chemours has reported that GenX chemicals have been discharged into the Cape Fear River for several decades as a byproduct of other manufacturing processes (NCDEQ, 2017). Additionally, several studies evaluated the occurrence of GenX chemicals in surface waters, with studies conducted in North America, Europe, Asia, and across multiple continents (see Appendix B, Table B-1). As noted in the final toxicity assessment for GenX chemicals (U.S. EPA, 2021a), GenX chemicals were first detected in North Carolina's Cape Fear River and its tributaries in the summer of 2012 (Pritchett et al., 2019; Strynar et al., 2015). Since that finding, U.S. studies of surface waters, some of which are source waters for PWSs, have reported results of sampling efforts from contaminated areas near the Cape Fear River (McCord et al., 2018; Sun et al., 2016) and in Ohio and West Virginia (Galloway et al., 2020).

In studies of the Cape Fear River basin by McCord et al. (2018) and Sun et al. (2018), surface water concentrations of GenX chemicals ranged from below the NCDHHS PHG of 140 ng/L to a maximum level of 4,560 ng/L. Sun et al. (2016) analyzed surface water from two sites upstream of a DWTP and one site downstream. They reported a median HFPO dimer acid concentration of 304 ng/L with a maximum of 4,560 ng/L in the source water of the plant. HFPO dimer acid levels did not exceed the quantitation limit (10 ng/L) at the two upstream locations. In source water samples collected from the Cape Fear River near a DWTP downstream of a fluorochemical manufacturer, McCord et al. (2018) reported initial HFPO dimer acid concentrations of approximately 700 ng/L. After the manufacturer diverted waste stream emissions from one of its manufacturing lines, the measured concentrations decreased to levels below the NCDHHS PHG (140 ng/L).

In Ohio and West Virginia, Galloway et al. (2020) sampled rivers and streams located upstream, downstream, and downwind to the north and northeast of the Chemours Washington Works facility outside Parkersburg, West Virginia. The downwind sampling was intended to explore potential airborne deposition. Some of the downstream sampling sites were in the vicinity of landfills. Reported levels of HFPO dimer acid in these waters ranged from non-detectable levels to a maximum of 227 ng/L. The highest HFPO dimer acid concentrations were measured downwind of the facility (i.e., to the northeast). The study observed an exponentially declining trend of HFPO dimer acid concentrations in surface water with distance from the facility in this direction and attributed its occurrence in surface water to air dispersion of emissions from the

facility. The most distant site where HFPO dimer acid was detected was 24 km north of the facility.

In one study of sites located in highly industrialized commercial waterways (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources), Pan et al. (2018) detected HFPO dimer acid in 100% of samples from sites in the Delaware River (n=12), reporting median and maximum concentrations of 2.02 ng/L and 8.75 ng/L, respectively, in surface waters.

Globally, GenX chemicals occurrence has been reported in surface waters from Germany (Heydebreck et al., 2015; Pan et al., 2018), China (Heydebreck et al., 2015; Li et al., 2020a; Pan et al., 2017, 2018; Song et al., 2018), the Netherlands (Gebbink et al., 2017; Heydebreck et al., 2015; Pan et al., 2018), the United Kingdom (Pan et al., 2018), South Korea (Pan et al., 2018), and Sweden (Pan et al., 2018). HFPO dimer acid was also detected with a mean concentration of 30 picograms per liter (pg/L; 0.030 ng/L) in Artic seawater samples, suggesting long-range transport (Joerss et al., 2020).

In one study of surface water collected from industrialized areas in Europe (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources), Pan et al. (2018) reported HFPO dimer acid detections in 100% of samples from the Thames River in the United Kingdom (n=6 sites), the Rhine River in Germany and the Netherlands (n=20 sites), and the Malaren Lake in Sweden (n=10 sites). Across these three river systems, median HFPO dimer acid concentrations ranged from 0.90 to 1.38 ng/L and the highest concentration detected was 2.68 ng/L.

Heydebreck et al. (2015) detected HFPO dimer acid at 17% of sampling locations on the industrialized non-estuarine reaches of the Rhine River, with a maximum concentration of 86.08 ng/L; however, HFPO dimer acid was not detected at locations on the Elbe River.

Gebbink et al. (2017) evaluated surface water samples upstream and downstream of a fluorochemical production plant in the Netherlands and reported only one of three samples upstream of the plant with detectable HFPO dimer acid concentrations (22 ng/L; method quantification limit [MQL] = 0.2 ng/L). Downstream of the fluorochemical plant, HFPO dimer acid was detected in 100% of samples, with a mean concentration of 178 ng/L and a range of 1.7 to 812 ng/L. Vughs et al. (2019) analyzed surface water from 11 water suppliers in the Netherlands and Belgium, some of which were located in the vicinity of a fluoropolymer manufacturing plant. The authors reported HFPO dimer acid detections in 77% of surface water samples (n=13) with a mean concentration of 2.2 ng/L and a maximum of 10.2 ng/L; however, only three samples in the study had HFPO dimer acid concentrations exceeding 1 ng/L.

Of the five studies conducted in China, one study evaluated surface water samples from an industrialized region (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources) (Pan et al., 2018), one study evaluated surface water river and reservoir samples in an industrialized river basin with potential PFAS point sources (Li et al., 2020a), and three studies examined samples from sites along the Xiaoqing river at locations upstream, downstream, or in the vicinity of known PFAS sources (Heydebreck et al., 2015; Pan et al., 2017; Song et al., 2018). GenX chemicals were detected in freshwater systems sampled in all five studies, though HFPO dimer acid concentrations appeared to be positively correlated

with proximity to known PFAS point sources. Song et al. (2018), Pan et al. (2017), and Heydebreck et al. (2015) sampled sites in the Xiaoqing River system, including one of its tributaries, nearby a known fluoropolymer production facility. These three studies reported maximum HFPO dimer acid concentrations of 9,350, 2,060, and 3,060 ng/L, respectively. HFPO dimer acid concentrations in samples collected upstream of the facility did not exceed 3.64 ng/L. Other Chinese freshwater systems evaluated in the other two studies (Li et al., 2020a; Pan et al., 2018) generally reported maximum concentrations similar to those from the upstream Xiaoqing River system sites (≤ 10.3 ng/L), except for one site in Tai Lake which was reported to have a maximum HPFO dimer acid concentration of 143 ng/L. Similarly, in a study that sampled an industrialized river in South Korea (authors did not report whether sampling sites were in the vicinity of known PFAS point sources), HFPO dimer acid was found in 100% of samples and the maximum concentration found was 2.49 ng/L (Pan et al., 2018).

1.4.3 Exposure in Humans

As described in the *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"* (U.S. EPA, 2021a), PFAS including GenX chemicals were analyzed in 2,682 urine samples of children ≥ 6 years of age collected as part of the 2013–2014 National Health and Nutrition Examination Survey (NHANES) (Calafat et al., 2019). GenX chemicals were detected (limit of detection of 0.1 µg/L) in the urine in approximately 1.2% of the population, though this limit of detection is 10-fold greater than the lifetime HA, which may lead to the low rate of urine positivity. The finding for GenX chemicals was similar to PFOA and PFOS which were only detected in paired urine samples for < 0.1% of the same population. In serum samples, PFOA and PFOS were detected in > 98% of this same study population (HFPO dimer acid was not measured), demonstrating that serum is a better biomarker than urine for PFAS.

The Chemours Company submitted a report to EPA of their analysis of HFPO dimer acid assessment in 24 human plasma samples. The results of their analysis are publicly available in a truncated study report that does not appear to be peer-reviewed or be the results of an epidemiology study. The results of their analysis found HFPO dimer acid at concentrations ranging from 1.0 ng/mL (reporting limit) to 51.2 ng/mL in plasma samples (DuPont-C30031_516655, 2017). HFPO dimer acid was not detected above the analytical reporting limit of less than 1.0 ng/mL in seven of the samples. However, it is important to note that interpretation of these results is difficult given that the publicly available information is lacking study design details, study participant characteristics, or exposure detail (e.g., "some of these workers are in areas with potential for exposure, others are not.")

Concern in the Cape Fear Watershed communities about the detection of GenX chemicals in water led to the initiation of a human exposure study in this area.[8] In blood samples from 344 Wilmington, North Carolina residents collected between November 2017 and May 2018 (including repeat sampling of 44 participants), GenX chemicals were not detected above the analytical reporting limit of 2 ng/mL in any of the blood samples collected (Kotlarz et al., 2020). It is difficult to draw conclusions about GenX exposure because discharge control of GenX chemicals from the nearby Chemours Fayetteville Works plant began in June of 2017 and by

[8] See GenX Exposure Study website, located at https://genxstudy.ncsu.edu/

September of 2017, the facility stopped discharging process wastewater containing PFAS into the Cape Fear River. Also, it is unknown whether study participants were drinking tap water, bottled water, or filtered tap water at the time of sample collection. GenX chemicals were not detected in a study from the Cape Fear River that measured concentrations of GenX chemicals and other PFAS in the urine and serum of nearby residents who had high concentrations of GenX in their drinking water wells (Pritchett et al., 2019). The authors indicated that it was not known if residents were using the well water or bottled water, but this finding does support the shorter $t_{1/2}$ in humans for GenX chemicals in comparison to other PFAS.

2.0 Problem Formulation and Scope

2.1 Conceptual Model

A conceptual model provides useful information to characterize and communicate the potential health risks related to GenX chemicals exposure from drinking water and to outline the scope of the HA. The sources of GenX chemicals, the routes of exposure for biological receptors of concern (e.g., various human activities related to tap water ingestion such as drinking, food preparation, and consumption), the potential health effects, and exposed populations including sensitive populations and life stages are depicted in the conceptual diagram below (Figure 1).

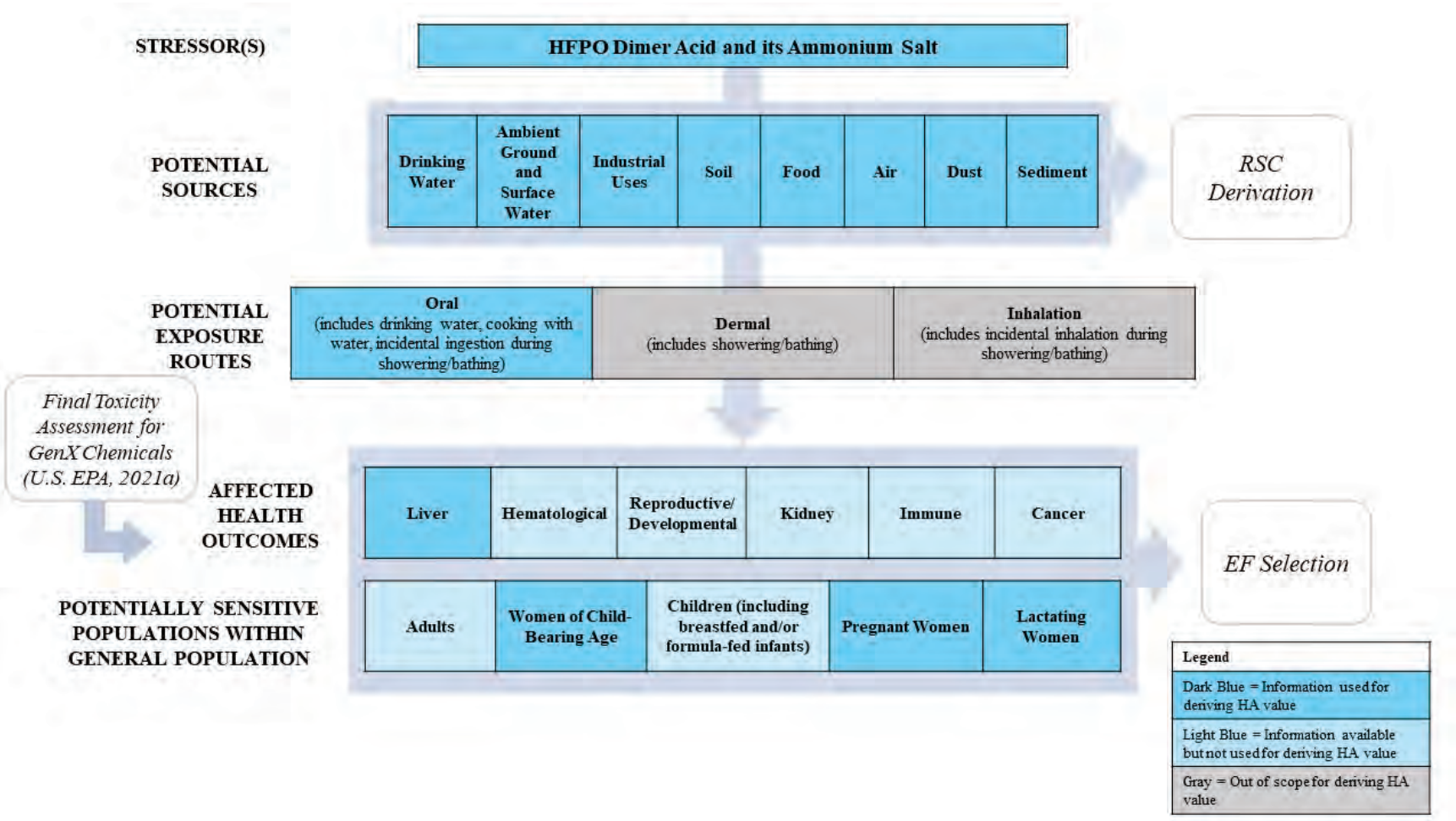

Figure 1. Conceptual Model for the Development of the Drinking Water Health Advisory for GenX Chemicals.

The conceptual model is intended to explore potential links between exposure to a contaminant or stressor and the adverse health outcomes, and to outline the information sources used to identify or derive the input values used for the HA derivation, which are the RfD, relative source contribution (RSC), and exposure factor (EF). The conceptual model also illustrates the scope of the GenX chemicals HA, which considers the following factors:

Stressors: The scope of this drinking water HA includes the two main GenX chemicals, the HFPO dimer acid and its ammonium salt, consistent with the scope of the 2021 toxicity assessment for GenX chemicals (U.S. EPA 2021a). The HFPO dimer acid and its ammonium salt are the two current commercial products of the GenX technology.

Potential Sources of Exposure: The scope of exposure sources considered for the HA derivation is limited to drinking water from public water facilities or private wells. Sources of exposure to GenX chemicals include both ground and surface waters used for drinking. To develop the RSC, information about non-drinking water sources was identified to determine the portion of the RfD attributable to drinking water. Non-drinking water sources of GenX chemicals for which studies were identified include foods, indoor dust, soil, air, and sediment. Consumer products and biosolids are other potential sources of exposure but relevant studies were not identified (see Section 3.3.1). Since GenX chemicals are replacements for PFOA, they could be present in consumer products (e.g., stain- and water-repellent textiles). Information on specific products containing GenX chemicals is not available, but they may be present in consumer products within the home, workplace, schools, and daycare centers.

Potential Exposure Routes: Oral exposure to GenX chemicals from contaminated drinking water sources (e.g., via drinking water, cooking with water, and incidental ingestion from showering) is the focus of the HA. The drinking water HA value does not apply to other exposure routes. However, information on other potential routes of exposure including dermal exposure (contact of exposed parts of the body with water containing GenX chemicals during bathing, showering, etc.) and inhalation exposure (during bathing or showering, using a humidifier or vaporizer, etc.) was considered to develop the RSC.

Affected Health Outcomes: The toxicity assessment for GenX chemicals (U.S. EPA, 2021a) considered all publicly available human, animal, and mechanistic studies of effects after exposure to GenX chemicals. The evaluation identified associations between GenX chemicals exposure and the following health outcomes: hepatic, hematological, developmental/ reproductive, renal, immune and cancer.

Potentially Sensitive Populations and Life Stages: The receptors are humans in the general population who could be exposed to GenX chemicals from tap water through ingestion at their homes and other places (e.g., workplaces, schools, daycare centers). Within the general population, there are potentially sensitive populations or life stages that may be more susceptible due to increased exposure and/or response. Potentially sensitive populations include pregnant women, women of childbearing age, and lactating women.

2.2 Analysis Plan

2.2.1 Health Advisory Guidelines

Assessment endpoints for HA guidelines or values can be developed, depending on the available data, for both short-term (one-day and ten-day) and lifetime exposure using information on the noncarcinogenic and carcinogenic toxicological endpoints of concern. Where data are available, HAs can reflect sensitive populations or life stages that may be more susceptible and/or more highly exposed.

One-Day HA is protective of noncancer effects for up to 1 day of exposure and is typically based on an *in vivo* toxicity study with a duration of 7 days or less. It is typically calculated for an infant.

Ten-Day HA is protective of noncancer effects for up to 10 days of exposure and is typically based on an *in vivo* toxicity study with a duration of 7 to 30 days. It is typically calculated for an infant.

Lifetime HA is designed to be protective of noncancer effects over a lifetime of exposure and is typically based on a chronic *in vivo* experimental animal toxicity study and/or human epidemiological data.

10^{-6} Cancer Risk Concentration is the concentration of a carcinogen in water at which the population is expected to have a one in a million (10^{-6}) excess cancer risk above background after exposure to the contaminant over a lifetime. It is calculated for carcinogens classified as known or likely human carcinogens (U.S. EPA, 1986, 2005b). Cancer risk concentrations are not derived for substances for which there is suggestive evidence of carcinogenic potential unless the cancer risk has been quantified.

2.2.2 Sources of Toxicity Information for Health Advisory Development

The final toxicity assessment for GenX chemicals, entitled *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"* published in October 2021 (U.S. EPA, 2021a), serves as the basis of the toxicity information and chronic RfD used to derive the lifetime noncancer HA for GenX chemicals. It also synthesizes and describes other information on GenX chemicals including physiochemical properties and toxicokinetics. This final toxicity assessment was published after a rigorous process of literature review, draft assessment development, agency and interagency review, an independent peer review, public comment, an independent expert review of data from two studies by the National Toxicology Program, and a second independent peer review.

2.2.3 Approach and Scope for Health Advisory Derivation

2.2.3.1 Approach for Deriving Noncancer HAs

The following equations (Eqs. 1–3) are used to derive the HAs.[9] Lifetime HAs and 10^{-6} cancer risk concentrations are only derived for chemicals without an existing National Primary Drinking Water Regulation.

$$\textbf{One-Day HA} = \left(\frac{\text{POD}}{\text{UF}_\text{C} * \text{DWI-BW}}\right) \quad \text{(Eq. 1)}$$

POD is typically derived from a toxicity study of duration 7 days or less

$$\textbf{Ten-Day HA} = \left(\frac{\text{POD}}{\text{UF}_\text{C} * \text{DWI-BW}}\right) \quad \text{(Eq. 2)}$$

POD is typically derived from a toxicity study of duration 7–30 days

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \quad \text{(Eq. 3)}$$

RfD is typically derived from a chronic study

Where:

POD is the point of departure, typically a lowest observed adverse effect level (LOAEL), a no observed adverse effect level (NOAEL), or a benchmark dose (BMD) (lower confidence limit; BMDL) from the critical study.

UF_C is the composite UF or total UF value after multiplying individual UFs. UFs are established in accordance with EPA best practices (U.S. EPA, 2002) and consider uncertainties related to the following: variation in sensitivity among the members of the human population (i.e., inter-individual variability), extrapolation from animal data to humans (i.e., interspecies uncertainty), extrapolation from data obtained in a study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure), extrapolation from a LOAEL rather than from a NOAEL, and extrapolation when the database is incomplete. For GenX chemicals, the value of UF_C was determined in the final toxicity assessment (U.S. EPA, 2021a).

DWI-BW is the 90th percentile drinking water intake (DWI), adjusted for body weight (bw), for the selected population in units of liter per kilogram body weight per day (L/kg bw-day). The DWI-BW considers direct and indirect consumption of tap water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea and coffee). For GenX chemicals, the value of this parameter is based on the critical study identified in the GenX

[9] https://www.epa.gov/system/files/documents/2022-01/dwtable2018.pdf

chemicals final toxicity assessment (U.S. EPA, 2021a), and is identified in Chapter 3 of EPA's Exposure Factors Handbook (EFH) (U.S. EPA, 2019a).

RfD is the chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime. The value of this parameter was derived in the final GenX chemicals toxicity assessment and is based on the critical effect and study identified in that assessment (U.S. EPA, 2021a).

RSC is the relative source contribution—the percentage of the total oral exposure attributed to drinking water sources (U.S. EPA, 2000a) where the remainder of the exposure is allocated to other routes or sources. The RSC is calculated by examining other sources of exposure (e.g., air, food, soil) and pathways of exposure in addition to drinking water using the methodology described for calculation of an RSC described in U.S. EPA (2000a) and Section 3.3.2.

2.2.3.2 Scope of Noncancer Health Advisory Values

Adequate data are available to derive a lifetime HA for GenX chemicals. EPA's final toxicity assessment for GenX chemicals derived subchronic and chronic RfDs but not an acute or short-term RfD (U.S. EPA, 2021a). Due to the lack of an available short duration (30 day or less exposure duration) toxicity value for GenX chemicals, EPA did not develop a one-day or ten-day HA value. Specifically, EPA did not derive an RfD for durations of 7-day or less exposure period on which to base a one-day HA or an RfD for a 7-to-30-day exposure on which to base a ten-day HA for GenX chemicals in the toxicity assessment (U.S. EPA, 2021a). Information about the available acute and short-term toxicity studies for HFPO dimer acid and its ammonium salt can be found in Sections 4.1 and 4.2 and Appendix B of the toxicity assessment (U.S. EPA, 2021a).

2.2.3.3 Approach and Scope for Deriving Cancer Risk Concentrations

The following equations (Eqs. 4–5) are used to derive cancer risk concentrations.

Calculated for non-mutagenic carcinogens[10] only:

$$\mathbf{10^{-6}\ Cancer\ Risk\ Concentration} = \frac{1\text{x}10^{-6}}{\text{CSF} * \text{DWI-BW}} \qquad \text{(Eq. 4)}$$

Calculated for mutagenic carcinogens only:

$$\mathbf{10^{-6}\ Cancer\ Risk\ Concentration} = \frac{1\text{x}10^{-6}}{\text{CSF}} * \sum_{i}\left(\frac{F_i * ADAF_i}{\text{DWI-BW}_i}\right) \qquad \text{(Eq. 5)}$$

[10] https://www.epa.gov/system/files/documents/2022-01/dwtable2018.pdf

Where:

CSF is the cancer slope factor—an upper bound, approximating a 95 percent confidence limit of the increased cancer risk from a lifetime of oral exposure to a stressor. The value for this parameter is derived in the final toxicity assessment when data are available.

$DWI\text{-}BW_i$ is the 90th percentile bw-adjusted DWI in units of L/kg bw-day for each age group (i), considered when calculating cancer risk concentrations for mutagenic carcinogens.

$ADAF_i$ is the age-dependent adjustment factor for each age group (i), used when calculating cancer risk concentrations for carcinogens that act via a mutagenic mode of action (U.S. EPA, 2005a,b).

F_i is the fraction of life spent in each age group (i), used when calculating cancer risk concentrations for mutagens (U.S. EPA, 2005a).

2.2.3.4 Scope of Cancer Risk Concentration Derivation

For cancer toxicity, EPA's toxicity assessment for GenX chemicals (U.S. EPA, 2021a) evaluated the weight of the evidence for cancer among the available cancer studies for GenX chemicals exposure per EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b). Based on the evaluation of the limited (i.e., one study) data for GenX chemicals, EPA concluded that there is *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans. EPA's conclusion is based on the findings of female hepatocellular adenomas and hepatocellular carcinomas and male combined pancreatic acinar adenomas and carcinomas observed in the chronic 2-year study in rats (for more information see U.S. EPA [2021a]). The single cancer bioassay for HFPO dimer acid ammonium salt showed increased incidence of liver tumors (females) and combined pancreatic acinar adenomas and carcinomas (males) in rats at the high doses only. A CSF was not derived in the toxicity assessment for GenX chemicals (U.S. EPA, 2021a). This is consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) which state that when the available evidence is suggestive for carcinogenicity, a quantitative risk estimate is generally not derived unless there exists a well-conducted study that could facilitate an understanding of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a). In the toxicity assessment for GenX chemicals, EPA concluded that the available human and animal studies are not sufficient to establish a reasonable understanding of the magnitude and uncertainty of potential risks for exposure to GenX chemicals and tumor incidence, and therefore do not justify a quantitative cancer assessment (U.S. EPA, 2021a). Consistent with EPA's guidelines, a CSF was not derived in the toxicity assessment for GenX chemicals (U.S. EPA, 2021a). Therefore, EPA did not derive a 10^{-6} cancer risk concentration in this HA for GenX chemicals.

2.2.4 Exposure Factors for Deriving Health Advisory

2.2.4.1 Exposure Factor Selection

An EF, such as body weight-adjusted drinking water intake (DWI-BW), is one of the input values for deriving a drinking water HA. EFs are factors related to human activity patterns, behavior, and characteristics that help determine an individual's exposure to a contaminant. EPA's EFH[11] is a resource for conducting exposure assessments and provides EFs based on

[11] EPA's EFH is available at https://www.epa.gov/expobox/about-exposure-factors-handbook

information from publicly available, peer-reviewed studies. Chapter 3 of the EFH presents EFs in the form of DWIs and DWI-BWs for various populations or life stages within the general population (U.S. EPA, 2019a). The use of EFs in HA calculations is intended to protect sensitive populations and life stages within the general population from adverse effects resulting from exposure to a contaminant.

When developing HAs, the goal is to protect all ages of the general population including potentially sensitive populations or life stages such as children. The approach to select the EF for the drinking water HA includes a step to identify sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can make it difficult to identify the most sensitive population (e.g., not all windows of exposure or health outcomes have been assessed in studies of GenX chemicals), the critical effect and POD that form the basis for the RfD can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In such cases, EPA can select the corresponding DWI-BW for that sensitive population or life stage from the EFH (U.S. EPA, 2019a) to derive the HA. When multiple populations or life stages are identified based on the critical effect or other health effects data (from animal or human studies), EPA selects the population or life stage with the greatest DWI-BW because it is the most health protective. For deriving lifetime HAs, the RSC corresponding to the sensitive life stage is also determined (see Section 3.3), and the most health-protective RSC is selected when data are available for multiple sensitive populations or life stages. In the absence of information indicating a sensitive population or life stage, the DWI-BW corresponding to all ages of the general population may be selected.

To derive a chronic HA, EPA typically uses DWI normalized to body weight (i.e., DWI-BW in L of water consumed/kg bw-day) for all ages of the general population or for a sensitive population or life stage, when identified. The Joint Institute for Food Safety and Applied Nutrition's Food Commodity Intake Database (FCID) Consumption Calculator Tool[12] includes the EFs from EPA's EFH and can also be used to estimate DWI-BW for specific populations or life stages across a designated age range. EPA uses the 90th percentile DWI-BW to ensure that the HA is protective of the general population as well as sensitive populations or life stages (U.S. EPA, 2000a, 2016a). In 2019, EPA updated its EFs for DWI and DWI-BW based on newly available science (U.S. EPA, 2019a).

Table 3 shows EPA EFs for some sensitive populations or life stages. Other populations or life stages may also be considered depending on the available information regarding sensitivity to health effects after exposure to a contaminant.

[12] Joint Institute for Food Safety and Applied Nutrition's FCID, Commodity Consumption Calculator is available at https://fcid.foodrisk.org/percentiles

Table 3. EPA Exposure Factors for Drinking Water Intake

Population or Life Stage	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
General population (all ages)	0.0338	90th percentile direct and indirect consumption of community water, consumer-only two-day average, all ages.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)
Children	0.143	90th percentile direct and indirect consumption of community water, consumer-only two-day average, birth to < 1 year.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)
Formula-fed infants	0.249	90th percentile direct and indirect consumption of community water, formula-consumers only, 1 to < 3 months. Includes water used to reconstitute formula plus all other community water ingested.	Kahn et al. (2013), Estimates of Water Ingestion in Formula by Infants and Children Based on CSFII 1994–1996 and 1998[a,b]
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Lactating women	0.0469	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[c] (U.S. EPA, 2019a)

Notes: CSFII = continuing survey of food intake by individuals; L/kg bw-day = liter per kilogram body weight per day.

[a] The sample size does not meet the minimum reporting requirements as described in the Third Report on Nutrition Monitoring in the United States (LSRO, 1995).

[b] Chapter 3.2.3 in U.S. EPA (2019a) cites Kahn et al. (2013) as the source of drinking water ingestion rates for formula-fed infants. While U.S. EPA (2019a) provides the 95th percentile total direct and indirect water intake values, Office of Water/Office of Science and Technology (OW/OST) policy is to utilize the 90th percentile DWI-BW. OW/OST was able to identify the 90th percentile DWI-BW in Kahn et al. (2013) and report the value in this table.

[c] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports: Human Nutrition Information Service (HNIS)/National Center for Health Statistics (NCHS) Analytical Working Group Recommendations (NCHS, 1993).

2.2.4.2 Determining Proportion of RfD Attributable to Drinking Water

To account for aggregate risk from exposures and exposure pathways other than oral ingestion of drinking water, EPA applies an RSC when calculating HAs to ensure that total human exposure to a contaminant does not exceed the daily exposure associated with the RfD. The RSC represents the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee, tea, or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD is allocated to other potential exposure sources (U.S. EPA, 2000a). The purpose of the RSC is to ensure that the level of a contaminant (e.g., HA value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD (U.S. EPA, 2000a).

To determine the RSC, EPA follows the Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment in EPA's guidance, *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a). EPA considers whether there are significant known or potential uses/sources other than drinking water, the adequacy of data and strength of evidence available for each relevant exposure medium and pathway, and whether adequate information on each source is available to quantitatively characterize the exposure profile. The RSC is developed to reflect the exposure to the general population or a sensitive population within the general population.

Per EPA's guidance, in the absence of adequate data to quantitatively characterize exposure to a contaminant, EPA typically recommends an RSC of 20%. When scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, thereby allocating the remaining 20% to other potential exposure sources (U.S. EPA, 2000a).

To inform the RSC determination, available information on all exposure sources and routes for GenX chemicals was identified using the literature search and screening method described in Appendix A. To identify information on GenX chemicals exposure routes and sources to inform RSC determination, EPA considered primary literature published between 2003–2020 and collected by EPA ORD as part of an effort to evaluate evidence for pathways of human exposure to eight PFAS, including GenX chemicals. To consider more recently published information on exposure to GenX chemicals, EPA incorporated the results of a date-unlimited gray literature search that was conducted in February 2022 as well as an ad hoc process to identify relevant and more recently published peer-reviewed scientific literature. The literature resulting from the search and screening process included only final (not draft) documents and articles that were then reviewed to inform the RSC for GenX chemicals.

3.0 Health Advisory Input Values

3.1 Toxicity Assessment Values

Table 4 summarizes the peer-reviewed chronic noncancer toxicity values for HFPO dimer acid and its ammonium salt from EPA's *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"* (U.S. EPA, 2021a).

Table 4. Chronic Noncancer Toxicity Information for GenX Chemicals for Deriving the Lifetime HA

Health Assessment	GenX Chemicals Exposure in Critical Study	RfD (mg/kg bw-day)	Critical Effect	Principal Study
Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals" (U.S. EPA, 2021a)	Pre-mating day 14 through lactation day 21	$3x10^{-6}$	Constellation of liver lesions (defined by the National Toxicology Program Pathology Working Group to include cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental females	Oral reproductive and developmental toxicity study (Dupont 18405-1037, 2010)

Note: mg/kg bw-day = milligram per kilogram body weight per day.

As noted in EPA's toxicity assessment for GenX chemicals (U.S. EPA, 2021a), HFPO dimer acid and its ammonium salt, chronic and reproductive and developmental oral animal toxicity studies are available in rats and mice. Repeated-dose toxicity data are available for oral exposure. The available studies report liver toxicity (e.g., increased relative liver weight, hepatocellular hypertrophy, apoptosis, and single-cell/focal necrosis), kidney toxicity (e.g., increased relative kidney weight), immune effects (e.g., antibody suppression), hematological effects (e.g., decreased red blood cell count, hemoglobin, and hematocrit), reproductive/developmental effects (e.g., increased early deliveries, placental lesions, changes in maternal gestational weight gain, and delays in genital development in offspring), and cancer (e.g., liver and pancreatic tumors) after exposure to GenX chemicals. The available toxicity study findings demonstrate that the liver is particularly sensitive to HFPO dimer acid and HFPO dimer acid ammonium salt exposure.

The critical study selected for deriving the noncancer subchronic and chronic RfDs for HFPO dimer acid and/or its ammonium salt was the oral reproductive/developmental toxicity study in mice that reported a NOAEL of 0.1 milligrams per kilogram body weight per day (mg/kg bw-day) based on liver effects (a constellation of lesions, including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) in females (DuPont-18405-1037, 2010; NTP, 2019). This endpoint was selected because the available health effects studies indicate that the liver is the most sensitive target of toxicity from exposure to GenX chemicals. Liver effects were observed in both male and female mice and rats after different doses and durations of exposures. These adverse liver effects occurred at the lowest doses and shortest durations of exposure to GenX chemicals among the available data (U.S. EPA, 2021). Importantly, EPA determined that the liver lesions observed in the rodent are relevant to human health (see U.S. EPA [2021a] for more information). Using EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), EPA modeled the dose-response relationship in the range of observed data. Additionally, EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011) was used to allometrically

scale a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans. Allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UFs). From BMD modeling of the DuPont-18045-1037 study, the resulting POD_{HED} is 0.01 mg/kg bw-day. For the chronic RfD, a composite UF of 3,000 was applied based on a 10X for intraspecies variability (UF_H), 3X for interspecies differences (UF_A), 10X for extrapolation from a subchronic to a chronic dosing duration (UF_S), and 10X for database deficiencies (UF_D) to yield a chronic RfD of 0.000003 mg/kg bw-day or 0.003 micrograms per kilogram body weight per day (µg/kg bw-day) (see U.S. EPA [2021a] for more details).

3.2 Exposure Factors

To identify potentially sensitive populations or life stages, EPA considered the sensitive life stage of exposure associated with the critical effect on which the chronic RfD was based. In the critical study selected in the toxicity assessment for GenX chemicals, parental female mice (approximately 10 weeks old at the start of the study) were dosed daily for 2 weeks prior to pairing, throughout gestation, and through to lactation day 20 for a total dosing duration of 53 to 65 days (Dupont 18405-1037, 2010). Therefore, exposure to GenX chemicals in the critical study corresponds to three potentially sensitive adult female life stages, women of childbearing age, pregnancy, and lactation (Table 5). For the calculation of the chronic HA for HFPO dimer acid and its ammonium salt, EPA interpreted the observation of adverse liver effects in parental females after exposure during pre-mating, pregnancy, and lactation as indicative of potentially sensitive populations relevant to the chronic exposure scenario. The available data do not permit a more precise identification of the most sensitive or critical window for GenX chemicals and the adverse liver effects because studies. However, after 10–16 days of dosing during the gestation period in mice, Blake et al. (2020) reported no significant changes in the observation of maternal liver necrosis or liver serum enzymes changes (i.e., alkaline phosphatase, alanine aminotransferase) in the 2 mg/kg bw-day dose group suggesting gestational dosing alone may be insufficient to produce adverse liver effects. These studies suggest the potential for critical windows of exposure across three potentially sensitive life stages: pre-conception or young adulthood, pregnancy, and lactation.

Given the available information, EPA identified three potentially sensitive life stages for GenX chemicals exposure—women of childbearing age (13 to < 50 years), pregnant women, and lactating women (Table 5). The Eq. used to calculate a drinking water lifetime HA (Eq. 3; also see Section 2.2.3) calculates the concentration of a contaminant in water based on the DWI for the sensitive population identified from the available studies (Chapter 3 in U.S. EPA, 2019a). Since all three life stages may represent critical windows of exposure to GenX chemicals and the DWI is higher for lactating women than for women of childbearing age or pregnant women, the DWI for lactating women was selected and is anticipated to be protective of the other two sensitive life stages.

Table 5. EPA Exposure Factors for Drinking Water Intake for Different Candidate Sensitive Populations Based on the Critical Effect and Study

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Lactating women	**0.0469**	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[a] (U.S. EPA, 2019a)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day. The DWI-BW used to calculate the GenX chemicals' lifetime HA is in bold.

[a] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports: HNIS/ NCHS Analytical Working Group Recommendations (NCHS, 1993).

3.3 Relative Source Contribution

As stated in the analysis plan, EPA collected and evaluated information about GenX chemicals exposure routes and sources to inform RSC determination. Results from the literature search are described below.

3.3.1 Non-Drinking Water Sources and Routes

EPA presents information below from studies performed in the United States as well as studies published globally for this emerging contaminant to be as comprehensive as possible, given that the overall information is limited. While the studies from non-U.S. countries inform an understanding of global exposure sources and trends, the RSC determination is based on the available data for the Unites States.

3.3.1.1 Dietary Sources

HFPO dimer acid was included in a suite of individual PFAS selected as part of PFAS-targeted reexaminations of samples collected for the U.S. Food and Drug Administration's (FDA's) Total Diet Study (U.S. FDA, 2020a,b, 2021a,b, 2022a,b); however, it was not detected in any of the food samples tested. It should be noted that FDA indicated that the sample sizes were limited and that the results should not be used to draw definitive conclusions about PFAS levels or presence in the general food supply (U.S. FDA, 2022c). HFPO dimer acid was not detected in cow milk samples collected from a farm with groundwater known to be contaminated with PFAS;

however, it was detected in produce (collard greens, cabbage) collected from an area near a PFAS production plant in FDA studies of the potential exposure to the U.S. population to PFAS (U.S. FDA 2018, 2021c). GenX chemicals were detected at low levels in 14% of vegetable garden crops (endive, beets, celery, lettuce, and tomatoes) grown near a PFAS manufacturing facility in the Netherlands (Mengelers et al., 2018; NCDEQ, 2018c).

Feng et al. (2021) measured HFPO dimer acid in food samples collected from up to ten home gardens or farms in villages within 15 km of a large fluoropolymer facility located on the Dongzhulong River in Shandong Province, China. The authors detected HFPO dimer acid in wheat (mean concentration: 5.53 nanograms per gram dry weight [ng/g dw]; range: 2.27–9.19 ng/g dw; detection frequency [DF] 100%), maize (mean concentration: 1.17 ng/g dw; range: not detected (ND)–1.94 ng/g dw; DF 80%), and vegetable samples (mean concentration: 20.1 ng/g dw; range: ND–67.2 ng/g dw; DF 82%). In fish collected at two sites along the Dongzhulong River, HFPO dimer acid was detected at concentrations of 43.9 and 3.23 ng/g dw at sites approximately 3 km and 15 km downstream of the fluoropolymer facility, respectively. HFPO dimer acid was not found in eggs (home-produced and store-bought), store-bought meat or seafood, or milk from domestic goats (Feng et al., 2021). Except for the fish sampled at two sites, the study did not report HFPO dimer acid concentrations in food according to sampling location or proximity to the fluoropolymer facility.

GenX chemicals were not target chemicals in EPA's National Lake Fish Tissue Study or EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study and they were not target chemicals in EPA's 2008–2009 or 2013–2014 National Rivers and Streams Assessment studies (Stahl et al., 2014; U.S. EPA, 2009a, 2020a, 2021e). GenX chemicals were detected in a redear sunfish fillet composite sample collected from a privately-owned lake near a PFAS manufacturing facility in North Carolina at a concentration of 270 nanograms per kilogram (ng/kg) (wet weight tissue) (U.S. EPA, 2021a; NCDEQ, 2018c). GenX chemicals were not included in the National Oceanic and Atmospheric Administration's National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). Li et al. (2021) found HFPO dimer acid in fish collected from a Xiaoqing River estuary impacted by PFAS discharge from fluoropolymer manufacturing industry, at concentrations ranging from ND to 3.47 ng/g dw (mean concentration: 0.93 ng/g dw).

3.3.1.2 Consumer Products

Although no specific studies on the occurrence of GenX chemicals in consumer products were identified, DuPont began transitioning to GenX processing aid technology in 2009 to work toward eliminating long-chain PFAS as part of the company's commitment under the 2010/2015 PFOA Stewardship Program (U.S. EPA, 2021a). It is unknown if GenX chemicals in consumer products have increased as a result of this transition.

3.3.1.3 Indoor Dust

Feng et al. (2021) detected HFPO dimer acid in indoor dust samples taken from homes from 10 villages within 15 km of a large fluoropolymer facility in Shandong Province, China, at concentrations ranging from ND to 841 ng/g (mean concentration 159 ng/g; DF 72%). Contaminated dust was found in homes as far as 15 km from the fluoropolymer facility and HFPO dimer acid concentrations were highest in homes nearest to the facility. Although only

one study on the occurrence of GenX chemicals in indoor dust was identified, PFAS have been detected in indoor dust and on window films (ATSDR, 2021).

3.3.1.4 Air

PFAS have been released to air from wastewater treatment plants, waste incinerators, and landfills (U.S. EPA, 2016a). GenX chemicals could be transported in the vapor phase or with particulates (U.S. EPA, 2021a). When released to air or volatilized from water, GenX chemicals are stable and short- and long-range transport has occurred (D'Ambro et al., 2021; Galloway et al., 2020). Galloway et al. (2021) analyzed HFPO dimer acid concentrations in soil samples downwind of and surface water samples upstream of the Chemours Washington Works facility outside of Parkersburg, West Virginia, and results suggest atmospheric transport of HFPO dimer acid emissions. Additionally, a study that modeled the atmospheric transport of a PFAS mixture containing GenX chemicals from a fluoropolymer manufacturing facility in North Carolina (D'Ambro et al., 2021) predicted that only 2.5% of total GenX (consisting of HFPO dimer acid and HFPO dimer acid fluoride) would be deposited within 150 km of the facility (U.S. EPA, 2021a).

HFPO dimer acid and its ammonium salt are persistent in air (half-lives longer than 6 months), and they are not readily broken down by biodegradation, direct photolysis, or hydrolysis (U.S. EPA, 2021a). In the vapor phase, HFPO dimer acid and its ammonium salt are expected to undergo hydroxyl radical-catalyzed indirect photolysis slowly, with a predicted average hydroxylation rate of 8.50×10^{-13} cubic centimeters (cm^3)/molecule - second (U.S. EPA, 2021a, 2022a,b). Based on a measured vapor pressure of 2.7 mm Hg at 20°C for HFPO dimer acid, volatilization is expected to be an important fate process for this chemical (U.S. EPA, 2021a). EPA's Toxics Release Inventory reported release data for HFPO dimer acid and its ammonium salt in 2020 (U.S. EPA, 2022c). GenX chemicals are not listed as hazardous air pollutants (U.S. EPA, 2022d).

GenX chemicals have been identified in air emissions. NCDEQ estimates for the Chemours Fayetteville Works plant, located in the North Carolina Cape Fear watershed, indicate that annual emissions of GenX chemicals could have exceeded 2,700 pounds per year during the reporting period (2017–2018) (NCDEQ, 2018a). Rainwater samples collected within a seven-mile radius of this facility were reported to have detectable levels of GenX chemicals (NCDEQ, 2018b), with the highest concentration of 810 ng/L found in a rainwater sample collected five miles from the facility. The three samples collected seven miles from the plant had GenX chemicals concentrations ranging from 45.3 to 60.3 ng/L (NCDEQ, 2018b).

3.3.1.5 Soil

When HFPO dimer acid and its ammonium salt are deposited on or applied to soil, they are expected to run off into surface waters or rapidly leach to groundwater (U.S. EPA, 2021a). PFAS can also be taken up from contaminated soil by plants (ATSDR, 2021). No specific studies on the occurrence of GenX chemicals in biosolids were identified.

Two studies reported GenX chemicals concentrations in soil. In the United States, Galloway et al. (2020) analyzed 13 soil samples for HFPO dimer acid at locations in Ohio and West Virginia that were upstream and downwind of the Chemours Washington Works facility in order to

evaluate HFPO dimer acid contamination due to atmospheric deposition. HFPO dimer acid was detected in 5 out of 13 samples, with a maximum concentration of 8.14 ng/g dw. In China, Li et al. (2020a) collected and analyzed residential soil samples throughout the country from 31 provincial-level administrative regions (consisting of 26 provinces, 4 municipalities, and 1 special administrative region). HFPO dimer acid was detected in 40.5% of soil samples at concentrations up to 967 picograms per gram (pg/g) dw and a mean level of 19.1 pg/g dw. PFOA was detected in these soils more frequently (96.6%) and at higher mean levels (354 pg/g dw), leading the authors to conclude that HFPO dimer acid consumption was still limited at the national scale of China, despite its use as a PFOA replacement.

One study measured concentrations of GenX chemicals in and/or on grass and leaves collected from sites various distances from a fluoropolymer manufacturing plant in the Netherlands (Brandsma et al., 2019). GenX chemicals concentrations ranged from 86 ng/g in leaves from a site closest to the plant to ND furthest from the plant. A similar pattern was observed for grass samples, except the maximum GenX chemicals concentration was lower (27 ng/g). The study authors note that it hadn't rained for five days prior to sample collection.

Semerád et al. (2020) investigated occurrence of HFPO dimer acid in sewage sludge from 43 facilities in the Czech Republic. HFPO dimer acid was detected in 7 of 43 samples at concentrations ranging from 0.3 to 1.2 ng/g dw. The authors raised concerns about the agriculture use of sludge containing PFAS for growing crops.

3.3.1.6 Sediment

HFPO dimer acid and its ammonium salt are expected to remain in water and exhibit low partitioning to sediment (U.S. EPA, 2021a). One study evaluated the occurrence of GenX chemicals in sediments from the North and Baltic Seas in Europe, and reported that HFPO dimer acid was not detected in any of the 24 sediment samples taken in the North and Baltic Seas in the vicinity of Germany (Joerss et al. (2019). An additional four studies analyzed sediments in China (Li et al., 2020b, 2021; Song et al., 2018; Wang et al., 2019a). Of the four studies, Wang et al. (2019a) analyzed sediment from the South China Sea coastal region in the area of the highly industrialized Pearl River Delta and reported that HFPO dimer acid was below the LOQ in all 53 samples. Li et al. (2020b) analyzed 20 sediment samples from eight rivers and three reservoirs in the Hai River Basin in the vicinity of several industrialized areas. HFPO dimer acid was reportedly detected at minimal levels, but the authors did not report actual concentrations. Song et al. (2018) analyzed concentrations of HFPO dimer acid in 24 sediment samples from the Xiaoqing River in the vicinity of a fluoropolymer production facility. The study reported a maximum HFPO dimer acid concentration in sediment of 22.3 ng/g dw, with median and mean levels below the LOQ. Li et al. (2021) also analyzed sediment samples from five sites of the Xiaoqing River estuary, and reported a mean HFPO dimer acid concentration of 0.23 ng/g dw.

3.3.2 RSC Determination

In summary, based on the physical properties, detected levels, and limited available exposure information for GenX chemicals, multiple non-drinking water sources (foods, indoor dust, air, soil, and sediment) are potential exposure sources. Following the Exposure Decision Tree in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a), potential sources other than drinking water ingestion were identified

(Box 8A in the Decision Tree). However, the available information is limited. The available information does not allow for the quantitative characterization of the relative levels of exposure among these different sources (Box 8B in the Decision Tree).

EPA also considered the exposure information specifically for the identified sensitive population (lactating women). However, the literature search did not identify non-drinking water exposure information specific to lactating women that could be used quantitatively to derive the RSC. Since neither the available data for the general population (all ages) nor the sensitive population enabled quantitative characterization of relative exposure sources and routes, EPA applied the default RSC of 0.2 (see Section 2.2.4.2 above; EPA, 2000a), which means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential exposure sources such as food, indoor dust, soil, and sediment.

4.0 Lifetime Noncancer Health Advisory Derivation

The lifetime HA for HFPO dimer acid and its ammonium salt is calculated as follows:

$$\textbf{Lifetime HA} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} \qquad \text{(Eq. 3)}$$

$$\textbf{Lifetime HA} = \left(\frac{0.000003\ \frac{\text{mg}}{\text{kg bw} - \text{day}}}{0.0469\ \frac{\text{L}}{\text{kg bw} - \text{day}}}\right) * 0.2$$

$$\textbf{Lifetime HA} = 0.00001\ \frac{\text{mg}}{\text{L}}$$

$$= 0.01\ \frac{\mu\text{g}}{\text{L}}$$

$$= 10\ \frac{\text{ng}}{\text{L}}$$

EPA is issuing a lifetime noncancer drinking water HA for GenX chemicals of 10 ng/L (ppt). The lifetime health advisory for GenX chemicals used a chronic RfD from the final EPA toxicity assessment (U.S. EPA, 2021a) based on the critical effect of adverse liver effects in adults (parental females) from a subchronic study (53–64 day exposure, depending on the time of conception). In the assessment, a 10X UF for subchronic to chronic exposure was used to derive the chronic RfD (U.S. EPA, 2021a). Because the critical effect identified for GenX chemicals is not a developmental effect and the chronic RfD was used to develop the lifetime HA, the GenX chemicals health advisory is more appropriate for the chronic exposure scenarios than shorter duration exposure scenarios. However, application of the GenX chemicals health advisory to a shorter-term risk assessment scenario would provide a conservative, health protective approach in the absence of other information.

5.0 Analytical Methods

EPA developed two liquid chromatography/tandem mass spectrometry (LC/MS/MS) analytical methods to quantitatively monitor drinking water for targeted PFAS that include HFPO dimer acid: EPA Method 533 (U.S. EPA, 2019b) and EPA Method 537.1, Version 2.0 (U.S. EPA, 2020b). The methods discussed below can be used to accurately and reasonably quantitate HFPO dimer acid at single digit ng/L levels that are nearly three times lower than the HFPO dimer acid lifetime HA of 10 ng/L.

EPA Method 533 monitors for 25 select PFAS with published measurement accuracy and precision data for HFPO dimer acid in reagent water, finished groundwater, and finished surface water and a single laboratory-derived minimum reporting level or approximate quantitation limit for HFPO dimer acid at 3.7 ng/L (0.0037 µg/L). For further details about the procedures for this analytical method, please see *Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry* (U.S. EPA, 2019b).

EPA Method 537.1 (an update to EPA Method 537 [EPA, 2009c]) monitors for 18 select PFAS with published measurement accuracy and precision data for HFPO dimer acid in reagent water, finished groundwater, and finished surface water and a single laboratory-derived minimum reporting level or approximate quantitation limit for HFPO dimer acid at 4.3 ng/L (0.0043 µg/L). For further details about the procedures for this analytical method, please see *Method 537.1, Version 2.0, Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)* (U.S. EPA, 2020b).

Drinking water analytical laboratories have different performance capabilities dependent upon their instrumentation (manufacturer, age, usage, routine maintenance, operating configuration, etc.) and analyst experience. Some laboratories will effectively generate accurate, precise, quantifiable results at lower concentrations than others. Organizations leading efforts that include the collection of data need to establish data quality objectives (DQOs) to meet the needs of their program. These DQOs should consider establishing reasonable quantitation limits that laboratories can routinely meet, without recurring quality control (QC) failures that will necessitate repeating sample analyses, increase costs, and potentially reduce laboratory capacity. Establishing a quantitation limit that is too high may result in important lower-concentration results being overlooked.

EPA's approach to establishing DQOs within the UCMR program serves as an example. EPA established minimum reporting limits (MRLs) for UCMR 5,[13] and requires laboratories approved to analyze UCMR samples to demonstrate that they can make quality measurements at or below the established MRLs. EPA calculated the UCMR 5 MRLs using quantitation-limit data from multiple laboratories participating in an MRL-setting study. The laboratories' quantitation limits represent their lowest concentration for which future recovery is expected, with 99% confidence, to be between 50 and 150%. The UCMR 5-derived and promulgated MRL for HFPO dimer acid is 0.005 µg/L (5 ng/L).

[13] Information about UCMR 5 is available at https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule

6.0 Treatment Technologies

This section summarizes available drinking water treatment technologies that have been demonstrated to remove GenX chemicals. This section is not meant to provide specific guidance for operation or design criteria. Sorption based treatment processes including granular activated carbon (GAC), anion exchange (AIX), and powdered activated carbon (PAC) as well as high pressure membranes such as nanofiltration (NF) and reverse osmosis (RO) have been shown to successfully remove GenX chemicals from drinking water to below the 5 ppt EPA UCMR5 reporting limit (Heidari et al., 2021). Care should be taken when introducing one of these processes into a well-functioning treatment train, as there can be unintended consequences related to interactions with other treatment types and for systems unfamiliar with proper operation and potential hazards. These treatment processes may have additional benefits on finished water quality by removing other contaminants and disinfection by-product (DBP) precursors. General information about these processes and treatment performance data summaries may be found in the Drinking Water Treatability Database.[14]

Non-treatment means of managing GenX chemicals such as changing source waters, consolidation, or source water protection are also viable options for reducing GenX chemical concentrations in finished drinking water. One available resource for protecting source water from PFAS, including GenX chemicals, is the PFAS-Source water Protection Guide and Toolkit,[15] which shares effective strategies for addressing PFAS contamination risk in source waters.

Conventional water treatment methods such as coagulation, flocculation, sedimentation, and biologically active carbon filtration (where the column is operated for extended periods of time) are ineffective at removing GenX chemicals (Sun et al., 2016). Ozonation has increased concentrations of some GenX chemicals at full-scale DWTPs, possibly due to precursor compound oxidation (Sun et al. 2016). Medium pressure ultra-violet lamps and chlorination can possibly decrease concentrations of GenX compounds but only to a very limited extent and the observed results could be due to temporal and spatial fluctuations within the DWTPs monitored (Sun et al., 2016). These processes are generally not considered as viable GenX chemicals, or more broadly PFECA, treatment options. Boiling water will concentrate GenX chemicals and should not be considered as an emergency action.

6.1 Sorption Technologies

Sorption is where substances present in liquids are removed by accumulation on a solid phase (Crittenden et al., 2012). There are two main sorption technologies that are in use for PFAS removal and have been demonstrated to remove GenX: activated carbon and ion exchange. Activated carbon comes in two key forms distinguished by size, PAC and GAC.

There are select considerations that are similar across all sorption technologies. Common key criteria include influent water quality and desired effluent quality. Influent water quality can greatly impact the ability of sorption technologies to treat drinking water. Desired effluent quality can drive both operational and capital expenditures. Pilot scale testing is highly recommended to ensure the design effectiveness will be maximized for given source waters.

[14] More information regarding treatment processes is available at https://tdb.epa.gov/tdb/findtreatmentprocess
[15] The PFAS Source Water Protection Guide and Toolkit are available for download at https://www.asdwa.org/pfas/

EPA's ICR Manual for Bench- and Pilot-Scale Treatment Studies (U.S. EPA, 1996) contains guidance on conducting pilot studies for contactors which are used for GAC and ion exchange.

Sorption technologies are largely reversible: PFAS can detach from sorbents and re-enter the drinking water under certain conditions. In addition, direct competition with stronger sorbing constituents can lead to effluent PFOS concentrations temporarily exceeding influent concentration (known as chromatographic peaking). An implication for treatment plants is that the effluent GenX chemicals concentrations can temporarily exceed influent concentrations. Competitive sorption is especially important in co-removal systems where other PFAS are present. When GenX was co-removed with PFOA, the total GenX quantity removed decreased significantly. After an initial loading period absorbed GenX desorbed and then was replaced by PFOA (Wang et al. 2019b). Competitive sorption may be controlled by changing or regeneration of the sorptive media at appropriate intervals.

The majority of studies found that natural or dissolved organic matter (NOM/DOM) interferes with PFAS sorption, in general, and its presence dramatically lowers treatment efficacy (McNamara et al., 2018; Pramanik et al., 2015; Yu et al., 2012). The lowered treatment effectiveness was found to be less pronounced for GenX chemicals than for perfluoroalkyl carboxylic acid (PFCA) C7 and above for GAC (Park et al., 2020).

GAC can typically be regenerated when treatment performance reaches an unacceptable level.. Regeneration can be on or off site. On-site regeneration typically requires a higher spatial footprint and capital outlay. Given water quality and other considerations, regenerated media can become totally exhausted or "poisoned" with other contaminants not removed during regeneration and must be replaced. However, for GAC, the loss of approximately 10 percent of the media due to abrasion withing the reactivation process can result in a somewhat steady state for performance as new GAC is added each time to replace the lost GAC. Most AIX resins in current use for PFAS are single use resins and not designed to be regenerated.

6.1.1 Activated Carbon

Activated carbon is a highly porous media with high internal surface areas (U.S. EPA, 2017b). Activated carbon can be made from a variety of materials. Designs that work with a carbon made from one source material activated in a specific way may not be optimized for other carbon types. It is normally used in either a granular or powdered form for water treatment. Installing activated carbon as a treatment method may have ancillary benefits on finished water quality, particularly with disinfectant byproduct control as well as taste and odor.

With activated carbon, more non-polar and larger compounds tend to be more easily removed than smaller more polar compounds. Adsorption of acids and bases on activated carbon is dependent on the pH. Adsorption of neutral forms, as opposed to anionic forms, are generally stronger so lowering the pH increases GenX chemical sorption. However, the acid dissociation constant (pK_a) of HFPO dimer acid is 2.84 and lowering the pH is not practical for drinking water applications (Park et al. 2020; U.S. EPA 2021a). GenX forms a fast, weak electrostatic bond with adsorbents and can be substituted by PFOA or other long-chain PFAS which adsorb preferentially on activated carbon due in part to their higher hydrophobicity (Heidari et al., 2021; Wang et al., 2019b). These differences in physical chemical properties are consistent with the

faster adsorption kinetics but less tight binding of GenX than PFOA and result in GenX chemicals partitioning more quickly onto activated carbon.

Based on findings with emerging PFCA PFOA replacements, cations such as aluminum, calcium, and sodium increase PFAS sorption to activated carbons (Pereira et al., 2018) at low pH. Anions such as fluorine, chlorine, nitrate, sulfate, and phosphate have not yet been shown to correlate with GenX removal despite expectations that these anions would inhibit GenX treatment (Wu et al., 2020).

Activated carbon has a maximum sorbent capacity and must be replaced or regenerated. For carbon regenerated off-site, several organizations recommend that spent carbon should be segregated and traceable from the time it leaves the drinking water facility through all steps at the reactivation facility, and then returned to the same site (National Science Foundation [NSF]/American National Standards Institute [ANSI] Standard 61 [NSF/ANSI, 2021]).

Before adding activated carbon to an existing treatment train, there are effects which should be considered. For instance, activated carbon may change system pH or, release leachable metals (particularly arsenic and antimony) when new carbon media is first used without acid washing, and may require disinfection. Activated carbon may also cause unintended consequences with disinfection efficacy depending on process placement. Activated carbon can also shift the bromide-to-total organic carbon ratio and increase brominated (Br)-DBP concentrations as well as concentrations relative to chlorinated DBPs (Krasner et al., 2016). Despite increased Br-DBP, studies have indicated a decreased overall DBP risk (Wang et al., 2019c).

6.1.1.1 Powdered Activated Carbon

PAC is the same material as GAC but has a smaller particle size and is applied differently. PAC is typically dosed intermittently although it can be employed continuously. PAC dosage and type, along with dosing location, contact time, and water quality, often influence process cost as well as treatment efficiency (Heidari et al., 2021). Sometimes PAC is combined with other processes, particularly floc blanket reactors and membrane filters (low or high pressure), although this is not necessary. For more information on employing PAC, please see the Drinking Water Treatability Database.[16]

With GenX, PAC was found to achieve equilibrium more quickly than GAC however, total removal capacity was similar (Wang et al., 2019b), although the steady state PAC application cannot match the benefits of column operation of GAC in terms of percent removal. Significant increases in GenX chemicals treatment efficiencies have been observed with smaller PAC particle sizes (Wang et al., 2019b). Compared to GAC, competing species such as PFOA displace GenX chemicals more rapidly on PAC (Wang et al. 2019b) which is consistent with GenX being less tightly bound and more mobile than PFOA. For PFAS, information to date indicates that increasing PAC dose increases removal to a point and then starts to decrease. Jar testing is used to empirically determine the optimal PAC dosage; doses between 45–100 mg/L are generally suitable for GenX Chemicals (Dudley, 2012; Hopkins et al., 2018; Sun et al., 2016). These doses are high and drinking water utilities would have difficulty in maintaining them for extended periods of time. Standardized jar testing procedures have been published

[16] https://tdb.epa.gov/tdb/treatmentprocess?treatmentProcessId=2109700949

(ASTM, 2019; AWWA, 2011). The AWWA published standard for PAC is ANSI/AWWA B600-16 (AWWA, 2016).

Other key operational parameters determining PAC efficiency include contact time and loading rate. Contact time in most plants is generally between 30 minutes and 2 hours. Sun et al. (2016) found that the full PAC capacity for GenX chemicals is unlikely to be used in this time. While PAC can be regenerated it rarely makes sense to do so because of the associated costs, presence of coagulants and particulates in the sludge, and degraded removal capacities post-reactivation (Clifford et al., 1983).

PAC poses additional safety considerations including depleting oxygen in confined or partially enclosed areas, fire hazards including spontaneous combustion when stored with hydrocarbons or oxidants, and inhalation hazards. PAC is also a good electrical conductor and can create dangerous conditions when it accumulates (AWWA, 2016).

6.1.1.2 Granulated Activated Carbon

As a result of GenX chemicals being only moderately absorbable, GAC contactors are normally placed as a post-filter step. Key design criteria include empty bed contact time (EBCT), superficial velocity, and carbon type. Typical EBCTs for GenX chemicals removal are 10–20 minutes and superficial linear velocities are normally 5–15 meters per hour (m/hr). Normal height-to-diameter ratios are around 1.5 to 2.0; lower ratios can run into problems with too shallow beds and require more space, and higher ratios induce greater pressure drops. AWWA has published a GAC standard (ANSI/AWWA B604-18; AWWA, 2018a); there is also an AWWA published standard for GAC reactivation (ANSI/AWWA B605-18; AWWA, 2018b).

6.1.2 Ion Exchange

Ion exchange involves the exchange of an ion in the aqueous phase for an ion on the exchange resin. Once the resin has exchanged all its ions for contaminants, it can either be disposed (single use) or regenerated (i.e., restoring its ions for further use).

Resins are either cationic or anionic; cationic resins remove positively charged ions such as sodium or calcium and anionic resins remove negatively charged ions such as sulfates and nitrates. Cationic exchange resins do not remove GenX chemicals. The pK_a of HPFO-DA is 2.84; this means that in drinking water applications GenX chemicals will predominately exist in an anionic form and are strong acids (U.S. EPA, 2021a). Based on the pK_a strongly basic anionic exchange resins will be the most relevant. Key design parameters for GAC are also key design parameters for AIX, although there are slight differences in operation. AIX typically uses 2-to-5-minute EBCTs, allowing for lower capital costs and a smaller footprint; generally smaller height-to-diameter ratios are used in exchange columns compared to GAC. Columns used in pilot studies and scaled directly to full-scale if loading rates and EBCTs are kept constant (Crittenden 2012). For more information about AIX, please see Dixit et al. (2021), Tarleton (2014), or Tanaka (2015), Crittenden et al. (2012), or the EPA Drinking Water Treatability Database (2022).

Strong base acrylate resins contaminated with HFPO dimer acid have been greater than 95% regenerated with a 10% sodium chloride solution (Dixit et al., 2020). Sodium hydroxide may be added to the sodium chloride solution to combat organic fouling; this is referred to as ‘brine

squeeze' and helps in solubilizing NOM and unplugging pores (Dixit et al., 2021). Once PFAS-contaminated spent brine is recovered, it must be treated or disposed. Resin regeneration may not be practical for water utilities from safety and/or cost perspectives (Liu and Sun, 2021).

Before adding AIX to an existing treatment train, there are effects which should be considered. For instance, AIX can increase water corrosivity which may increase heavy metals through leaching, can release organic leachables such as the amines from which they are made, and will increase concentrations of the counter-ion used (typically chloride).

6.2 High Pressure Membranes

NF and RO are high-pressure processes where water is forced through a membrane. The water that transverses the membrane is known as permeate or produce water, and has few solutes left in it; the remaining water is known as concentrate, brine, retentate, or reject water and forms a waste stream with concentrated solutes. NF has a less dense active layer than RO, which enables lower operating pressures but also makes it less effective at removing contaminants. NF and RO tend to take up less space than sorption separation technologies. However, both NF and RO also tend to have higher operating expenses, use a significant amount of energy, and generate concentrate waste streams which require disposal. Generally, NF and RO require pre- and posttreatment processes. Higher expenses typically associated with NF and RO are only rarely competitive from an economic perspective for removing a specific contaminant; however, for waters requiring significant treatment and where concentrate disposal options are reasonably available, NF and RO may be the best option.

PFAS removal fluxes are generally 1–50 liters per square meter per hour (L/[m^2·hr]) at 5–85 bar operating pressure (Mastropietro et al., 2021). Temperature can dramatically impact flux; it is common to normalize flux to a specific reference temperature for operational purposes (U.S. EPA, 2005c). It is also common to normalize flux to pressure ratios to identify productivity changes attributable to fouling (U.S. EPA, 2005c). It is important to note that outside-in and inside-out systems operating at the same flux produce differing quantities of finished water so membrane systems with differing configurations cannot be directly compared based on flux. Total flow per module and cost per module are more important decision support indicators for capital planning. Unlike low pressure membranes, NF and RO systems are not manufactured as proprietary equipment and membranes from one manufacturer are typically interchangeable with those from others (U.S. EPA, 2005c).

High-pressure membranes may have important unintended effects when added onto a well-functioning treatment train. For instance, high-pressure membranes may remove beneficial minerals and increase corrosivity. Increased water corrosivity may increase heavy metals such as iron, lead, and copper through leaching. For more information, see AWWA (2007).

6.3 Point-of-Use Devices for Individual Household PFAS Removal

Although the focus of this treatment technologies section is the different available options for removal of PFOA at DWPs, centralized treatment technologies can also often be used in a decentralized fashion as point-of-entry (POE) (where the distribution system meets a service connection) or point-of-use (POU) (at a specific tap or application) treatment in cases where centralized treatment is impractical or individual consumers wish to further reduce their individual household risks. Many home drinking water treatment units are certified by

independent third-party accreditation organizations against ANSI standards to verify contaminant removal claims. NSF International has developed protocols for NSF/ANSI Standards 53 (sorption) and 58 (RO) that establish minimum requirements for materials, design, and construction, and performance of point-of-use systems. Previously, NSF P473 was designed to certify PFOA reduction technologies below EPA's 2016 HA of 70 ppt for PFOA; in 2019, these standards were retired and folded into NSF/ANSI 53 and 58. When properly maintained, these certified systems may reduce other PFAS, including GenX chemicals, although removal should not be automatically inferred for PFAS not specified within the protocol. It has been reported that home under-the-sink RO filters effectively removed GenX chemicals in Cape Fear, North Carolina (Hopkins et al., 2018). GenX specific certification procedures may be developed by standards organizations, such as NSF and the Water Quality Association. Individuals or systems interested in POU or POE treatment should check with standards organizations for the most recent certification procedures.

6.4 Treatment Technologies Summary

Non-treatment management options, such as changing source waters, source water protection, or consolidation, are viable strategies for reducing GenX chemicals concentrations in finished drinking water. Should treatment be necessary, activated carbon, AIX, NF, or RO have been shown to successfully remove HFPO dimer acid from drinking water to below the 4 ppt reporting limit for UCMR 5. These processes are the best means for removing GenX chemicals from drinking water and can be used in central treatment plants or in POU/POE applications. Some treatment processes have been shown to increase GenX chemicals concentrations, most likely through precursor oxidation. These treatment technologies often require pre- as well as posttreatment and may help remove other unwanted contaminants along with DBP precursors. Each technology may also introduce unintended consequences to an existing treatment train. Additionally, these treatment processes are separation technologies and produce waste streams with GenX chemicals on or in them. Boiling water will concentrate GenX chemicals and should not be considered as an emergency action.

7.0 Consideration of Noncancer Health Risks from PFAS Mixtures

EPA recently released a *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (U.S. EPA, 2021f) that is currently undergoing Science Advisory Board (SAB) review. That draft document describes a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on current, available EPA chemical mixtures approaches and methods (U.S. EPA, 2000b). Examples are presented for three approaches—Hazard Index (HI), Relative Potency Factor (RPF), and Mixture BMD—to demonstrate application to PFAS mixtures. To use these approaches, specific input values and information for each PFAS are needed or can be developed. These approaches may help to inform PFAS evaluation(s) by federal, state, and tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential noncancer human health hazards and risks associated with PFAS mixtures.

The HI approach, for example, could be used to assess the potential noncancer risk of a mixture of four component PFAS for which HAs, either final or interim (iHA), are available from EPA

(PFOA, PFOS, GenX chemicals, and perfluorobutane sulfonic acid [PFBS]). In the HI approach described in the draft framework (U.S. EPA 2021f), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (U.S. EPA, 1986). The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Eq. 6). In the context of PFAS in drinking water, a mixture PFAS HI can be calculated when health-based water concentrations (e.g., HAs, Maximum Contaminant Level Goals [MCLGs]) for a set of PFAS are available or can be calculated. In this example, HQs are calculated by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HA (e.g., expressed as ng/L) (Eqs. 7, 8). The component chemical HQs are then summed across the PFAS mixture to yield the mixture PFAS HIs based on interim and final HAs.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

(Eq. 6)

$$HI = HQ_{PFOA} + HQ_{PFOS} + HQ_{GenX} + HQ_{PFBS}$$

(Eq. 7)

$$HI = \left(\frac{[PFOA_{water}]}{[PFOA_{iHA}]}\right) + \left(\frac{[PFOS_{water}]}{[PFOS_{iHA}]}\right) + \left(\frac{[GenX_{water}]}{[GenX_{HA}]}\right) + \left(\frac{[PFBS_{water}]}{[PFBS_{HA}]}\right)$$

(Eq. 8)

Where:

HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS
RfV = human health-based toxicity value for component (i) PFAS
HQ_{PFAS} = hazard quotient for a given PFAS
$[PFAS_{water}]$ = concentration of a given PFAS in water
$[PFAS_{HA}]$ = HA value, interim or final, for a given PFAS

In cases when the mixture PFAS HI is greater than 1, this indicates an exceedance of the health protective level and indicates potential human health risk for noncancer effects from the PFAS mixture in water. When component health-based water concentrations (in this case, HAs) are below the analytical method detection limit, as is the case for PFOA and PFOS, such individual component HQs exceed 1, meaning that any detectable level of those component PFAS will result in an HI greater than 1 for the whole mixture. Further analysis could provide a refined assessment of the potential for health effects associated with the individual PFAS and their contributions to the potential joint toxicity associated with the mixture. For more details of the approach and illustrative examples of the RPF approach and Mixture BMD approaches please see U.S. EPA (2021f).

8.0 Health Advisory Characterization

EPA is issuing a lifetime noncancer drinking water HA for GenX chemicals of 10 ng/L or 10 ppt based on the best available science. This is the first HA for GenX chemicals. The input values for the HA are: 1) the final chronic RfD for GenX chemicals from the toxicity assessment (U.S. EPA, 2021a); 2) the RSC based on exposure information collected from a literature search and following EPA's Exposure Decision Tree (U.S. EPA, 2000a) and presented herein; and 3) the DWI-BW, described herein, selected for the sensitive population or life stage. The final toxicity assessment for GenX chemicals was developed from a systematic review of the available scientific information on health effects (U.S. EPA, 2021a) and reflects response to public comment, two expert peer reviews, and recommendations from an independent evaluation by the National Toxicology Program's Pathology Working Group of two liver toxicity studies.

Uncertainties in the lifetime noncancer HA value are due in part to the relatively small database of health effects information, based on animal studies, for GenX chemicals (U.S. EPA, 2021a). There were no human epidemiology studies identified during the literature search conducted as part of the toxicity assessment (U.S. EPA, 2021a). The mechanistic information for GenX chemicals was reviewed as part of the toxicity assessment (see Section 6 of EPA, 2021a). Multiple potential modes of action have been identified for effects of GenX chemicals exposure on the liver (the critical effect), including peroxisome proliferator-activated receptor alpha (PPARα) activation and cytotoxicity. Mechanisms and modes of action have not been elucidated for the other health outcomes associated with GenX chemicals exposure (e.g., developmental/reproductive effects). However, the current data gaps in the GenX chemicals health effects information were accounted for in the derivation of the final RfD by applying relevant UFs including a 10X UF_D.

Regarding EPA's RSC selection, uncertainties exist due to the current lack of information to allow for a quantitative exposure characterization among exposure sources including for lactating women, the sensitive population selected for deriving the HA. There is also uncertainty in the EF that EPA selected since it is possible that additional toxicity information may reveal more sensitive populations or life stages for GenX chemicals. This final HA is based on a recent toxicity assessment and recent literature searches of the publicly available scientific information regarding health effects, exposure, analytical methods, and treatment technologies for GenX chemicals.

8.1 Comparative Analysis of Exposure Factors for Different Populations

The exposure duration in the critical study identified in the toxicity assessment for GenX chemicals (U.S. EPA, 2021a) is from pre-mating, through gestation, and to day 21 of lactation and the adverse liver effects were observed in the dams (not their offspring). Therefore, three potentially sensitive life stages of adult females—pregnant women, women of childbearing age (13 to < 50 years), and lactating women were identified (Table 5). The DWI-BW for lactating women was selected since it is the most health protective.

To evaluate whether all ages of the general population would be protected by the resulting lifetime HA value for GenX chemicals, based on the DWI-BW for lactating women, EPA calculated HAs using the 90th percentile DWI-BW for four populations: the general population (all ages), pregnant women, women of childbearing age, and lactating women. The HA values (rounded to one significant figure) using the EF for general population, pregnant women, or

women of childbearing age are all 0.00002 mg/L (20 ppt) which is higher than the GenX HA value calculated using the EF for lactating women (0.00001 mg/L [10 ppt]) (Table 6). The comparison of the four candidate HA values indicates that the lifetime noncancer HA derived using the DWI-BW for lactating women is protective of the other candidate sensitive populations or life stages as well as the general population (all ages).

Table 6. Comparison of HA Values Using EPA Exposure Factors for Drinking Water Intake for Different Candidate Populations.

Population	DWI-BW (L/kg bw-day)	HA two sig figs/ HA one sig fig (mg/L)	Description of Exposure Metric	Source
General population, all ages	0.0338	0.000018/ 0.00002	90th percentile direct and indirect consumption of community water, consumer-only two-day average, all ages.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (U.S. EPA, 2019a)
Pregnant women	0.0333	0.000018/ 0.00002	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Women of childbearing age	0.0354	0.000017/ 0.00002	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (U.S. EPA, 2019a)
Lactating women	**0.0469**	0.000013/ **0.00001**	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[a] (U.S. EPA, 2019a)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day. Sig fig = significant figure. The DWI-BW used to calculate the GenX chemicals' lifetime HA is in bold. EPA HAs are rounded to one significant figure.

[a] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports: HNIS/ NCHS Analytical Working Group Recommendations (NCHS, 1993).

8.2 Related Compounds of Emerging Concern

This HA addresses the two chemicals that are the two current commercial products of the GenX technology: the HFPO dimer acid and its ammonium salt. During the synthesis of HFPO dimer acid, which is manufactured from hexafluoropropene oxide (HFPO), other chemicals including

the HFPO trimer acid (HFPO-TA) and HFPO tetramer acid (HFPO-TeA) can be produced in the synthesis process (Geng et al., 2016). These same HFPO chemicals are byproducts of longer chain perfluoropolyether synthesis. Health effects are indicated from *in vivo* and *in vitro* studies of the liver (Sheng et al., 2018) and the endocrine system after exposure to HFPO-TA and the HFPO-TeA (Xin et al., 2019). While some information is available on the occurrence and bioaccumulation of HFPO-TA (Pan et al., 2017), more research is needed to improve our understanding of the exposure information and health effects for HFPO-TA and HFPO-TeA.

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Appendix A: Relative Source Contribution – Literature Search and Screening Methodology

In support of U.S. Environmental Protection Agency's (EPA's) human health toxicity assessment for hexafluoropropylene oxide dimer acid (HPFO) and its ammonium salt (GenX chemicals) (EPA, 2021a), literature searches were conducted of four databases (PubMed, Toxline, Web of Science (WOS), and Toxic Substances Control Act Test Submissions (TSCATS) to identify publicly available literature using Chemical Abstracts Service Registry Number (CASRN), synonyms, and additional relevant search strings (see EPA (2021a) for details). Due to the limited search results, additional databases were searched for information on physicochemical properties, health effects, toxicokinetics, and mechanism of action. The initial date-unlimited database searches were conducted in July 2017 and January/February 2018, with updates completed in February 2019, October 2019, and March 2020. In addition, available information on toxicokinetics; acute, short-term, subchronic, and chronic toxicity; developmental and reproductive toxicity; neurotoxicity; immunotoxicity; genotoxicity; and cancer in animals was submitted with premanufacture notices to EPA by DuPont/Chemours, the manufacturer of GenX chemicals, as required under Toxic Substances Control Act pursuant to a consent order (EPA, 2009b) or reporting requirements (15 U.S.C. § 2607.8(e)). The results of the literature searches of publicly available sources and submitted studies from DuPont/Chemours are available through EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

The GenX chemicals literature search results and all studies submitted from DuPont/Chemours were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and non-human (i.e., those not identified as human) studies. Studies identified as human studies were further categorized into seven major PFAS pathways (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) as well as an additional category for Human Exposure Measures. Non-human studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill.

Application of the SWIFT-Review tags identified 52 studies for title and abstract screening. An additional three references were identified through gray literature sources that were included to supplement the search results. Title and abstract screening to determine relevancy followed the populations, exposures, comparators, and outcomes (PECO) criteria in Table A-1:

Table A-1. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults (including women of childbearing age) and/or children in the general populations from any country
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater, human blood/serum/urine, indoor air, landfill, sediment, soil, surface water (freshwater), wastewater/biosolids/sludge

PECO Element	Inclusion Criteria
Comparator	Not applicable
Outcome	Measured concentrations of GenX chemicals (or measured emissions from food packaging and consumer products only)

The title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree if a study should be included or excluded, a third reviewer was consulted to make a final decision. The title and abstract screening resulted in 24 studies tagged as relevant (i.e., data on occurrence of GenX chemicals in one of the media of interest were presented in the study) that were further screened with full-text review using the same inclusion criteria. Of these 24 studies, 4 contain only human biomonitoring data and are not discussed further here. Based on full-text review, 15 studies were identified as relevant and are summarized below. At the full-text review stage, two additional studies were identified as only containing biomonitoring data.

To supplement the primary literature database, EPA also searched the following gray literature sources in February 2022 for information related to relative exposure of GenX chemicals for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- EPA's (2021a) *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"*
- Agency for Toxic Substances and Disease Registry's (ATSDR's) *Toxicological Profiles*
- Centers for Disease Control and Prevention's (CDC's) national reports on human exposures to environmental chemicals
- EPA's CompTox Chemicals Dashboard
- EPA's fish tissue studies
- EPA's Toxics Release Inventory
- EPA's Unregulated Contaminant Monitoring Rule data
- Relevant documents submitted under the Toxics Substances Control Act and relevant reports from EPA's Office of Chemical Safety and Pollution Prevention
- U.S. Food and Drug Administration's (FDA's) *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada
- National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science data collections
- National Science Foundation direct and indirect food and/or certified drinking water additives
- PubChem compound summaries
- Relevant sources identified in the relative source contribution discussions (section 5) of EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level*

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- Additional sources, as needed

EPA has included available information from these gray literature sources for GenX chemicals relevant to their uses, chemical and physical properties, and for occurrence in drinking water (directly or indirectly in beverages like coffee, tea, commercial beverages, or soup), ambient air, foods (including fish and shellfish), incidental soil/dust ingestion, and consumer products. EPA has also included available information specific to GenX chemicals below on any regulations that may restrict levels of GenX chemicals in media (e.g., water quality standards, air quality standards, food tolerance levels).

EPA incorporated 3 references (Feng et al., 2021; Li et al., 2021; and Semerád et al., 2020) that were not identified in the contractor's RSC literature search strategy; these references were provided by Chemours as part of their outreach to EPA on uses and sources for GenX chemicals in April 2022.

Appendix B: Compilation of Data on HFPO Dimer Acid Occurrence in Surface Water Collected from Primary Literature

This appendix includes a table resulting from the efforts to identify and screen primary literature (i.e., peer-reviewed journal articles), described in Appendix A, as well as extract data that may be relevant to informing the RSC derivation for GenX chemicals.

Table B-1. Compilation of Studies Describing of HFPO Dimer Acid Occurrence in Surface Water

Study	Location	Site Details	Results
North America			
Sun et al. (2016)	United States (North Carolina, Cape Fear River Basin)	Source waters of three community drinking water treatment plants, two upstream and one downstream of a PFAS manufacturing plant (LOQ = 10 ng/L)	Community A (upstream): DF 0% Community B (upstream): DF NR, median (range) = ND (ND-10 ng/L) Community C (downstream): DF NR, mean = 631 ng/L, median (range) = 304 (55–4,560) ng/L
McCord et al. (2018)	United States (North Carolina, Cape Fear River Basin)	Source water of a drinking water treatment plant near the industrial waste outfall of a fluorochemical manufacturer, before and after the manufacturer diverted a waste stream (exact values NR, estimated values from Figure 3)	Before waste diversion (estimated): DF NR, measured concentration = ~ >700 ng/L After waste diversion (estimated): DR NR, measured concentration = < 140 ng/L
Galloway et al. (2020)	United States (Ohio and West Virginia, Ohio River Basin)	Rivers and tributaries located upstream, downstream, and downwind of a fluoropolymer production facility; some sample locations potentially impacted by local landfills	DF = 21/24 unique sites with detections > LOQ, median[a] (range) = 46.7 (ND–227) ng/L

Study	Location	Site Details	Results
Europe			
Gebbink et al. (2017)	The Netherlands	Upstream and downstream of the Dordrecht fluorochemical production plant; two control sites	Control sites: DF 0% Upstream of plant (n=3): DF[a] 33%, point = 22 ng/L Downstream of plant (n=13): DF 100%, mean[a] (range) = 178 (1.7–812) ng/L (MQL = 0.2)
Vughs et al. (2019)	The Netherlands and Belgium	Thirteen surface water samples collected from eleven water suppliers, some near a fluoropolymer manufacturing plant. The study did not map the distribution of reported concentrations by geographic location or with respect to distance from the fluoropolymer manufacturing plant.	DF 77%, mean (range) = 2.2 (ND–10.2) ng/L (LOQ = 0.2 ng/L)
Asia			
Pan et al. (2017)	China (Xiaoqing River and tributary)	Upstream and downstream of a fluoropolymer production plant in an industrialized region	Upstream of plant in the Xiaoqing River (n=6): DF[a] 100%, median[a] (range) = 2.10 (1.61–3.64) ng/L Tributary directly receiving plant effluent (n=4): DF[a] 100%, median[a] (range) = 1,855 (2.34-2,060) ng/L Downstream of plant in the Xiaoqing River receiving tributary waters (n=8): DF[a] 100%, median[a] (range) = 311 (118–960) ng/L
Song et al. (2018)	China (Xiaoqing River)	Near the Dongyue group industrial park, including a fluoropolymer production plant	DF NR, mean, median (range) = 519, 36.7 (<LOQ–9,350) ng/L (n=25 sites; LOQ=0.24 ng/L)

Study	Location	Site Details	Results
Li et al. (2020a)	China (Hai River Basin)	40 surface water samples from 8 rivers and 3 reservoirs – many of the rivers flowed through industrialized areas, some with potential PFAS point sources	DF[b] 80%, mean (range) = 0.316 (<MDL–2.6) ng/L (MDL = 0.0132 ng/L)
Multiple Continents			
Heydebreck et al. (2015)	Germany (Elbe and Rhine Rivers), the Netherlands (Rhine-Meuse delta)	All sampling locations in industrialized areas	Rhine River (n=23): DF[a] 17%, range = ND–86.08 ng/L Elbe River (n=22): DF 0%
	China (Xiaoqing River)	Some sampling locations were downstream of PFAS point sources	Xiaoqing River (n=20): DF[a] 65%, range = ND–3,060 ng/L
Pan et al. (2018)	United States (Delaware River)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Delaware River (n=12): DF 100%, mean, median (range) = 3.32, 2.02 (0.78–8.75) ng/L
	United Kingdom (Thames River), Germany and the Netherlands (Rhine River), Sweden (Malaren Lake)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Thames River (n=6): DF 100%, mean, median (range) = 1.12, 1.10 (0.70–1.58) ng/L Rhine River (n=20): DF 100%, mean, median (range) = 0.99, 0.90 (0.59–1.98) ng/L Malaren Lake (n=10): DF 100%, mean, median (range) = 1.47, 1.38 (0.88–2.68) ng/L

Study	Location	Site Details	Results
	South Korea (Han River), China (Liao, Huai, Yellow, Yangtze, and Pearl Rivers; Chao and Tai Lakes)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Han River (n=6): DF 100%, mean, median (range) = 1.38, 1.16 (0.78–2.49) ng/L Liao River (n=6): DF 100%, mean, median (range) = 1.44, 0.88 (0.62–4.51) ng/L Huai River (n=9): DF 100%, mean, median (range) = 1.66, 1.40 (0.83–3.62) ng/L Yellow River (n=15): DF 67%, mean, median (range) = 1.01, 1.30 (< LOQ–1.74) ng/L Yangtze River (n=35): DF 94%, mean, median (range) = 0.73, 0.67 (< LOQ–1.54) ng/L Pearl River (n=13): DF 100%, mean, median (range) = 1.51, 0.70 (0.21–10.3) ng/L Chao Lake (n=13): DF 100%, mean, median (range) = 1.92, 1.81 (0.93–3.32) ng/L Tai Lake (n=15): DF 100%, mean, median (range) = 14.0, 0.77 (0.38–143.7) ng/L (LOQ = 0.05 ng/L; MDL = 0.38 ng/L)
	All locations	Sampling sites were not proximate to known point sources of any fluorochemical facilities	All locations (n=160): DF 96%, mean, median (range) = 2.55, 0.95 (0.18–144) ng/L (LOQ = 0.05 ng/L; MDL = 0.38 ng/L)

Notes:
DF = detection frequency; LOQ = limit of quantification; ND = not detected.; ng/L = nanograms per liter; NR = not reported; MQL = method quantification limit; MDL = method detection limit.
[a] The DF, median and/or mean was not reported in the study and was calculated in this synthesis. Mean values were only calculated if DF = 100%.
[b] The DF in Li et al. (2020a) was reported as 82.5% in the main article. The DF of 80% shown in this table is based on the supporting information data, which show only 32/40 samples with data > MDL.
[c] The Xiaoqing River results reported in Heydebreck et al. (2015) included samples from Laizhou Bay. EPA considered freshwater samples only.

Attachment 9

METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

Technical questions concerning this analytical method should be addressed to:

Steven C. Wendelken, Ph.D.
U.S. EPA, Office of Ground Water and Drinking Water, Standards and Risk Management Division,
Technical Support Center, 26 W. Martin Luther King Dr. Cincinnati, OH 45268
Phone: (513) 569-7491
wendelken.steve@epa.gov

Questions concerning this document or policy should be addressed to: safewater@epa.gov

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Authors

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Disclaimer

This analytical method may support a variety of monitoring applications, which include the analysis of multiple short-chain per- and polyfluoroalkyl substances (PFAS) that cannot be measured by Method 537.1. This publication meets an agency commitment identified within the 2019 EPA PFAS Action Plan. Publication of the method, in and of itself, does not establish a requirement, although the use of this method may be specified by the EPA or a state through independent actions. Terms such as "must" or "required," as used in this document, refer to procedures that are to be followed to conform with the method. References to specific brands and catalog numbers are included only as examples and do not imply endorsement of the products. Such reference does not preclude the use of equivalent products from other vendors or suppliers.

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1 Scope and Application

This is a solid phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of select per- and polyfluoroalkyl substances (PFAS) in drinking water. Method 533 requires the use of MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance selectivity. Accuracy and precision data have been generated in reagent water and drinking water for the compounds included in the Analyte List.

This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of LC-MS/MS instrumentation, and the interpretation of the associated data.

Analyte List

Analyte[a]	Abbreviation	CASRN
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF3ONS	756426-58-1
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorobutanesulfonic acid	PFBS	375-73-5
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluoroheptanoic acid	PFHpA	375-85-9
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Perfluorononanoic acid	PFNA	375-95-1
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
Perfluoroundecanoic acid	PFUnA	2058-94-8

a. Some PFAS are commercially available as ammonium, sodium, and potassium salts. This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts.

1.1 Detection of PFAS Isomers

Both branched and linear PFAS isomers may be found in the environment. This method includes procedures for summing the contribution of multiple isomers to the final reported concentration. In those cases where standard materials containing multiple isomers are commercially available, laboratories should obtain such standards for the method analytes.

1.2 Lowest Concentration Minimum Reporting Limits

The lowest concentration minimum reporting level (LCMRL) is the lowest concentration for which the future recovery is predicted to fall between 50 and 150% with high confidence (99%). Single-laboratory LCMRLs determined for the method analytes during method development are reported in **Table 7**. It should be noted that most of the LCMRL values determined during the second laboratory evaluation were lower than the values listed in **Table 7**. The values that a laboratory can obtain are dependent on the design and capability of the instrumentation used. The procedure used to determine the LCMRL is described elsewhere.[1,2] Laboratories using this method are not required to determine LCMRLs, but they must demonstrate that they are able to meet the minimum reporting level (MRL) (Sect. 3.15) for each analyte per the procedure described in Section 9.1.4.

1.3 Method Flexibility

The laboratory may select LC columns, LC conditions, and MS conditions different from those used to develop the method. At a minimum, the isotope dilution standards and the isotope performance standards specified in the method must be used, if available. The laboratory may select the aqueous sample volume within the range of 100–250 mL that meets their objectives. During method development, 250 mL aqueous samples were extracted using a 500 mg solid phase extraction (SPE) sorbent bed volume. The ratio of sorbent mass to aqueous sample volume may not be decreased. If a laboratory uses 100 mL aqueous samples, the sorbent mass must be at least 200 mg. Changes may not be made to sample preservation, the quality control (QC) requirements, or the extraction procedure. The chromatographic separation should minimize the number of compounds eluting within a retention window to obtain a sufficient number of scans across each peak. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. Method modifications should be considered only to improve method performance. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the Initial Demonstration of Capability (IDC, Sect. 9.1), verify that all QC acceptance criteria in this method (Sect. 9.2) are met, and verify method performance in a representative sample matrix (Sect. 9.3.2).

2 Method Summary

A 100–250 mL sample is fortified with isotopically labeled analogues of the method analytes that function as isotope dilution standards. The sample is passed through an SPE cartridge containing polystyrene divinylbenzene with a positively charged diamino ligand to extract the method analytes and isotope dilution analogues. The cartridge is rinsed with sequential washes of aqueous ammonium acetate followed by methanol, then the compounds are eluted from the solid phase sorbent with methanol containing ammonium hydroxide. The extract is concentrated to dryness with nitrogen in a heated water bath. The extract volume is adjusted to 1.0 mL with 20% water in methanol (v/v), and three isotopically labeled isotope performance standards are added. Extracts are analyzed by LC-MS/MS

in the MRM detection mode. The concentration of each analyte is calculated using the isotope dilution technique. For QC purposes, the percent recoveries of the isotope dilution analogues are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution analogues.

3 Definitions

3.1 Analysis Batch

A set of samples that are analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the Analysis Batch and the number of field samples.

3.2 Calibration Standard

A solution of the method analytes, isotope dilution analogues, and isotope performance standards prepared from the Primary Dilution Standards and stock standards. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

3.3 Continuing Calibration Check (CCC)

A calibration standard that is analyzed periodically to verify the accuracy of the existing calibration.

3.4 Extraction Batch

A set of up to 20 field samples (not including QC samples) extracted together using the same lot of solid phase extraction devices, solvents, and fortifying solutions.

3.5 Field Duplicates (FD)

Separate samples collected at the same time and sampling location, shipped and stored under identical conditions. Method precision, including the contribution from sample collection procedures, is estimated from the analysis of Field Duplicates. Field Duplicates are used to prepare Laboratory Fortified Sample Matrix and Laboratory Fortified Sample Matrix Duplicate QC samples. For the purposes of this method, Field Duplicates are collected to support potential repeat analyses (if the original field sample is lost or if there are QC failures associated with the analysis of the original field sample).

3.6 Field Reagent Blank (FRB)

An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are introduced into the sample from shipping, storage, and the field environment.

3.7 Isotope Dilution Analogues

Isotopically labeled analogues of the method analytes that are added to the sample prior to extraction in a known amount. Note: Not all target PFAS currently have an isotopically labelled analogue. In these cases, an alternate isotopically labelled analogue is used as recommended in **Table 5**.

3.8 Isotope Dilution Technique

An analytical technique for measuring analyte concentration using the ratio of the peak area of the native analyte to that of an isotopically labeled analogue, added to the original sample in a known amount and carried through the entire analytical procedure.

3.9 Isotope Performance Standards

Quality control compounds that are added to all standard solutions and extracts in a known amount and used to measure the relative response of the isotopically labelled analogues that are components of the same solution. For this method, the isotope performance standards are three isotopically labeled analogues of the method analytes. The isotope performance standards are indicators of instrument performance and are used to calculate the recovery of the isotope dilution analogues through the extraction procedure. In this method, the isotope performance standards are not used in the calculation of the recovery of the native analytes.

3.10 Laboratory Fortified Blank (LFB)

An aliquot of reagent water to which known quantities of the method analytes and isotope dilution analogues are added. The results of the LFB verify method performance in the absence of sample matrix.

3.11 Laboratory Fortified Sample Matrix (LFSM)

An aliquot of a field sample to which known quantities of the method analytes and isotope dilution analogues are added. The purpose of the LSFM is to determine whether the sample matrix contributes bias to the analytical results. Separate field samples are required for preparing fortified matrix so that sampling error is included in the accuracy estimate.

3.12 Laboratory Fortified Sample Matrix Duplicate (LFSMD)

A Field Duplicate of the sample used to prepare the LFSM that is fortified and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the method analytes are rarely found at concentrations greater than the MRL.

3.13 Laboratory Reagent Blank (LRB)

An aliquot of reagent water fortified with the isotope dilution analogues and processed identically to a field sample. An LRB is included in each Extraction Batch to determine if the method analytes or other interferences are introduced from the laboratory environment, the reagents, glassware, or extraction apparatus.

3.14 Lowest Concentration Minimum Reporting Level (LCMRL)

The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50% to 150% range is at least 99%.[1,2]

3.15 Minimum Reporting Level (MRL)

The minimum concentration that may be reported by a laboratory as a quantified value for a method analyte. For each method analyte, the concentration of the lowest calibration standard must be at or

below the MRL and the laboratory must demonstrate its ability to meet the MRL per the criteria defined in Section 9.1.4.

3.16 Precursor Ion

The gas-phase species corresponding to the method analyte that is produced in the electrospray ionization interface. During tandem mass spectrometry, or MS/MS, the precursor ion is mass selected and fragmented by collision-activated dissociation to produce distinctive product ions of smaller mass to charge (*m/z*) ratio. For this method, the precursor ion is usually the deprotonated molecule ($[M - H]^-$) of the method analyte, except for HFPO-DA. For this analyte, the precursor ion is formed by decarboxylation of HFPO-DA.

3.17 Primary Dilution Standard (PDS)

A solution that contains method analytes (or QC analytes) prepared from stock standards. PDS solutions are used to fortify QC samples and diluted to prepare calibration standards.

3.18 Product Ion

One of the fragment ions that is produced in MS/MS by collision-activated dissociation of the precursor ion.

3.19 Quality Control Standard (QCS)

A calibration standard prepared independently from the primary calibration solutions. For this method, the QCS is a repeat of the entire dilution scheme starting with the same stock materials (neat compounds or purchased stock solutions) used to prepare the primary calibration solutions. Independent sources and separate lots of the starting materials are not required, provided the laboratory has obtained the purest form of the starting materials commercially available. The purpose of the QCS is to verify the integrity of the primary calibration standards.

3.20 Quantitative Standard

A quantitative standard of assayed concentration and purity traceable to a Certificate of Analysis.

3.21 Stock Standard Solution

A concentrated standard that is prepared in the laboratory using assayed reference materials or that is purchased from a commercial source with a Certificate of Analysis.

3.22 Technical-Grade Standard

As defined for this method, a technical-grade standard includes a mixture of the branched and linear isomers of a method analyte. For the purposes of this method, technical-grade standards are used to identify retention times of branched and linear isomers of method analytes.

4 Interferences

4.1 Labware, Reagents and Equipment

Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts or

elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, deactivated syringes, SPE sample transfer lines, etc.[3] Laboratories must demonstrate that these items are not contributing to interference by analyzing LRBs as described in Section 9.2.1.

4.2 Sample Contact with Glass

Aqueous samples should not come in contact with any glass containers or pipettes as PFAS analytes can potentially adsorb to glass surfaces. Standards dissolved in organic solvent may be purchased in glass ampoules. These standards in organic solvent are acceptable and subsequent transfers may be performed using glass syringes and pipets. Following extraction, the eluate must be collected in a polypropylene tube prior to concentration to dryness. Concentration to dryness in glass tubes may cause poor recovery.

4.3 Matrix Interferences

Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and fulvic material may be co-extracted during SPE and high levels may cause enhancement or suppression in the electrospray ionization source.[4] Inorganic salts may cause low recoveries during the anion-exchange SPE procedure.

4.3.1 Co-extracted Organic Material

Under the LC conditions used during method development, matrix effects due to co-extracted organic material enhanced the ionization of 4:2 FTS appreciably. Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.3.2 Inorganic Salts

The authors confirmed acceptable method performance for matrix ion concentrations up to 250 mg/L chloride, 250 mg/L sulfate, and 340 mg/L hardness measured as $CaCO_3$. Acceptable performance was defined as recovery of the isotope dilution analogues between 50–200%.

4.3.3 Ammonium Acetate

Relatively large quantities of ammonium acetate are used as a preservative. The potential exists for trace-level organic contaminants in this reagent. Interferences from this source should be monitored by analysis of LRBs, particularly when new lots of this reagent are acquired.

4.3.4 SPE Cartridges

Solid phase extraction cartridges may be a source of interferences. The analysis of LRBs provides important information regarding the presence or absence of such interferences. Each brand and lot of SPE devices must be monitored to ensure that contamination does not preclude analyte identification and quantitation. SPE cartridges should be sealed while in storage to prevent ambient contamination of the SPE sorbent.

4.4 Bias Caused by Isotopically Labeled Standards

During method development, no isotopically labeled standard solution yielded any signal that gave the same mass and retention time as any native analyte. However, due to isotopic impurity, the $^{13}C_3$-PFBA isotope performance standard contained a small amount of $^{13}C_4$-PFBA, slightly contributing to the signal of the isotope dilution analogue. Further, due to natural abundance of ^{34}S, the native telomer sulfonates produced a small contribution to the $^{13}C_2$ labeled telomer sulfonate isotope dilution analogues. The effects on quantitation are insignificant. However, these cases are described below in Sections 4.4.2 and 4.4.3 to alert the user that these situations could occur.

4.4.1 Method Analytes

At the concentrations used to collect method performance data, the authors could not detect any contribution from the isotope dilution analogues or isotope performance standards to the corresponding native analyte response. However, the user should evaluate each source of isotopically labeled analogues and isotope performance standards to verify that they do not contain any native analyte at concentrations greater than 1/3 of the MRL.

4.4.2 Isotopic purity of $^{13}C_3$-PFBA

In this method, $^{13}C_3$-PFBA is used as an isotope performance standard and $^{13}C_4$-PFBA is used as an isotope dilution analogue. Both share the same product ion, *m/z* 172. Ten nanograms per liter of $^{13}C_4$-PFBA is added to the sample prior to extraction (10 ng/mL extract concentration assuming 100% recovery), and 10 ng/mL of $^{13}C_3$-PFBA is added to the final extract. Because the natural abundance of ^{13}C is 1.1%, there is a 1.1% contribution to the $^{13}C_4$-PFBA area from the lone, unlabeled ^{12}C atom in $^{13}C_3$-PFBA. The authors confirmed this contribution empirically. Users of this method may consider this bias to the area of the PFBA isotope dilution analogue insignificant.

4.4.3 Isotopic purity of $^{13}C_4$-PFBA

A trace amount of $^{13}C_3$-PFBA was detected in the $^{13}C_4$-PFBA. The contribution was no greater than 1%. The contribution of the isotope performance standard to the isotope dilution analogue is insignificant.

4.4.4 Telomer Sulfonates

Each of the three telomer sulfonates in the analyte list (4:2FTS, 6:2FTS, and 8:2FTS) are referenced to their $^{13}C_2$ isotope dilution analogue. The mass difference between the telomer sulfonates and the isotope dilution analogues is 2 mass units. The single sulfur atom in each of the unlabeled molecules has a naturally occurring M+2 isotope (^{34}S) at 4.25%. Thus, the precursor ions of the $^{13}C_2$ isotopically labeled analogues and the naturally occuring ^{34}S analogues present in the native analytes have the same nominal masses. The product ions of the telomer sulfonate isotope dilution analogues listed in **Table 6** would contain a small contribution from the ^{34}S analogue of the native telomer sulfonates. At the concentrations used in this study, the contribution of the ^{34}S analogue to the isotope dilution analogue was not greater than 2.7%. Alternate product ions may be used if there is sufficient abundance.

5 Safety

Each chemical should be treated as a potential health hazard and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding

safe handling of chemicals used in this method. A reference file of safety data sheets should be made available to all personnel involved in the chemical analysis.

6 Equipment and Supplies

References to specific brands and catalog numbers are included as examples only and do not imply endorsement of the products. Such reference does not preclude the use of equivalent products from other vendors or suppliers. Due to potential adsorption of analytes onto glass, polypropylene containers were used for sample preparation and extraction steps. Other plastic materials (e.g., polyethylene) that meet the QC requirements of Section 9 may be substituted.

6.1 Sample Containers

Polypropylene bottles with polypropylene screw caps (for example, 250 mL bottles, Fisher Scientific, Cat. No. 02-896-D or equivalent).

6.2 Polypropylene Vials

These vials are used to store stock standards and PDS solutions (4 mL, VWR Cat. No. 16066-960 or equivalent).

6.3 Centrifuge Tubes

Conical polypropylene centrifuge tubes (15 mL) with polypropylene screw caps for storing standard solutions and for collection of the eluate during the extraction procedure (Thomas Scientific Cat. No. 2602A10 or equivalent).

6.4 Autosampler Vials

Polypropylene autosampler vials (ThermoFisher, Cat. No. C4000-14) with polypropylene caps (ThermoFisher, Cat. No. C5000-50 or equivalent). Note: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, creating the potential for evaporation to occur after injection. Multiple injections from the same vial are not permissible unless the cap is replaced immediately after injection.

6.5 Micro Syringes

Suggested sizes include 10, 25, 50, 100, 250, 500 and 1000 µL.

6.6 Pipets

Polypropylene or glass pipets may be used for methanolic solutions.

6.7 Analytical Balance

Capable of weighing to the nearest 0.0001 g.

6.8 Solid Phase Extraction (SPE) Apparatus

6.8.1 SPE Cartridges

SPE cartridges containing weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand), particle size approximately 33 µm. The SPE sorbent must have a pKa above 8 so that it remains positively charged during extraction. SPE cartridges containing 500 mg sorbent

(Phenomenex Cat. No. 8B-S038-HCH) were used during method development. Use of 200 mg cartridges is acceptable for the extraction of 100 mL samples.

6.8.2 Vacuum Extraction Manifold

Equipped with flow and vacuum control [Supelco Cat. No. 57030-U, UCT Cat. No. VMF016GL (the latter requires UCT Cat. No. VMF02116 control valves), or equivalent systems]. Automated devices designed for use with SPE cartridges may be used; however, all extraction and elution steps must be the same as in the manual procedure. Care must be taken with automated SPE systems to ensure that Teflon tubing and other PTFE components commonly used in these systems, do not contribute to unacceptable analyte concentrations in LRBs.

6.8.3 Sample Delivery System

Use of large volume sampling lines, constructed with polyethylene tubing, are recommended, but not mandatory. Large volume sample transfer lines, constructed with PTFE tubing, are commercially available for standard extraction manifolds (Supelco Cat. No. 57275 or equivalent). The PTFE tubing can be replaced with 1/8" o.d. x 1/16" i.d. polyethylene tubing [Freelin-Wade (McMinnville, Oregon) LLDPE or equivalent] cut to an appropriate length. This prevents potential contamination from PTFE transfer lines. Other types of non-PTFE tubing may be used provided it meets the LRB and LFB QC requirements. PTFE tubing may be used, but an LRB must be run on each individual transfer line and the QC requirements in Section 9.2.1 must be met. In the case of automated SPE, the removal of PTFE lines may not be feasible; therefore, acceptable performance for the LRB must be met for each port during the IDC (Sect 9.1.1). LRBs must be rotated among the ports during routine analyses thereafter. Plastic reservoirs are difficult to rinse during elution and their use may lead to lower recovery.

6.9 Extract Concentration System

Extracts are concentrated by evaporation with high-purity nitrogen using a water bath set no higher than 60 °C [N-Evap, Model 11155, Organomation Associates (Berlin, MA), Inc., or equivalent].

6.10 Laboratory Vacuum System

Sufficient capacity to maintain a vacuum of approximately 15 to 20 inches of mercury for extraction cartridges.

6.11 pH Meter

Used to verify the pH of the phosphate buffer and to measure the pH of the aqueous sample prior to anion exchange SPE.

6.12 LC-MS/MS System

6.12.1 LC System

The LC system must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate. On some LC systems, PFAS may build up in PTFE transfer lines when the system is idle for more than one day. To prevent long delays in purging high levels of PFAS from the LC solvent lines, it may be useful to replace PTFE tubing with PEEK™ tubing and the PTFE solvent frits with stainless steel frits. These modifications were not used on the LC system used for method development. However, a delay column, HLB Direct Connect 2.1 x 30 mm (Waters 186005231),

was placed in the mobile phase flow path immediately before the injection valve. This direct connect column may have reduced the co-elution of PFAS originating from sources prior to the sample loop from the PFAS injected in the sample. It may not be possible to remove all PFAS background contamination.

6.12.2 Analytical Column

C18 liquid chromatography column (2 x 50 mm) packed with 3 µm C18 solid phase particles (Phenomenex Part Number 00B-4439-B0 or equivalent).

6.12.3 Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)

The mass spectrometer must be capable of electrospray ionization in the negative ion mode. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is needed to ensure adequate precision. Some ESI-MS/MS instruments may not be suitable for PFAS analysis. See the procedures in Section 10.1.2.1 to ensure that the selected MS/MS platform is capable of monitoring all the required MS/MS transitions for the method analytes.

6.12.4 MS/MS Data System

An interfaced data system is required to acquire, store, and output MS data. The computer software must have the capability of processing stored data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the abundance of any specific ion between specified time or scan number limits. The software must be able to construct a linear regression or quadratic regression calibration curve and calculate analyte concentrations using the internal standard technique.

7 Reagents and Standards

Reagent grade or better chemicals must be used. Unless otherwise indicated, all reagents must conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available. Other grades may be used if the reagent is demonstrated to be free of analytes and interferences and all requirements of the IDC are met when using these reagents.

7.1 Reagent Water

Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than one-third of the MRL for each method analyte. It may be necessary to flush the water purification unit to rinse out any build-up of PFAS in the system prior to collection of reagent water.

7.2 Methanol

CH_3OH, CASRN 67-56-1, LC grade (Fisher Scientific, Cat. No. A456 or equivalent).

7.3 Ammonium Acetate

$NH_4C_2H_3O_2$, CASRN 631-61-8, HPLC grade, molecular weight equals 77.08 g/mole.

7.3.1 20 mM Ammonium Acetate

Chromatographic mobile phase. To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water. This solution is volatile and must be replaced at least once per week. More frequent replacement may be necessary if unexplained losses in sensitivity or retention time shifts are encountered.

7.3.2 1 g/L Ammonium Acetate

Used to rinse SPE cartridges after loading the aqueous sample and prior to the methanol rinse. Prepare in reagent water.

7.4 Concentrated Ammonium Hydroxide Reagent

NH_4OH, CASRN 1336-21-6, approximately 56.6% in water as ammonium hydroxide (w/w), approximately 28% in water as ammonia, approximately 14.5 N (Fisher Scientific, Cat. No. A669, Certified ACS Plus grade, or equivalent).

7.5 Solution of Ammonium Hydroxide in Methanol

Used for elution of SPE cartridges. Dilute 2 mL of concentrated ammonium hydroxide (56.6% w/w) in 100 mL methanol. This solution should be made fresh on the day of extraction.

7.6 Sodium Phosphate Dibasic (Na_2HPO_4)

Used for creating the aqueous buffer for conditioning the SPE cartridges. Dibasic sodium phosphate may be purchased in either the anhydrous or any hydrated form. The formula weight will vary based on degree of hydration.

7.7 Sodium Phosphate Monobasic (NaH_2PO_4)

Used for creating the aqueous buffer for conditioning the SPE cartridges. Monobasic sodium phosphate may be purchased in either the anhydrous or any hydrated form. The formula weight will vary based on degree of hydration.

7.8 0.1 M Phosphate Buffer pH 7.0

Mix 500 mL of 0.1 M dibasic sodium phosphate with approximately 275 mL of 0.1 M monobasic sodium phosphate. Verify that the solution pH is approximately 7.0.

7.9 Nitrogen

7.9.1 Nitrogen Nebulizer Gas

Nitrogen used as a nebulizer gas in the ESI interface and as collision gas in some MS/MS platforms should meet or exceed the instrument manufacturer's specifications.

7.9.2 Nitrogen used for Concentrating Extracts

Ultra-high-purity-grade nitrogen should be used to concentrate sample extracts.

7.10 Argon

Used as collision gas in MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen may be used as the collision gas if recommended by the instrument manufacturer.

7.11 Sodium Hydroxide

May be purchased as pellets or as aqueous solution of known concentration. Added to methanolic solutions of PFAS to prevent esterification.

7.12 Acetic Acid (glacial)

May be necessary to adjust pH of aqueous samples. The pH of the aqueous sample containing 1 g/L ammonium acetate must be between 6 and 8.

7.13 Standard Solutions

7.13.1 Stability of Methanolic Solutions

Fluorinated carboxylic acids will esterify in anhydrous acidic methanol. To prevent esterification, standards must be stored under basic conditions. If base is not already present, this may be accomplished by the addition of sodium hydroxide (approximately 4 mole equivalents) when standards are diluted in methanol. When calculating molarity for solutions containing multiple PFAS, the molecular weight can be estimated as 250 atomic mass units (amu). It is necessary to include sodium hydroxide in solutions of both isotopically labeled and native analytes. The amount of sodium hydroxide needed may be calculated using the following equation:

$$\frac{Total\ PFAS\ mass\ (g) \times 160(\frac{g}{mol})}{250\ (\frac{g}{mol})} = Mass\ of\ NaOH\ Required\ (g)$$

7.13.2 Preparation of Standards

When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Sorption of PFAS analytes in methanol solution to glass surfaces after prolonged storage has not been evaluated. PFAS analyte and isotopically labeled analogues commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be stored in polypropylene containers.
Solution concentrations listed in this section were used to develop this method and are included as examples. Alternate concentrations may be used as necessary depending on instrument sensitivity and the calibration range used. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. Laboratories should use standard QC practices to determine when standards need to be replaced. The analyte supplier's guidelines may be helpful when making this determination.

7.14 Storage Temperatures for Standards Solutions

Store stock standards at less than 4 °C unless the vendor recommends otherwise. The Primary Dilution Standards may be stored at any temperature, but cold storage is recommended to prevent solvent evaporation. During method development, the PDS was stored at –20 °C and no change in analyte concentrations was observed over a period of 6 months.

7.15 Isotope Performance Standards

This method requires three isotope performance standards listed in the table below. These isotopically labeled compounds were chosen during method development to include the analogues of three method analytes: two carboxylates with different chain lengths and a sulfonate.

Obtain the isotope performance standards as certified standard solutions, if available, or as the neat compounds. During method development, the isotope performance standards were obtained from Wellington Laboratories (Guelph, ON, Canada) as certified stocks in basic methanol. Note that Chemical Abstracts Registry Numbers are not currently available for these compounds. The concentrations of the stocks supplied by Wellington are listed in the table below.

Isotope Performance Standards	Abbreviation	Wellington Stock, µg/mL	PDS, ng/µL
Perfluoro-*n*-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	50	1.0
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA	50	1.0
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS	50[a]	3.0

a. 47.8 µg/mL as the anion.

All the isotope performance standards listed in this section must be used, if available. Additional isotope performance standards may be used provided they are isotopically labeled analytes or labeled analytes with similar functional groups as the method analytes. Linear isomers are recommended to simplify peak integration. Method modification QC requirements must be met (Sect. 9.3) whenever additional isotope performance standards are used.

7.15.1 Isotope Performance Standard PDS

Prepare the isotope performance standard PDS in methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. The PDS concentrations used to develop the method are listed in the table above (Sect. 7.15). During collection of method performance data, the final extracts were fortified with 10 µL of the PDS to yield a concentration of 10 ng/mL for $^{13}C_3$-PFBA and $^{13}C_2$-PFOA, and 30 ng/mL for $^{13}C_4$-PFOS (28.7 ng/mL as the anion).

7.16 Isotope Dilution Analogues

Obtain the isotopically labeled analogues listed in the table in this section as individual certified standard solutions or as certified standard mixes. All listed isotope dilution analogues must be used, if available. Linear isomers are recommended to simplify peak integration. During method development, the isotope dilution analogues were obtained from Wellington Laboratories (Guelph, ON, Canada) as certified stocks in basic methanol. These analogues were chosen during method development because they encompass most of the functional groups, as well as the molecular weight range of the method analytes. Note that Chemical Abstracts Registry Numbers are not currently available for these isotopically labeled analogues.

Isotope Dilution Standards	Abbreviation	PDS, ng/µL[a]
Perfluoro-*n*-[1,2,3,4-$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	0.50
Perfluoro-*n*-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	0.50
Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	$^{13}C_3$-PFBS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	$^{13}C_2$-4:2FTS	2.0
Perfluoro-*n*-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	0.50
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	0.50
Perfluoro-*n*-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	0.50
Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-13C_2]-octane sulfonate	$^{13}C_2$-6:2FTS	2.0
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	0.50
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	0.50
Sodium perfluoro-[$^{13}C_8$]octanesulfonate	$^{13}C_8$-PFOS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	$^{13}C_2$-8:2FTS	2.0
Perfluoro-*n*-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	0.50
Perfluoro-*n*-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	0.50
Perfluoro-*n*-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	0.50

[a] Concentrations used during method development.

As additional isotopically labelled PFAS analogues become commercially available they may be integrated into the method provided they have similar functional groups as the method analytes or are isotopically labeled analogues of the method analytes. Method modification QC requirements must be met (Sect. 9.3) whenever new analogues are proposed.

7.16.1 Isotope Dilution Analogue PDS

Prepare the isotope dilution analogue PDS in methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. The PDS concentrations used during method development are listed in the table above. Method performance data were collected using 20 µL of this PDS to yield concentrations of 40–160 ng/L in the 250 mL aqueous samples. Note that the concentrations of sulfonates in the isotope dilution analogue PDS is based on the weight of the salt. It is not necessary to account for difference in the formula weight of the salt compared to the free acid for sample quantitation.

7.17 Analyte Standard Materials

Analyte standards may be purchased as certified standard solutions or prepared from neat materials of assayed purity. If available, the method analytes should be purchased as technical-grade (as defined in Sect. 3.22) to ensure that linear and branched isomers are represented. Standards or neat materials that contain only the linear isomer can be substituted if technical-grade analytes are not available as quantitative standards.

During method development, analyte standards were obtained from AccuStandard, Inc. (New Haven, CT), Absolute Standards (Hamden, CT), Wellington Laboratories (Guelph, Ontario, Canada), Santa Cruz Biotechnology (Dallas, TX), and Synquest Laboratories, Inc. (Alachua, FL). Stock standards are made by dilution in methanol containing 4 mole equivalents of sodium hydroxide as described in Section 7.13.1

7.17.1 PFOA

A quantitative standard for PFOA is currently available only for the linear isomer; however, a technical-grade standard (Sect. 3.22) is available for PFOA that contains the linear and branched isomers (Wellington Labs, Cat. No. T-PFOA, or equivalent). This product or a similar technical-grade PFOA standard must be used to identify the retention times of the branched and linear PFOA isomers. However, the linear-only PFOA standard must be used for quantitation until a quantitative PFOA standard containing the branched and linear isomers becomes commercially available.

7.17.2 PFHxS and PFOS

Technical grade, quantitative PFHxS and PFOS standards containing branched and linear isomers must be used when available.

7.17.3 Correction for Analytes Obtained in the Salt Form

This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be commercially available as neat materials or as certified stock standards as their corresponding ammonium, sodium, or potassium salts. These salts are acceptable standards provided the measured mass, or concentration, is corrected for the salt content. The equation for this correction is provided below.

$$mass(acid\ form) = mass(salt\ form) \times \frac{MWacid}{MWsalt}$$

7.17.4 Analyte PDS

The analyte PDS is used to prepare the calibration standards and to fortify the LFBs, LFSMs and LFSMDs with the method analytes. Prepare the analyte PDS by combining and diluting the analyte stock standards in 100% methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. Select nominal analyte concentrations for the PDS such that between 5 and 100 µL of the PDS is used to fortify samples and prepare standard solutions. More than one PDS concentration may be necessary to meet this requirement. During method development, the analyte PDS was prepared at an identical concentration for all analytes, 0.5 ng/µL. The user may modify the concentrations of the individual analytes based on the confirmed MRLs and the desired monitoring range. If the PDS is stored cold, warm the vials to room temperature and vortex prior to use.

7.17.5 Calibration Standards

Prepare a series of calibration standards of at least five levels by diluting the analyte PDS into methanol containing 20% reagent water. The lowest calibration standard must be at or below the MRL for each analyte. The calibration standards may also be used as Continuing Calibration Checks (CCCs). Using the PDS solutions, add a constant amount of the isotope performance standards and the isotope dilution analogues to each calibration standard. The concentration of the isotope dilution analogues should match the concentration of the analogues in sample extracts, assuming 100% recovery through the extraction process. During method development, the concentrations of the isotope dilution analogues were 40 ng/mL extract concentration (160 ng/L in the aqueous sample) for 4:2FTS, 6:2FTS and 8:2FTS, and 10 ng/mL (40 ng/L) for all others. The analyte calibration ranged from approximately 0.50 ng/mL to 25 ng/mL extract concentration.

8 Sample Collection, Preservation, and Storage

8.1 Sample Bottles

Samples must be collected in plastic bottles: polypropylene bottles fitted with polypropylene screw-caps, or polyethylene bottles with polypropylene screw caps. Discard sample bottles after a single use. The bottle volume should approximate the volume of the sample. Subsampling from a single bottle is not permitted except as described in Section 12.5.

8.2 Sample Preservation

Based on sample volume, add ammonium acetate to each sample bottle as a solid (prior to shipment to the field or immediately prior to sample collection) to achieve a 1g/L concentration of ammonium acetate. Ammonium acetate will sequester free chlorine to form chloramine.

8.3 Sample Collection

8.3.1 Precautions against Contamination

Workers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. Users should seek to minimize accidental contamination of the samples.

8.3.2 Collection Procedure

Open the tap and allow the system to flush until the water temperature has stabilized. Collect samples from the flowing system. Samples do not need to be collected headspace free. After collecting the sample, cap the bottle and agitate by hand until the preservative is dissolved. Keep the sample sealed from time of collection until extraction.

8.4 Field Reagent Blanks (FRB)

Each sample set must include an FRB. A sample set is defined as samples collected from the same site and at the same time. The same lot of preservative must be used for the FRBs as for the field samples.

8.4.1 Analysis of Reagent Water used for FRBs

Reagent water used for the FRBs must be analyzed prior to shipment to ensure the water has minimal residual PFAS. Extract an LRB prepared with reagent water using the same lot of sample bottles destined for shipment to the sampling site and ensure that analyte concentrations are less than one-third the MRL, as described in Section 9.2.1. This will ensure that any significant contamination detected in the FRBs originated from exposure in the field.

8.4.2 Field Reagent Blank Procedure

In the laboratory, fill the FRB sample bottle with the analyzed reagent water (Sect. 8.4.1), then seal and ship to the sampling site with the sample bottles. For each FRB shipped, a second FRB sample bottle containing only preservative must also be shipped. At the sampling site, open the FRB bottle and pour the reagent water into the second sample bottle containing preservative; seal and label this bottle as the FRB with the date, time and location of the site.

8.5 Sample Shipment and Storage

Samples must be shipped on ice. Samples are valid if any ice remains in the cooler when it is received at the laboratory or bottles are received within 2 days of collection and below 10 ^{0}C. Once at the laboratory, samples must be stored at or below 6 °C until extraction. Samples must not be frozen.

8.6 Sample and Extract Holding Times

Analyze samples as soon as possible. Samples must be extracted within 28 days of collection. Extracts are generally stored at room temperature and must be analyzed within 28 days after extraction.

9 Quality Control

QC procedures include the IDC and ongoing QC requirements. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to satisfy method objectives. The QC criteria discussed in the following sections are summarized in **Table 16** and **Table 17**. These QC requirements are considered the minimum for an acceptable QC program. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1 Initial Demonstration of Capability

The IDC must be successfully performed prior to analyzing field samples. The IDC must be repeated if changes are made to analytical parameters not previously validated during the IDC. This may include, for example, changing the sample volume, selecting alternate quantitation ions, extending the calibration range, adding additional isotope performance standards, or adding additional isotope dilution analogues. Prior to conducting the IDC, the analyst must meet the calibration requirements outlined in Section 10. The same calibration range used during the IDC must be used for the analysis of field samples.

9.1.1 Demonstration of Low System Background

Analyze an LRB immediately after injecting the highest calibration standard in the selected calibration range. Confirm that the blank is free from contamination as defined in Section 9.2.1. If an automated extraction system is used, an LRB must be extracted on each port to fulfil this requirement.

9.1.2 Demonstration of Precision

Prepare, extract, and analyze seven replicate LFBs in a valid Extraction Batch (seven LFBs and an LRB). Fortify the LFBs near the midpoint of the initial calibration curve. The percent relative standard deviation (%RSD) of the concentrations of the replicate analyses must be less than 20% for all method analytes.

9.1.3 Demonstration of Accuracy

Using the same set of replicate data generated for Section 9.1.2, calculate the average percent recovery. The average recovery for each analyte must be within a range of 70–130%.

9.1.4 Minimum Reporting Level (MRL) Confirmation

Establish a target concentration for the MRL (Sect. 3.15) based on the intended use of the method. If there is a programmatic MRL requirement, the laboratory MRL must be set at or below this level. In doing so, one should consider that establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements.

Perform initial calibration following the procedures in Section 10.3. The lowest calibration standard used to establish the initial calibration (as well as the low-level CCC) must be at, or below, the MRL. Confirm the laboratory's ability to meet the MRL following the procedure outlined below.

9.1.4.1 Prepare and Analyze MRL Samples

Fortify, extract, and analyze seven replicate LFBs at, or below, the proposed MRL concentration.

9.1.4.2 Calculate MRL Statistics

Calculate the mean and standard deviation for each analyte in these replicates. Determine the Half Range for the Prediction Interval of Results (HR_{PIR}) using the following equation:

$$HR_{PIR} = 3.963S$$

Where,

S = the standard deviation and 3.963 is a constant value for seven replicates.[1]

Calculate the Upper and Lower Limits for the Prediction Interval of Results (*PIR* = *Mean* ± HR_{PIR}) as shown below. These equations are only defined for seven replicate samples.

$$Upper\ PIR\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100$$

$$Lower\ PIR\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100$$

9.1.4.3 MRL Acceptance Criteria

The laboratory's ability to meet the MRL is confirmed if the *Upper PIR Limit* is less than, or equal to, 150%; and the *Lower PIR Limit* is greater than, or equal to, 50%. If these criteria are not met, the MRL has been set too low and must be confirmed again at a higher concentration.

9.1.5 Calibration Verification

Analyze a QCS (Sect. 9.2.9) to confirm the accuracy of the primary calibration standards.

9.2 Ongoing QC Requirements

This section describes the ongoing QC elements that must be included when processing and analyzing field samples.

9.2.1 Laboratory Reagent Blank (LRB)

Analyze an LRB with each Extraction Batch. Background concentrations of method analytes must be less than one-third the MRL. If method analytes are detected in the LRB at concentrations greater than or equal to this level, then all positive field sample results (i.e., results at or above the MRL) for those analytes are invalid for all samples in the Extraction Batch. Subtracting blank values from sample results is not permitted.

9.2.1.1 Estimating Background Concentrations

Although quantitative data below the MRL may not be accurate enough for data reporting, such data are useful in determining the magnitude of background interference. Therefore, the analyte concentrations in the LRB may be estimated by extrapolation when results are below the MRL.

9.2.1.2 Influence of Background on Selection of MRLs

Because background contamination can be a significant problem, some MRLs may be background limited.

9.2.1.3 Evaluation of Background when Analytes Exceed the Calibration Range

After analysis of a sample in which method analytes exceed the calibration range, one or more LRBs must be analyzed (to detect potential carryover) until the system meets the LRB acceptance criteria. If this occurs during an automated sequence, examine the results of samples analyzed following the sample that exceeded the calibration range. If the analytes that exceeded the calibration range in the previous sample are detected at, or above, the MRL, these samples are invalid. If the affected analytes do not exceed the MRL, these subsequent samples may be reported.

9.2.2 Continuing Calibration Check (CCC)

Analyze CCC standards at the beginning of each Analysis Batch, after every tenth field sample, and at the end of the Analysis Batch. See Section 10.4 for concentration requirements and acceptance criteria for CCCs.

9.2.3 Laboratory Fortified Blank

An LFB is required with each Extraction Batch. The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch.

9.2.3.1 LFB Concentration Requirements

Fortify the low concentration LFB near the MRL. The high concentration LFB must be near the high end of the calibration range.

9.2.3.2 Evaluate Analyte Recovery

Results for analytes fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) must be within 50–150% of the true value. Results for analytes fortified at all other concentrations must be within 70–130% of the true value. If the LFB results do not meet these criteria, then all data for the problem analytes must be considered invalid for all samples in the Extraction Batch.

9.2.4 Isotope Performance Standard Areas

The analyst must monitor the peak areas of the isotope performance standards in all injections of the Analysis Batch. The isotope performance standard responses (as indicated by peak area) in any chromatographic run must be within 50–150% of the average area measured during the initial calibration. Random evaporation losses have been observed with the polypropylene caps causing high-biased isotope performance standard areas. If an isotope performance standard area for a sample does not meet these criteria, reanalyze the extract in a subsequent Analysis Batch. If the isotope performance standard area fails to meet the acceptance criteria in the repeat analysis, extraction of the sample must be repeated, provided the sample is still within holding time.

9.2.5 Isotope Dilution Analogue Recovery

Calculate the concentration of each isotope dilution analogue in field and QC samples using the average area in the initial calibration and the internal standard technique. Calculate the percent recovery (%R) for each analogue as follows:

$$\%R = \frac{A}{B} \times 100$$

Where,
A = measured concentration of the isotope dilution analogue, and
B = fortification concentration of the isotope dilution analogue.

The percent recovery for each analogue must be within a range of 50–200%.

9.2.5.1 Corrective Action for Failed Analogue Recovery

If an isotope dilution analogue fails to meet the recovery criterion, evaluate the area of the isotope performance standard to which the analogue is referenced and the recovery of the analogues in the CCCs. If necessary, recalibrate and service the LC-MS/MS system. Take corrective action, then analyze the failed extract in a subsequent Analysis Batch. If the repeat analysis meets the 50–200% recovery criterion, report only data for the reanalyzed extract. If the repeat analysis fails the recovery criterion after corrective action, extraction of the sample must be repeated provided a sample is available and still within the holding time.

9.2.6 Laboratory Fortified Sample Matrix (LFSM)

Within each Extraction Batch, analyze a minimum of one LFSM. The native concentrations of the analytes in the sample matrix must be determined in a separate field sample and subtracted from the measured values in the LFSM. If various sample matrices are analyzed regularly, for example, drinking water processed from ground water and surface water sources, collect performance data for each source.

9.2.6.1 Prepare the LFSM

Prepare the LFSM by fortifying a Field Duplicate with an appropriate amount of the analyte PDS (Sect. 7.17.4) and isotope dilution analogue PDS (Sect. 7.16.1). Generally, select a spiking concentration that is greater than or equal to the native concentration for the analytes. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data when selecting a fortifying concentration.

9.2.6.2 Calculate the Percent Recovery

Calculate the percent recovery (%R) using the equation:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where,
A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

In order to obtain meaningful percent recovery results, correct the measured values in the LFSM and LFSMD for the native levels in the unfortified samples, even if the native values are less than the MRL.

9.2.6.3 Evaluate Analyte Recovery in the LFSM

Results for analytes fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) must be within 50–150% of the true value. Results for analytes fortified at all other concentrations must be within 70–130% of the true value. If the accuracy for any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs and in the LFB, the recovery is judged matrix biased. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix".

9.2.7 Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD)

Within each Extraction Batch, analyze a minimum of one Field Duplicate or one Laboratory Fortified Sample Matrix Duplicate. If the method analytes are not routinely observed in field samples, analyze an LFSMD rather than an FD.

9.2.7.1 Calculate the RPD for the LFSM and LFSMD

If an LFSMD is analyzed instead of a Field Duplicate, calculate the RPD using the equation:

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100$$

9.2.7.2 Acceptance Criterion for the RPD of the LFSM and LFSMD

RPDs for duplicate LFSMs must be less than, or equal to, 30% for each analyte. Greater variability may be observed when the matrix is fortified at analyte concentrations near or at the MRL (within a factor of two times the MRL concentration). LFSMs at these concentrations must have RPDs that are less than or equal to 50%. If the RPD of an analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCCs and in the LFB, the precision is judged matrix influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix".

9.2.7.3 Calculate the RPD for Field Duplicates

Calculate the relative percent difference (RPD) for duplicate measurements. (FD1 and FD2) using the equation:

$$RPD = \frac{|\mathrm{FD}_1 - \mathrm{FD}_2|}{(\mathrm{FD}_1 + \mathrm{FD}_2)/2} \times 100$$

9.2.7.4 Acceptance Criterion for Field Duplicates

RPDs for Field Duplicates must be less than, or equal to, 30% for each analyte. Greater variability may be observed when Field Duplicates have analyte concentrations that are near or at the MRL (within a factor of two times the MRL concentration). At these concentrations, Field Duplicates must have RPDs that are less than or equal to 50%. If the RPD of an analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC and in the LFB, the precision is judged matrix influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix"

9.2.8 Field Reagent Blank (FRB)

The purpose of the FRB is to ensure that PFAS measured in the field samples were not inadvertently introduced into the sample during sample collection and handling. The FRB is processed, extracted, and analyzed in exactly the same manner as a field sample. Analysis of the FRB is required only if a field

sample contains a method analyte or analytes at, or above, the MRL. If a method analyte found in the field sample is present in the FRB at a concentration greater than one-third of the MRL, then the results for that analyte are invalid for all samples associated with the failed FRB.

9.2.9 Calibration Verification using QCS

A QCS must be analyzed during the IDC, and then quarterly thereafter. For this method, the laboratory is not required to obtain standards from a source independent of the primary calibration standards. Instead, the laboratory should acquire the best available quantitative standards (Sect. 3.20) and use these to prepare both the primary calibration standards and the QCS. The QCS must be an independent dilution beginning with the common starting materials. Preparation by a second analyst is recommended. The acceptance criterion for the QCS is 70–130% of the true value. If the accuracy for any analyte fails the recovery criterion, prepare fresh standard dilutions and repeat the Calibration Verification.

9.3 Method Modification QC Requirements

The analyst is permitted to modify the chromatographic and MS/MS conditions. Examples of permissible method modifications include alternate LC columns, MRM transitions, and additional QC analytes proposed for use with the method. Any method modifications must be within the scope of the established method flexibility and must retain the basic chromatographic elements of this method (Sect. 2). The following are required after a method modification.

9.3.1 Repeat the IDC

Establish an acceptable initial calibration (Sect. 10.3) using the modified conditions. Repeat the procedures of the IDC (Sect. 9.1).

9.3.2 Document Performance in Representative Sample Matrices

The analyst is also required to evaluate and document method performance for the modifications in real matrices that span the range of waters that the laboratory analyzes. This additional step is required because modifications that perform acceptably in the IDC, which is conducted in reagent water, could fail ongoing method QC requirements in real matrices. This is particularly important for methods subject to matrix effects, such as LC-MS/MS-based methods. For example, a laboratory may routinely analyze finished drinking water from municipal treatment plants that process ground water, surface water, or a blend of surface and ground water. In this case, the method modification requirement could be accomplished by assessing precision (Sect. 9.1.2) and accuracy (Sect. 9.1.3) in finished drinking waters derived from a surface water with moderate to high total organic carbon (e.g., 2 mg/L or greater) and from a hard ground water (e.g., 250 mg/L as calcium carbonate ($CaCO_3$) equivalent, or greater).

10 Calibration and Standardization

Demonstration and documentation of acceptable MS calibration and initial analyte calibration are required before performing the IDC and prior to analyzing field samples. The initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.1 MS/MS Optimization

10.1.1 Mass Calibration

Calibrate the mass spectrometer with the calibration compounds and procedures specified by the manufacturer.

10.1.2 MS Parameters

During the development of this method, instrumental parameters were optimized for the precursor and product ions listed in **Table 6**. Product ions other than those listed may be selected; however, the analyst should avoid using ions with lower mass or common ions that may not provide sufficient discrimination between the analytes of interest and co-eluting interferences.

10.1.2.1 Requirement for Branched Isomers

There have been reports that not all product ions in the linear PFOS are produced in all branched PFOS isomers.[5] (This phenomenon may exist for many of the PFAS.) For this method, the *m/z* 80 product ion must be used for PFOS and PFHxS to minimize this problem and promote comparability between laboratories. Some MS/MS instruments, may not be able to scan a product ion with such a wide mass difference from the precursor ion. These instruments may not be used for this method if PFOS or PFHxS analysis is to be conducted.

10.1.2.2 Precursor Ion

Optimize the response of the precursor ion ($[M - H]^-$ or $[M - CO_2 - H]^-$) for each analyte following manufacturer's guidance. Analyte concentrations of 1.0 µg/mL were used for this step during method development. Vary the MS parameters (source voltages, source and desolvation temperatures, gas flows, etc.) until optimal analyte responses are determined. The electrospray parameters used during method development are listed in **Table 2**. The analytes may have different optimal parameters, requiring some compromise on the final operating conditions. See **Table 6** for ESI-MS conditions used to collect method performance data.

10.1.2.3 Product Ion

Optimize the product ion for each analyte following the manufacturer's guidance. Typically, the carboxylic acids have similar MS/MS conditions and the sulfonic acids have similar MS/MS conditions. See **Table 6** for MS/MS conditions used to collect method performance data.

10.2 Chromatographic Conditions

Establish LC operating parameters that optimize resolution and peak shape. Suggested LC conditions can be found in **Table 1**. Modifying the solvent composition of the standard or extract by increasing the aqueous content to better focus early eluting compounds on the column is not permitted. A decrease in methanol concentration could lead to lower or imprecise recovery of the more hydrophobic method analytes, while higher methanol concentration could lead to the precipitation of salts in some extracts. The peak shape of the early eluting compounds may be improved by increasing the volume of the injection loop or increasing the aqueous content of the initial mobile phase composition.

10.2.1 Minimizing PFAS Background

LC system components, as well as the mobile phase constituents, may contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep baseline levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, priming the mobile phase and flushing the column with at least 90% methanol before initiating a sequence may reduce background contamination.

10.2.2 Establishing Branched vs. Linear Isomer Profiles

Prepare and analyze the technical-grade standard of PFOA, discussed in Section 7.17.1, at a mid- to high-level concentration. Identify the retention times of the branched isomers of PFOA present in the technical-grade PFOA standard. When PFOA is chromatographed on a reversed-phase column, the branched isomers elute prior to the linear isomer. Repeat the procedure in this section for PFHxS and PFOS discussed in Section 7.17.2, and any other analytes for which technical-grade standards have been acquired. The branched isomer identification checks must be repeated any time chromatographic changes occur that alter analyte retention times.

10.2.3 Establish LC-MS/MS Retention Times and MRM Segments

Inject a mid- to high-level calibration standard under optimized LC-MS/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into segments that contain one or more chromatographic peaks. For maximum sensitivity, minimize the number of MRM transitions that are simultaneously monitored within each segment. Ensure that the retention time window used to collect data for each analyte is of sufficient width to detect earlier eluting branched isomers.
The retention times observed during collection of the method performance data are listed in **Table 3**, **Table 4**, and **Table 5**.

10.3 Initial Calibration

This method has three isotope performance standards that are used as reference compounds for the internal standard quantitation of the isotope dilution analogues. The suggested isotope performance standard reference for each isotope dilution analogue is listed in **Table 4**. The sixteen isotope dilution analogues are used as reference compounds to quantitate the native analyte concentrations. The suggested isotope dilution analogue references for the native analytes are listed in **Table 5**.

10.3.1 Calibration Standards

Prepare a set of at least five calibration standards as described in Section 7.17.5. The analyte concentrations in the lowest calibration standard must be at or below the MRL.

10.3.2 Calibration Curves of Native Analytes

Quantitate the native analytes using the internal standard calibration technique. The internal standard technique calculates concentration based on the ratio of the peak area of the native analyte to that of the isotope dilution analogue. Calibrate the LC-MS/MS and fit the calibration points with either a linear or quadratic regression. Weighting may be used. Forcing the calibration curve through the origin is mandatory for this method. Forcing zero allows for a better estimate of the background levels of

method analytes. The MS/MS instrument used during method development was calibrated using weighted (1/x) quadratic regression with forced zero.

10.3.3 Calibration of Isotope Dilution Analogues

The isotope dilution analogues are quantified using the internal standard calibration technique. Because isotope dilution analogues are added at a single concentration level to the calibration standards, calibrate for each of these using an average response factor.

10.3.4 Calibration of Isotope Performance Standards

Because Isotope performance standards are added at a single concentration level to the calibration standards, calibrate for each of these using an average response factor.

10.3.5 Calibration Acceptance Criteria

Evaluate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. For calibration levels that are less than or equal to the MRL, the result for each analyte should be within 50–150% of the true value. All other calibration points should be within 70–130% of their true value. If these criteria cannot be met, the analyst could have difficulty meeting ongoing QC criteria. In this case, corrective action is recommended such as reanalyzing the calibration standards, restricting the range of calibration, or performing instrument maintenance. If the cause for failure to meet the criteria is due to contamination or standard degradation, prepare fresh calibration standards and repeat the initial calibration.

10.4 Continuing Calibration

Analyze a CCC to verify the initial calibration at the beginning of each Analysis Batch, after every tenth field sample, and at the end of each Analysis Batch. The beginning CCC for each Analysis Batch must be at, or below, the MRL for each analyte. This CCC verifies instrument sensitivity prior to the analysis of samples. If standards have been prepared such that all low calibration levels are not in the same solution, it may be necessary to analyze two standards to meet this requirement. Alternatively, the nominal analyte concentrations in the analyte PDS may be customized to meet these criteria. Alternate subsequent CCCs between the mid and high calibration levels. Verify that the CCC meets the criteria in the following sections.

10.4.1 CCC Isotope Performance Standard Responses

The absolute area of the quantitation ion for each of the three isotope performance standards must be within 50–150% of the average area measured during the initial calibration. If these limits are exceeded, corrective action is necessary (Sect. 10.5).

10.4.2 CCC Isotope Dilution Analogue Recovery

Using the average response factor determined during the initial calibration and the internal standard calibration technique, calculate the percent recovery of each isotope dilution analogue in the CCC. The recovery for each analogue must be within a range of 70–130%. If these limits are exceeded, corrective action is necessary (Sect. 10.5).

10.4.3 CCC Analyte Responses

Calculate the concentration of each method analyte in the CCC. Each analyte fortified at a level less than or equal to the MRL must be within 50–150% of the true value. The concentration of the analytes in CCCs fortified at all other levels must be within 70–130%. If these limits are exceeded, then all data for the failed analytes must be considered invalid. Any field samples analyzed since the last acceptable CCC that are still within holding time must be reanalyzed after an acceptable calibration has been restored.

10.4.3.1 Exception for High Recovery

If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a method analyte, and field sample extracts show no concentrations above the MRL for that analyte, non-detects may be reported without re-analysis.

10.5 Corrective Action

Failure to meet the CCC QC performance criteria requires corrective action. Following a minor remedial action, such as servicing the autosampler or flushing the column, check the calibration with a mid-level CCC and a CCC at the MRL, or recalibrate according to Section 10.3. If isotope performance standard and calibration failures persist, maintenance may be required, such as servicing the LC-MS/MS system or replacing the LC column. These latter measures constitute major maintenance and the analyst must return to the initial calibration step (Sect. 10.3).

11 Procedure

This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. The data published in this method (Sect. 17) demonstrate acceptable performance using manual extraction. The authors did not evaluate automated extraction systems. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the LRBs should be rotated among the ports to ensure that all the valves and tubing meet the LRB requirements (Sect. 9.2.1).

11.1 Sample Bottle Rinse

Some of the PFAS adsorb to surfaces, including polypropylene. During the elution step of the procedure, sample bottles must be rinsed with the elution solvent whether extractions are performed manually or by automation.

11.2 Reuse of Extraction Cartridges

The SPE cartridges described in this section are designed for a single use. They may not be reconditioned for subsequent analyses.

11.3 Sample Preparation

11.3.1 Sample Volume

Determine sample volume. An indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 1 gram. After

extraction, proceed to Section 11.5 to complete the volume measurement. Some of the PFAS adsorb to surfaces, thus the sample may not be transferred to a graduated cylinder for volume measurement. The LRB, LFB and FRB must have the same volume as that of the field samples and may be prepared by measuring reagent water with a graduated cylinder.

11.3.2 Verifying Sample pH

Verify that the sample containing 1 g/L ammonium acetate has a pH between 6.0 and 8.0. Acetic acid may be added as needed to reduce the pH

11.3.3 Fortify QC Samples

Fortify LFBs, LFSMs, and LFSMDs, with an appropriate volume of Analyte PDS (Sect. 7.17.4). Cap and invert each sample several times to mix.

11.3.4 Addition of Isotope Dilution Analogues

Add an aliquot of the isotope dilution analogue PDS (Sect. 7.16.1) to each sample, then cap and invert to mix. During method development, a 20 µL aliquot of the PDS (0.50–2.0 ng/µL) was added to achieve a final concentration of 40 ng/L of the isotopically labeled carboxylates and perfluorinated sulfonates, and 160 ng/L of the telomer sulfonates.

11.4 Extraction Procedure

11.4.1 Cartridge Cleaning and Conditioning

Do not allow cartridge packing material to go dry during any of the conditioning steps. If the cartridge goes dry during the conditioning phase, the conditioning must be repeated. Rinse each cartridge with 10 mL of methanol. Next, rinse each cartridge with 10 mL of aqueous 0.1 M phosphate buffer (Sect. 7.8) without allowing the water to drop below the top edge of the packing. Close the valve and add 2–3 mL of phosphate buffer to the cartridge reservoir and fill the remaining volume with reagent water.

11.4.2 Cartridge Loading

Attach the sample transfer tubes (Sect. 6.8.3) and adjust the vacuum to approximately 5 inches Hg. Begin adding sample to the cartridge. Adjust the vacuum and control valves so that the approximate flow rate is 5 mL/min. Do not allow the cartridge to go dry before all the sample has passed through. Flow rates above 5 mL/min during loading may cause low analyte recovery.

11.4.3 Sample Bottle Rinse and Cartridge Drying

After the entire sample has passed through the cartridge, rinse the sample bottle with a 10 mL aliquot of 1 g/L ammonium acetate in reagent water. Draw the rinsate through the sample transfer tubes and the cartridges. Add 1 mL of methanol to the sample bottle and draw through the transfer tube and SPE cartridge. This step is designed to remove most of the water from the transfer line and cartridge resulting in the reduction of the salt and water present in the eluate. The methanol rinse may also reduce interferences by removing weakly retained organic material prior to elution. If plastic reservoirs are used instead of transfer lines, the reservoirs must be rinsed with the ammonium acetate solution and the 1 mL aliquot of methanol.

11.4.4 Cartridge Drying

Draw air or nitrogen through the cartridge for 5 min at high vacuum (15–20 in. Hg).

11.4.5 Sample Bottle and Cartridge Elution

After the drying step, release the vacuum on the extraction manifold and place a collection tube under each sample position. Rinse the sample bottles with 5 mL of the elution solvent, methanol with 2% ammonium hydroxide (v/v), then elute the analytes from the cartridges by pulling the elution solvent through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 5 mL aliquot of elution solvent. If plastic reservoirs are used instead of transfer lines, attempt to rinse the entire inner surface of the reservoir with the elution solvent.

11.4.6 Extract Concentration

Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (55–60 °C). Reconstitute the extract with 1.0 mL of 20% reagent water in methanol (v/v). Add the isotope performance standards to the extract and vortex.

11.4.7 Extract Transfer and Storage

Transfer the final extract to a polypropylene autosampler vial. Store extracts at room temperature. Recap vials as soon as possible after injection to prevent evaporation losses; the polypropylene caps do not reseal after puncture. Alternatively, extracts can be stored in the 15 mL collection tubes after extraction. A small aliquot can be removed for analysis if the autosampler vial and injection system accommodate small volumes.

11.5 Sample Volume Determination

Use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. If using weight to determine the volume, weigh the empty bottle to the nearest 1 gram and subtract this value from the weight recorded prior to extraction. Assume a sample density of 1.0 g/mL. Record the sample volumes for use in the final calculations of analyte concentrations.

11.6 Sample Analysis

11.6.1 Establish LC-MS/MS Operating Conditions

Establish MS/MS operating conditions per the procedures in Section 10.1 and chromatographic conditions per Section 10.2. Establish a valid initial calibration following the procedures in Section 10.3 or confirm that the existing calibration is still valid by analyzing a low-level CCC. If establishing an initial calibration for the first time, complete the IDC prior to analyzing field samples. Analyze field and QC samples in a properly sequenced Analysis Batch as described in Section 11.7.

11.6.2 Verify Retention Time Windows

The analyst must ensure that each method analyte elutes entirely within the assigned window during each Analysis Batch. Make this observation by viewing the quantitation ion for each analyte in the CCCs analyzed during an Analysis Batch. If an analyte peak drifts out of the assigned window, then data for

that analyte is invalid in all injections acquired since the last valid CCC. In addition, all peaks representing multiple isomers of an analyte must elute entirely within the same MRM window.

11.7 Analysis Batch Sequence

An Analysis Batch is a sequence of samples, analyzed within a 24-hour period, of no more than 20 field samples and includes all required QC samples (LRB, CCCs, the LFSM and LFSMD (or FD)). The required QC samples are not included in counting the maximum field sample total of 20. LC-MS/MS conditions for the Analysis Batch must be the same as those used during calibration.

11.7.1 Analyze Initial CCC

After a valid calibration is established, begin every Analysis Batch by analyzing an initial low-level CCC at or below the MRL. This initial CCC must be within 50–150% of the true value for each method analyte and must pass both the isotope performance standard area response criterion (Sect. 10.4.1) and the isotope dilution analogue recovery criterion (Sect. 10.4.2). The initial CCC confirms that the calibration is still valid. Failure to meet the QC criteria may indicate that recalibration is required prior to analyzing samples.

11.7.2 Analyze Field and QC Samples

After the initial CCC, continue the Analysis Batch by analyzing an LRB, followed by the field samples and QC samples. Analyze a mid- or high-level CCC after every ten field samples and at the end each Analysis Batch. Do not count QC samples (LRBs, FDs, LFSMs, LFSMDs) when calculating the required frequency of CCCs.

11.7.3 Analyze Final CCC

The last injection of the Analysis Batch must be a mid- or high-level CCC. The acquisition start time of the final CCC must be within 24 hours of the acquisition start time of the low-level CCC at the beginning of the Analysis Batch. More than one Analysis Batch within a 24-hour period is permitted. An Analysis Batch may contain field and QC samples from multiple extraction batches.

11.7.4 Initial Calibration Frequency

A full calibration curve is not required before starting a new Analysis Batch. A previous calibration can be confirmed by running an initial, low-level CCC followed by an LRB. If a new calibration curve is analyzed, an Analysis Batch run immediately thereafter must begin with a low-level CCC and an LRB.

12 Data Analysis and Calculations

Because environmental samples may contain both branched and linear isomers of the method analytes, but quantitative standards that contain branched isomers do not exist for all method analytes, integration and quantitation of the PFAS is dependent on the type of standard materials available.

12.1 Identify Peaks by Retention Times

At the conclusion of data acquisition, use the same software settings established during the calibration procedure to identify analyte peaks in the predetermined retention time windows. Confirm the identity of each analyte by comparison of its retention time with that of the corresponding analyte peak in an

initial calibration standard or CCC. Proceed with quantitation based on the type of standard available for each method analyte.

12.1.1 Method Analytes without Technical-Grade Standards

If standards containing the branched and linear isomers cannot be purchased (i.e., only the linear isomer is available), only the linear isomer can be identified and quantitated in field samples and QC samples because the retention time of the branched isomers cannot be confirmed.

12.1.2 PFHxS, PFOS, and other Analytes with Technical-Grade Standards

During method development, multiple chromatographic peaks, representing branched and linear isomers, were observed for standards of PFHxS and PFOS using the LC conditions in **Table 1**. For PFHxS and PFOS, all the chromatographic peaks observed in the standard must be integrated and the areas summed. Chromatographic peaks in all field samples and QC samples must be integrated in the same way as the calibration standard for analytes with quantitative standards containing the branched and linear isomers.

12.1.3 PFOA

For PFOA, identify the branched and linear isomers by analyzing a technical-grade standard that includes both linear and branched isomers as directed in Section 10.2.2 and ensure that all isomers elute within the same acquisition segment. Quantitate field samples and fortified matrix samples by integrating the total response, accounting for peaks that are identified as linear and branched isomers. Quantitate based on the initial calibration with the quantitative PFOA standard containing just the linear isomer.

12.2 Calculate Analyte Concentrations

Calculate analyte concentrations using the multipoint calibration and the measured sample volume. Report only those values that fall between the MRL and the highest calibration standard.

12.3 Calculate Isotope Dilution Analogue Recovery

Calculate the concentration of each isotope dilution analogue using the multipoint calibration and the measured sample volume. Verify that the percent recovery is within 50–200% of the true value.

12.4 Significant Figures

Calculations must use all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

12.5 Exceeding the Calibration Range

The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, a field duplicate of the sample must be extracted, if available. Dilute an aliquot of the field duplicate with reagent water to a final volume equal to that used for the IDC. Add ammonium acetate to a final concentration of 1 g/L and process the diluted sample. Report all concentrations measured in the original sample that do not exceed the calibration range. Report concentrations of analytes that exceeded the calibration range in the in the original sample based on measurement in a diluted sample. Incorporate the dilution factor into final concentration calculations

and the resulting data must be annotated as a dilution. This is the only circumstance when subsampling is permitted.

13 Method Performance

13.1 Precision, Accuracy, and LCMRL Results

Tables for these data are presented in Section 17. LCMRLs are presented in **Table 7**. Single-laboratory precision and accuracy data are presented for three water matrices: reagent water (**Table 8**), finished ground water (**Table 10**), and a drinking water matrix from a surface water source (**Table 12)**. The mean isotope dilution analogue recoveries measured in the replicate samples used in these studies are presented in **Table 9** for reagent water, **Table 11** for finished groundwater, and **Table 13** for the surface water matrix.

13.2 Analyte Stability Study

Chlorinated (finished) surface water samples were inoculated with microbial-rich water from an impacted surface source and fortified with 40 ng/L of the PFAS method analytes. These samples were stored as required in this method. The percent change from the initial analyzed concentration observed after 7, 14, 21, and 28 days is presented in Section 17, **Table 14**.

13.3 Extract Storage Stability

Extract storage stability studies were conducted on extracts obtained from the analyte stability study (Sect. 13.2). The percent change from the initial analyzed concentration observed after 14, 21, and 27 days storage is presented in Section 17, **Table 15**.

14 Pollution Prevention

For information about pollution prevention applicable to laboratory operations described in this method, consult: Less is Better, Guide to Minimizing Waste in Laboratories, a publication available from the American Chemical Society (accessed April 2019) at www.acs.org.

15 Waste Management

Laboratory waste management practices should be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16 References

1. US EPA. *Statistical Protocol for the Determination of the Single-Laboratory Lowest Concentration Minimum Reporting Level (LCMRL) and Validation of Laboratory Performance at or Below the Minimum Reporting Level (MRL)*; EPA 815-R-05-006; Office of Water: Cincinnati, OH, November 2004.
2. US EPA. *Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator*; EPA 815-R-11-001; Office of Water: Cincinnati, OH, December 2010.

3. Martin, J.W., et al. Analytical Challenges Hamper Perfluoroalkyl Research. *Environ. Sci. Technol*. 2004, Vol. 38, 248A–255A.
4. Cahill, J.D., et al. Determination of Pharmaceutical Compounds in Surface- and Ground-Water Samples by Solid-Phase Extraction and High-Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry. *J. Chromatography A*, 2004, 1041, 171–180.
5. Langlois, I. and Oehme, M. Structural Identification of Isomers Present in Technical Perfluorooctane Sulfonate by Tandem Mass Spectrometry. *Rapid Communication Mass Spectrometry*. 2006, Vol. 20, 844–850.

17 Tables, Figures and Method Performance Data

Table 1. HPLC Method Conditions[a]

Time (min)	% 20 mM ammonium acetate	% Methanol
Initial	95.0	5.0
0.5	95.0	5.0
3.0	60.0	40.0
16.0	20.0	80.0
18.0	20.0	80.0
20.0	5.0	95.0
22.0	5.0	95.0
25.0	95.0	5.0
35.0	95.0	5.0

a. Phenomenex Gemini® C18, 2 x 50 mm, 3.0 µm silica with TMS end-capping. Flow rate of 0.25 mL/min; run time 35 minutes; 10 µL injection into a 50 µL loop. The chromatogram in **Figure 1** was obtained under these conditions.

Table 2. ESI-MS Method Conditions

ESI Conditions for Waters (Milford, MA) Xevo TQD	
Polarity	Negative ion
Capillary needle voltage	-2.7 kV
Cone gas flow	40 L/hour
Nitrogen desolvation gas	800 L/hour
Desolvation gas temperature	300 °C

Table 3. Isotopically Labeled Isotope Performance Standards and Retention Times

Isotope Performance Standard	Peak # (Figure 1)	RT (min)
$^{13}C_3$-PFBA	1	4.14
$^{13}C_2$-PFOA	26	12.19
$^{13}C_4$-PFOS	32	13.73

Table 4. Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References

Isotopically Labeled Analyte	Peak # (Fig. 1)	RT (min)	Suggested Isotope Performance Standard
$^{13}C_4$-PFBA	2	4.14	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	5	6.13	$^{13}C_3$-PFBA
$^{13}C_3$-PFBS	7	6.62	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	12	8.12	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	14	8.35	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	17	9.06	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	19	10.34	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	21	10.61	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	24	12.05	$^{13}C_4$-PFOS
$^{13}C_8$-PFOA	27	12.19	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	30	13.70	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	33	13.73	$^{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	36	14.94	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	38	15.00	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	40	16.14	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	43	17.13	$^{13}C_2$-PFOA

Table 5. Method Analytes, Retention Times and Suggested Isotope Dilution Analogue References

Analyte	Peak # (Figure 1)	RT (min)	Isotope Dilution Analogue
PFBA	3	4.15	$^{13}C_4$-PFBA
PFMPA	4	4.84	$^{13}C_4$-PFBA
PFPeA	6	6.13	$^{13}C_5$-PFPeA
PFBS	8	6.62	$^{13}C_3$-PFBS
PFMBA	9	6.81	$^{13}C_5$-PFPeA
PFEESA	10	7.53	$^{13}C_3$-PFBS
NFDHA	11	8.01	$^{13}C_5$-PFHxA
4:2FTS	13	8.12	$^{13}C_2$-4:2FTS
PFHxA	15	8.36	$^{13}C_5$-PFHxA
PFPeS	16	8.69	$^{13}C_3$-PFHxS
HFPO-DA	18	9.06	$^{13}C_3$-HFPO-DA
PFHpA	20	10.42	$^{13}C_4$-PFHpA
PFHxS	22	10.62	$^{13}C_3$-PFHxS
ADONA	23	10.73	$^{13}C_4$-PFHpA
6:2FTS	25	12.04	$^{13}C_2$-6:2FTS
PFOA	28	12.19	$^{13}C_8$-PFOA
PFHpS	29	12.28	$^{13}C_8$-PFOS
PFNA	31	13.70	$^{13}C_9$-PFNA
PFOS	34	13.74	$^{13}C_8$-PFOS
9Cl-PF3ONS	35	14.53	$^{13}C_8$-PFOS
8:2 FTS	37	14.94	$^{13}C_2$-8:2FTS
PFDA	39	15.00	$^{13}C_6$-PFDA
PFUnA	41	16.14	$^{13}C_7$-PFUnA
11Cl-PF3OUdS	42	16.70	$^{13}C_8$-PFOS
PFDoA	44	17.13	$^{13}C_2$-PFDoA

Table 6. MS/MS Method Conditions[a]

Segment[b]	Analyte	Precursor Ion[c] (*m/z*)	Product Ion[c,d] (*m/z*)	Cone Voltage (v)	Collision Energy[e] (v)
1	PFBA	213	169	22	10
1	$^{13}C_3$-PFBA	216	172	22	10
1	$^{13}C_4$-PFBA	217	172	22	10
1	PFMPA	229	85	23	10
2	PFPeA	263	219	20	8
2	$^{13}C_5$-PFPeA	268	223	20	8
2	$^{13}C_3$-PFBS	302	80	45	30
2	PFBS	299	80	45	30
2	PFMBA	279	85	22	10
3	PFEESA	315	135	44	20
3	NFDHA	295	201	14	8
3	$^{13}C_2$-4:2FTS	329	309	40	18
3	4:2FTS	327	307	40	18
3	$^{13}C_5$-PFHxA	318	273	20	8
3	PFHxA	313	269	20	8
3	PFPeS	349	80	45	35
3	$^{13}C_3$-HFPO-DA	287[f]	169	15	5
3	HFPO-DA	285[f]	169	15	5
4	$^{13}C_4$-PFHpA	367	322	15	8
4	PFHpA	363	319	15	8
4	$^{13}C_3$-PFHxS[g]	402	80	45	40
4	PFHxS[h]	399	80	45	40
4	ADONA	377	251	15	10
5	$^{13}C_2$-6:2FTS	429	409	47	22
5	6:2FTS	427	407	47	22
5	$^{13}C_2$-PFOA	415	370	18	10
5	$^{13}C_8$-PFOA	421	376	18	10
5	PFOA	413	369	18	10
5	PFHpS	449	80	45	40
6	$^{13}C_9$-PFNA	472	427	17	10
6	PFNA	463	419	17	10
6	$^{13}C_4$-PFOS[g]	503	80	45	45
6	$^{13}C_8$-PFOS[g]	507	80	45	45
6	PFOS[h]	499	80	45	45
7	9Cl-PF3ONS	531	351	55	25
7	$^{13}C_2$-8:2FTS	529	509	53	28
7	8:2FTS	527	507	53	28
7	$^{13}C_6$-PFDA	519	474	22	10
7	PFDA	513	469	22	10

Segment[b]	Analyte	Precursor Ion[c] *(m/z)*	Product Ion[c,d] *(m/z)*	Cone Voltage (v)	Collision Energy[e] (v)
8	$^{13}C_7$-PFUnA	570	525	24	10
8	PFUnA	563	519	24	10
8	11Cl-PF3OUdS	631	451	60	30
8	$^{13}C_2$-PFDoA	615	570	22	10
8	PFDoA	613	569	22	10

a. An LC-MS/MS chromatogram of the analytes obtained using these parameters is shown in **Figure 1**.

b. Segments are time durations in which single or multiple scan events occur.

c. Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak (e.g., *m/z* 498.9→79.9 for PFOS). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

d. Ions used for quantitation purposes.

e. Argon used as collision gas.

f. HFPO-DA is not stable in the ESI source and the $[M - H]^-$ yields a weak signal under typical ESI conditions. The precursor ion used during method development was $[M - CO_2 - H]^-$.

g. The isotope dilution analogue used during method development was composed of the linear isomer exclusively.

h. Analyte has multiple resolved chromatographic peaks due to linear and branched isomers. All peaks summed for quantitation purposes. To reduce bias regarding detection of branched and linear isomers, the *m/z* 80 product ion must be used for this analyte.

Table 7. LCMRL Results

Analyte	LCMRL Fortification Levels (ng/L)	Calculated LCMRL (ng/L)
PFBA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	13
PFMPA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.8
PFPeA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.9
PFBS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.5
PFMBA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
PFEESA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.6
NFDHA	4.0, 6.0, 10, 14, 20, 41, 82	16
4:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.7
PFHxA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	5.3
PFPeS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	6.3
HFPO-DA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
PFHpA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.6
PFHxS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
ADONA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.4
6:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	14
PFOA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.4
PFHpS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	5.1
PFNA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.8
PFOS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.4
9Cl-PF3ONS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	1.4
8:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	9.1
PFDA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.3
PFUnA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.7
11Cl-PF3OUdS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	1.6
PFDoA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.2

Table 8. Precision and Accuracy Data for Reagent Water

Analyte	Low Fortification (ng/L)	Mean %R[a] (*n*=7)	%RSD[a]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	128	8.6	80	98.4	2.4
PFMPA	10	108	4.5	80	98.1	2.2
PFPeA	10	107	4.9	80	99.6	3.6
PFBS	10	102	9.1	80	96.2	2.9
PFMBA	10	111	6.8	80	101	3.4
PFEESA	10	107	10	80	98.8	4.0
NFDHA	10	110	15	80	98.5	5.4
4:2FTS	10	94.4	14	80	100	5.7
PFHxA	10	102	8.0	80	97	7.7
PFPeS	10	99.5	19	80	101	7.8
HFPO-DA	10	102	9.7	80	102	4.7
PFHpA	10	108	7.0	80	104	4.1
PFHxS	10	103	9.0	80	97.7	5.5
ADONA	10	96.3	3.1	80	96.8	5.6
6:2FTS	10	109	15	80	111	11
PFOA	10	108	7.4	80	98.5	6.9
PFHpS	10	98.8	8.9	80	102	7.0
PFNA	10	109	6.2	80	99.6	5.6
PFOS	10	104	8.7	80	98.0	4.3
9Cl-PF3ONS	10	99.7	4.6	80	103	6.8
8:2FTS	10	100	17	80	100	13
PFDA	10	100	4.2	80	100	1.8
PFUnA	10	102	10	80	97.3	8.1
11Cl-PF3OUdS	10	106	5.3	80	102	6.1
PFDoA	10	101	6.2	80	96.3	5.1

[a] %R = percent recovery; %RSD = percent relative standard deviation

Table 9. P&A in Reagent Water: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=7) P&A Low	%RSD[b,c]	Mean %R (*n*=5) P&A High	%RSD
$^{13}C_4$-PFBA	40	95.6	11	92.5	3.4
$^{13}C_5$-PFPeA	40	93.4	9.3	91.7	4.6
$^{13}C_3$-PFBS	40	98.6	9.6	107	6.6
$^{13}C_2$-4:2FTS	160	102	6.7	108	3.5
$^{13}C_5$-PFHxA	40	92.5	6.4	92.8	11
$^{13}C_3$-HFPO-DA	40	88.6	6.5	88.8	7.4
$^{13}C_4$-PFHpA	40	98.0	4.0	94.0	8.3
$^{13}C_3$-PFHxS	40	101	11	106	8.2
$^{13}C_2$-6:2FTS	160	109	9.5	99.8	4.7
$^{13}C_8$-PFOA	40	98.0	4.1	91.5	8.7
$^{13}C_9$-PFNA	40	97.1	4.9	92.1	8.4
$^{13}C_8$-PFOS	40	98.8	6.5	96.5	5.0
$^{13}C_2$-8:2FTS	160	106	13.9	108	8.7
$^{13}C_6$-PFDA	40	104	7.7	104	6.1
$^{13}C_7$-PFUnA	40	107	6.0	98.8	7.5
$^{13}C_2$-PFDoA	40	100	5.7	94.0	6.7

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 10. Precision and Accuracy Data for Finished Ground Water[a]

Analyte	Low Fortification (ng/L)	Mean %R[b] (*n*=5)	%RSD[b]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	127	15	80	98.0	4.0
PFMPA	10	100	8.3	80	103	9.8
PFPeA	10	105	11	80	105	5.1
PFBS	10	111	12	80	101	10
PFMBA	10	99.0	4.6	80	100	2.3
PFEESA	10	101	3.5	80	107	8.8
NFDHA	10	95.1	17	80	98.5	18
4:2FTS	10	70.5	20	80	116	9.2
PFHxA	10	104	18	80	111	17
PFPeS	10	87.5	5.0	80	106	6.2
HFPO-DA	10	105	7.4	80	103	7.5
PFHpA	10	102	6.8	80	101	6.4
PFHxS	10	86.6	18	80	108	6.8
ADONA	10	97.6	8.1	80	94.2	6.9
6:2FTS	10	99.9	15	80	100	12
PFOA	10	95.8	8.1	80	104	9.8
PFHpS	10	94.0	6.3	80	113	6.0
PFNA	10	95.1	7.2	80	108	3.3
PFOS	10	[c]	[c]	80	109	5.8
9Cl-PF3ONS	10	92.7	7.2	80	111	7.9
8:2FTS	10	108	19	80	102	3.2
PFDA	10	90.8	9.8	80	104	7.1
PFUnA	10	98.3	8.8	80	105	3.0
11Cl-PF3OUdS	10	94.6	8.3	80	110	9.3
PFDoA	10	92.7	7.8	80	102	6.3

a. Finished water from a ground water source. Hardness = 320 mg/L as $CaCO_3$. pH = 7.88 at 17 °C. Free Cl_2 = 0.64 mg/L. Total Cl_2 = 0.74 mg/L.

b. %R = percent recovery, corrected for native concentration; %RSD = percent relative standard deviation.

c. The spike level was below the ambient PFOS concentration of 25 ng/L.

Table 11. P&A in Finished Ground Water: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=6) P&A Low	%RSD[b,c]	Mean %R (*n*=6) P&A High	%RSD
$^{13}C_4$-PFBA	40	89.5	4.4	81.3	7.8
$^{13}C_5$-PFPeA	40	94.0	4.2	84.6	7.7
$^{13}C_3$-PFBS	40	103	1.7	93.6	8.5
$^{13}C_2$-4:2FTS	160	107	6.1	105	2.6
$^{13}C_5$-PFHxA	40	93.8	9.8	75.8	16
$^{13}C_3$-HFPO-DA	40	77.8	8.5	72.0	9.8
$^{13}C_4$-PFHpA	40	90.5	8.4	83.3	10
$^{13}C_3$-PFHxS	40	101	7.8	94.7	6.4
$^{13}C_2$-6:2FTS	160	101	5.2	101	4.5
$^{13}C_8$-PFOA	40	89.5	5.7	82.8	10
$^{13}C_9$-PFNA	40	103	6.6	78.0	11
$^{13}C_8$-PFOS	40	101	7.6	89.7	4.5
$^{13}C_2$-8:2FTS	160	97.2	7.4	94.0	8.0
$^{13}C_6$-PFDA	40	98.7	6.3	82.3	15
$^{13}C_7$-PFUnA	40	102	4.3	82.6	8.0
$^{13}C_2$-PFDoA	40	98.8	4.6	81.2	10

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the unfortified matrix sample and the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 12. Precision and Accuracy Data for a Surface Water Matrix[a]

Analyte	Low Fortification (ng/L)	Mean %R[b,c] (*n*=5)	%RSD[b]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	95.4	19	80	106	4.8
PFMPA	10	108	16	80	102	5.9
PFPeA	10	93	13	80	101	6.0
PFBS	10	111	17	80	98.3	2.7
PFMBA	10	93.0	12	80	103	3.0
PFEESA	10	95.6	15	80	99.1	2.4
NFDHA	10	102	14	80	101	2.5
4:2FTS	10	70.9	17	80	91.1	7.8
PFHxA	10	96.9	19	80	103	4.2
PFPeS	10	87.5	14	80	104	4.9
HFPO-DA	10	109	8.7	80	105	7.0
PFHpA	10	95.9	11	80	105	4.8
PFHxS	10	78.5	8.2	80	97.1	5.3
ADONA	10	94.3	7.9	80	95.8	6.0
6:2FTS	10	86.5	6.3	80	101	9.7
PFOA	10	91.9	9.8	80	98.7	4.9
PFHpS	10	88.4	14	80	106	3.4
PFNA	10	89.7	9.5	80	95.9	2.8
PFOS	10	95.1	11	80	105	8.0
9Cl-PF3ONS	10	82.4	5.0	80	94.1	3.9
8:2FTS	10	102	7.6	80	101	4.0
PFDA	10	87.3	12	80	98.5	8.0
PFUnA	10	96.9	5.4	80	95.2	2.7
11Cl-PF3OUdS	10	82.4	8.9	80	93.0	4.4
PFDoA	10	94.6	2.3	80	98.4	4.1

a. Surface water matrix was sampled after the clarifier and prior to granular activated carbon within the drinking water treatment plant and chlorinated in our laboratory. pH = 8.1 at 20 °C. Free Cl_2 = 0.98 mg/L. Total Cl_2 = 1.31 mg/L. Total Organic Carbon (TOC) = 3.8 mg/L C.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Corrected for native concentration.

Table 13. P&A in Surface Water Matrix: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=6) P&A Low	%RSD[b,c]	Mean %R (*n*=6) P&A High	%RSD
$^{13}C_4$-PFBA	40	86.9	18	86.3	6.5
$^{13}C_5$-PFPeA	40	105	15	102	5.7
$^{13}C_3$-PFBS	40	98.6	11	99.8	4.5
$^{13}C_2$-4:2FTS	160	136	13	138	6.3
$^{13}C_5$-PFHxA	40	88.8	16	84.8	4.5
$^{13}C_3$-HFPO-DA	40	78.4	14	75.4	13
$^{13}C_4$-PFHpA	40	91.6	12	89.3	6.0
$^{13}C_3$-PFHxS	40	98.2	6.5	96.0	9.6
$^{13}C_2$-6:2FTS	160	110	9.7	109	8.4
$^{13}C_8$-PFOA	40	90.1	14	86.6	4.5
$^{13}C_9$-PFNA	40	91.0	14	87.2	6.0
$^{13}C_8$-PFOS	40	98.8	15	95.6	5.0
$^{13}C_2$-8:2FTS	160	101	9.8	97.3	11
$^{13}C_6$-PFDA	40	92.0	16	86.6	10
$^{13}C_7$-PFUnA	40	92.2	16	90.0	5.6
$^{13}C_2$-PFDoA	40	91.2	14	90.8	10

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the unfortified matrix sample and the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 14. Aqueous Sample Holding Time Data[a]

Analyte	Fortified Conc. (ng/L)	Day Zero Mean (ng/L)	Day Zero %RSD	Day 7 %Change[b]	Day 7 %RSD	Day 14 %Change	Day 14 %RSD	Day 21 %Change	Day 21 %RSD	Day 28 %Change	Day 28 %RSD
PFBA	40	42	4.6	9.1	2.3	3.1	7.2	5.1	5.4	4.2	5.0
PFMPA	40	41	5.2	5.5	2.2	-7.8	5.1	1.0	6.3	-10	3.1
PFPeA	40	43	4.1	1.2	1.9	-2.2	6.5	-0.29	2.5	-6.5	5.8
PFBS	40	43	9.7	-1.9	3.6	-6.1	1.8	-4.0	2.5	-7.6	8.9
PFMBA	40	40	3.0	-2.5	3.7	-5.7	4.3	0.20	5.0	-6.6	6.3
PFEESA	40	39	3.2	2.6	5.7	-1.8	6.7	-2.4	4.5	-1.7	2.6
NFDHA	40	39	6.5	-4.0	7.2	-11	6.9	-3.8	5.2	-2.9	8.0
4:2FTS	40	43	9.7	-1.7	3.8	-2.6	9.6	-2.0	6.1	-0.34	5.3
PFHxA	40	42	5.2	-0.37	4.6	-2.61	5.6	-1.7	5.8	-2.3	7.6
PFPeS	40	41	3.2	5.6	7.5	-3.1	2.6	6.0	9.2	-11	9.4
HFPO-DA	40	42	5.1	6.2	4.8	3.2	9.2	2.1	2.1	-3.5	4.2
PFHpA	40	41	4.6	-0.042	2.4	-4.7	1.7	-2.9	3.6	-3.0	5.4
PFHxS	40	41	4.3	1.8	3.0	-1.8	1.8	-1.8	9.0	-0.99	6.8
ADONA	40	39	4.2	-4.3	3.1	-12	5.7	-6.2	5.9	-2.3	3.1
6:2FTS	40	41	7.5	-4.3	4.4	-0.74	9.4	2.5	6.0	-1.5	6.0
PFOA	40	41	5.4	-1.5	6.7	1.6	5.1	-2.0	4.9	-6.5	7.2
PFHpS	40	41	4.7	-2.4	5.4	1.2	3.1	0.30	3.2	2.9	7.2
PFNA	40	42	4.1	2.05	0.57	-6.0	4.9	-6.1	3.4	-9.5	3.4
PFOS	40	41	7.0	-2.1	4.7	-1.8	5.2	1.0	5.8	-1.6	5.3
9Cl-PF3ONS	40	40	3.5	1.6	4.8	-0.34	1.8	4.0	4.8	-2.6	10
8:2FTS	40	44	7.9	-0.36	2.5	-1.4	6.7	0.026	3.8	-3.6	6.9
PFDA	40	41	5.0	0.12	3.1	-2.7	3.8	-1.4	3.8	-2.4	7.0
PFUnA	40	39	3.9	-1.3	4.7	-12	1.2	3.7	3.1	-6.7	3.5
11Cl-PF3OUdS	40	40	4.9	-1.1	4.5	-9.4	5.1	-11.0	4.7	-12	7.3
PFDoA	40	39	4.4	9.5	6.5	-4.8	6.0	-3.4	5.8	-16	6.1

a. Finished water from a surface water source. pH = 8.84 at 18 °C; total organic carbon (TOC) = 0.75 mg/L C (mean of 2019 first quarter plant records); free chlorine = 0.87 mg/L, total chlorine = 1.04 mg/L. Day Zero: *n*=7. All other events: *n*=5.

b. %Change = percent change from Day Zero calculated as follows: (Day X mean concentration – Day Zero mean concentration) / Day Zero mean concentration * 100%, where X is the analysis day.

Table 15. Extract Holding Time Data[a]

Analyte	Fortified Conc. (ng/L)	Day Zero Mean (ng/L)	Day Zero %RSD	Day 14 %Change[b]	Day 14 %RSD	Day 21 %Change	Day 21 %RSD	Day 27 %Change	Day 27 %RSD
PFBA	40	42	4.6	-8.0	4.2	-4.4	0.89	-12	6.4
PFMPA	40	41	5.2	-3.9	4.5	-0.10	5.1	-3.9	12
PFPeA	40	43	4.1	-6.0	6.0	-0.55	4.8	-5.4	1.1
PFBS	40	43	9.7	2.6	2.0	6.6	2.3	2.9	3.6
PFMBA	40	40	3.0	-10	7.1	-4.8	5.3	-8.8	2.7
PFEESA	40	39	3.2	1.3	8.9	-3.6	2.1	-4.9	3.6
NFDHA	40	39	6.5	-10	3.9	-13	6.8	-11	3.1
4:2FTS	40	43	9.7	-4.7	8.5	-6.2	8.8	-7.3	8.5
PFHxA	40	42	5.2	-4.6	6.3	-20	3.0	-14	4.7
PFPeS	40	41	3.2	-6.7	8.6	-11	5.2	-10	4.5
HFPO-DA	40	42	5.1	-4.9	4.9	-4.7	5.1	-4.4	7.7
PFHpA	40	41	4.6	-1.9	1.9	-6.1	4.8	-8.7	7.8
PFHxS	40	41	4.3	-19	9.9	-21	8.4	-22	11
ADONA	40	39	4.2	-1.2	1.9	-7.8	6.4	-7.5	5.0
6:2FTS	40	41	7.5	-5.3	13	-7.6	5.8	-8.4	14
PFOA	40	41	5.4	-5.7	6.3	-2.2	4.2	-2.4	3.3
PFHpS	40	41	4.7	-8.7	7.3	-6.0	5.2	-3.2	4.2
PFNA	40	42	4.1	-5.8	5.6	0.17	3.2	-2.0	6.0
PFOS	40	41	7.0	-3.8	10	-4.2	2.5	-3.7	4.4
9Cl-PF3ONS	40	40	3.5	-5.8	7.7	-9.3	4.0	-8.6	4.7
8:2FTS	40	44	7.9	-4.7	6.3	-1.3	5.8	-6.4	2.9
PFDA	40	41	5.0	-3.7	5.3	-1.8	5.6	-4.8	3.1
PFUnA	40	39	3.9	6.2	4.0	0.63	7.5	-2.8	5.2
11Cl-PF3OUdS	40	40	4.9	-12	5.9	-18	4.6	-10	6.3
PFDoA	40	39	4.4	1.9	5.5	1.0	6.4	-2.6	3.3

a. Finished water from a surface water source. pH = 8.84 at 18 °C; total organic carbon (TOC) = approximately 0.75 mg/L C (2019 first quarter plant records); free chlorine = 0.87 mg/L, total chlorine = 1.04 mg/L. Day Zero: *n*=7. All other events: *n*=7.

b. %Change = percent change from Day Zero calculated as follows: (Day X mean concentration – Day Zero mean concentration) / Day Zero mean concentration * 100%, where X is the analysis day.

Table 16. Initial Demonstration of Capability (IDC) Quality Control Requirements

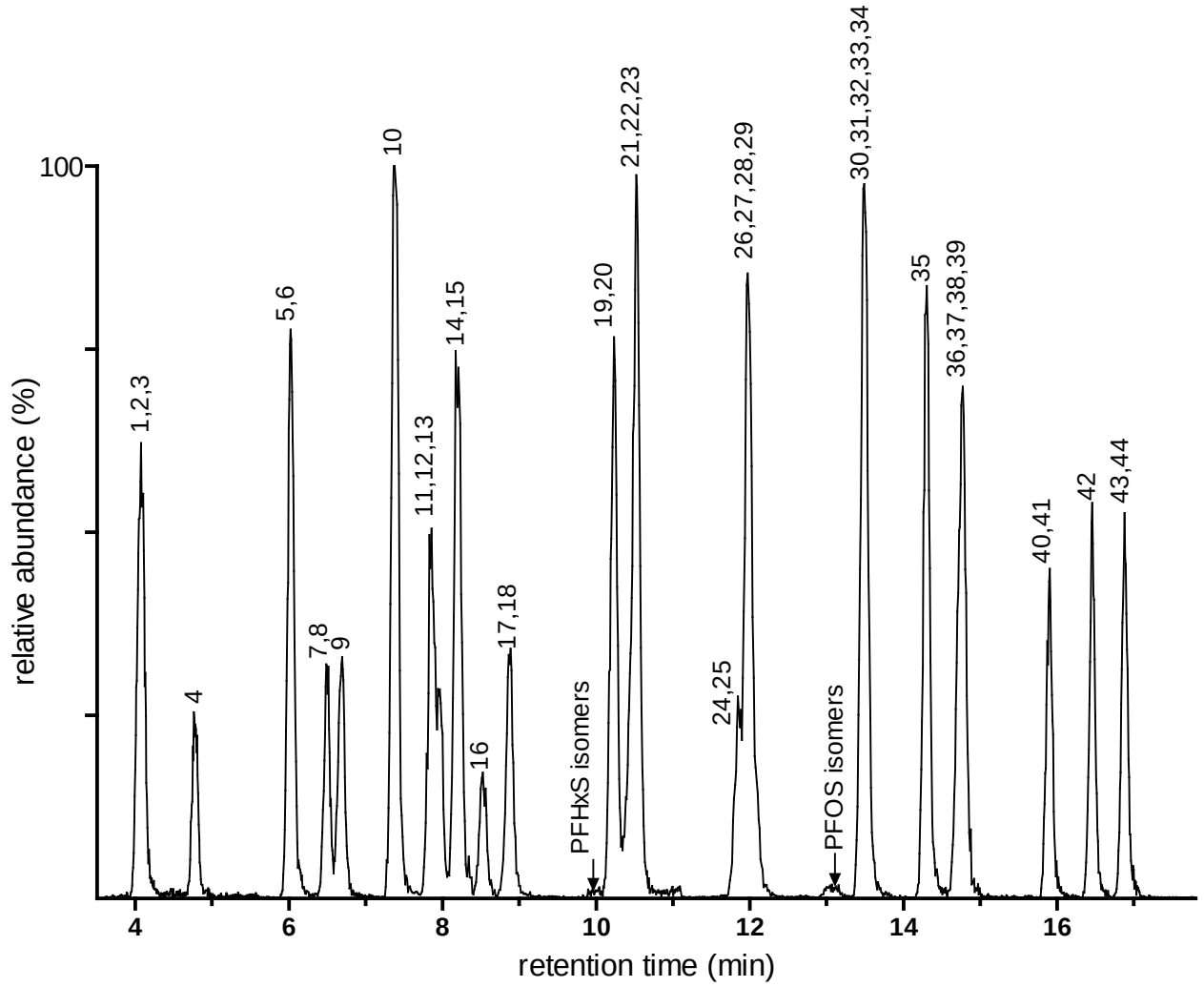
Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2.2	Establish retention times for branched isomers	Each time chromatographic conditions change	All isomers of each analyte must elute within the same MRM window.
Section 9.1.1	Demonstration of low system background	Analyze a Laboratory Reagent Blank (LRB) after the highest standard in the calibration range.	Demonstrate that the method analytes are less than one-third of the Minimum Reporting Level (MRL).
Section 9.1.2	Demonstration of precision	Extract and analyze 7 replicate Laboratory Fortified Blanks (LFBs) near the mid-range concentration.	Percent relative standard deviation must be ≤20%.
Section 9.1.3	Demonstration of accuracy	Calculate mean recovery for replicates used in Section 9.1.2.	Mean recovery within 70–130% of the true value.
Section 9.1.4	MRL confirmation	Fortify and analyze 7 replicate LFBs at the proposed MRL concentration. Confirm that the Upper Prediction Interval of Results (PIR) and Lower PIR meet the recovery criteria.	Upper PIR ≤150% Lower PIR ≥50%
Section 9.1.5	Calibration Verification	Analyze mid-level QCS.	Results must be within 70–130% of the true value.

Table 17. Ongoing Quality Control Requirements

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.3	Initial calibration	Use the isotope dilution calibration technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Evaluate the calibration curve as described in Section 10.3.5.	When each calibration standard is calculated as an unknown using the calibration curve, analytes fortified at or below the MRL should be within 50–150% of the true value. Analytes fortified at all other levels should be within 70–130% of the true value.
Section 9.2.1	Laboratory Reagent Blank (LRB)	Include one LRB with each Extraction Batch. Analyze one LRB with each Analysis Batch.	Demonstrate that all method analytes are below one-third the Minimum Reporting Level (MRL), and that possible interference from reagents and glassware do not prevent identification and quantitation of method analytes.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.3	Laboratory Fortified Blank	Include one LFB with each Extraction Batch.	For analytes fortified at concentrations ≤2 x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2 x the MRL.
Section 10.4	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low-level CCC (concentrations at or below the MRL for each analyte) at the beginning of each Analysis Batch. Subsequent CCCs are required after every tenth field sample and to complete the batch.	The lowest level CCC must be within 50–150% of the true value. All other levels must be within 70–130% of the true value.
Section 9.2.4	Isotope performance standards	Isotope performance standards are added to all standards and sample extracts.	Peak area counts for each isotope performance standard must be within 50–150% of the average peak area in the initial calibration.
Section 9.2.5	Isotope dilution analogues	Isotope dilution analogues are added to all samples prior to extraction.	50%–200% recovery for each analogue
Section 9.2.6	Laboratory Fortified Sample Matrix (LFSM)	Include one LFSM per Extraction Batch. Fortify the LFSM with method analytes at a concentration close to but greater than the native concentrations (if known).	For analytes fortified at concentrations ≤2 x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2 x the MRL.
Section 9.2.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD)	Include at least one LFSMD or FD with each Extraction Batch.	For LFSMDs or FDs, relative percent differences must be ≤30% (≤50% if analyte concentration ≤2 x the MRL).
Section 9.2.8	Field Reagent Blank (FRB)	Analyze the FRB if any analyte is detected in the associated field samples.	If an analyte detected in the field sample is present in the associated FRB at greater than one-third the MRL, the results for that analyte are invalid.
Section 9.2.9	Calibration Verification using QCS	Perform a Calibration Verification at least quarterly.	Results must be within 70–130% of the true value.

Figure 1. Example Chromatogram for Reagent Water Fortified with Method Analytes at 80 ng/L[a]



a. Numbered peaks are identified in **Table 3**, **Table 4**, and **Table 5**.

Attachment 10

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METHOD 537.1 DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Version 2.0
March 2020

J.A. Shoemaker and D.R. Tettenhorst, Office of Research and Development

J.A. Shoemaker and D.R. Tettenhorst, Office of Research and Development, Method 537.1, Rev 1.0 (2018)

J.A. Shoemaker (Office of Research and Development), P.E. Grimmett (Office of Research and Development), B.K. Boutin (National Council on Aging), Method 537, Rev 1.1 (2009)

CENTER FOR ENVIRONMENTAL SOLUTIONS & EMERGENCY RESPONSE
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 537.1

DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1. This is a solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water. Accuracy and precision data have been generated in reagent water and drinking water for the compounds listed in the table below.

Analyte[a]	Acronym	Chemical Abstract Services Registry Number (CASRN)
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6[b]
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9[c]
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1[d]
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4[e]

[a] Some PFAS are commercially available as ammonium, sodium and potassium salts. This method measures all forms of the analytes as anions while the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts (see Section 7.2.3 regarding correcting the analyte concentration for the salt content).
[b] HFPO-DA and the ammonium salt of HFPO-DA are components of the GenX processing aid technology and both are measured as the anion of HFPO-DA by this method.
[c] 11Cl-PF3OUdS is available in salt form (e.g. CASRN of potassium salt is 83329-89-9).
[d] 9Cl-PF3ONS analyte is available in salt form (e.g. CASRN of potassium salt is 73606-19-6)
[e] ADONA is available as the sodium salt (no CASRN) and the ammonium salt (CASRN is 958445-44-8).

1.2. Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The single laboratory lowest concentration MRL (LCMRL) is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99%), between 50 and 150% recovery. Single laboratory LCMRLs for analytes in this method range from 0.53-6.3 ng/L and are listed in Table 5. The procedure used to determine the LCMRL is described elsewhere.[1]

1.3. Laboratories using this method will not be required to determine the LCMRL for this method, but will need to demonstrate that their laboratory MRL for this method meets requirements described in Section 9.2.6.

1.4. Determining the Detection Limit (DL) for analytes in this method is optional (Sect. 9.2.8). Detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[2] The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance.

1.5. This method is intended for use by analysts skilled in solid phase extractions, the operation of LC/MS/MS instruments, and the interpretation of the associated data.

1.6. METHOD FLEXIBILITY – In recognition of technological advances in analytical systems and techniques, the laboratory is permitted to modify the evaporation technique, separation technique, LC column, mobile phase composition, LC conditions and MS and MS/MS conditions (Sect. 6.12, 9.1.1, 10.2, and 12.1). **Changes may not be made to sample collection and preservation (Sect. 8), the sample extraction steps (Sect. 11.4), or to the quality control requirements (Sect. 9).** Method modifications should be considered only to improve method performance. Modifications that are introduced in the interest of reducing cost or sample processing time, but result in poorer method performance, should not be used. Analytes must be adequately resolved chromatographically to permit the mass spectrometer to dwell on a minimum number of compounds eluting within a retention time window. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the initial demonstration of capability (IDC, Sect. 9.2), verify that all Quality Control (QC) acceptance criteria in this method (Sect. 9) are met, and that acceptable method performance can be verified in a real sample matrix (Sect. 9.3.6).

NOTE: The above method flexibility Section is intended as an abbreviated summation of method flexibility. Sections 4-12 provide detailed information of specific portions of the method that may be modified. If there is any perceived conflict between the general method flexibility statement in Section 1.6 and specific information in Sections 4-12, Sections 4-12 supersede Section 1.6.

2. SUMMARY OF METHOD

A 250-mL water sample is fortified with surrogates and passed through an SPE cartridge containing polystyrenedivinylbenzene (SDVB) to extract the method analytes and surrogates. The compounds are eluted from the solid phase sorbent with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 96:4% (vol/vol) methanol:water and addition of the internal standards. A 10-µL injection is made into an LC equipped with a C18 column that is interfaced to an MS/MS. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to all Field and QC Samples to monitor the extraction efficiency of the method analytes.

3. DEFINITIONS

3.1. ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period, including no more than 20 Field Samples, that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.2. CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution, internal standard(s), and the surrogate(s). The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. COLLISIONALLY ACTIVATED DISSOCIATION (CAD) – The process of converting the precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

3.4. CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes, internal standard(s) and surrogate(s). The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5. DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.8), and accurate quantitation is not expected at this level.2

3.6. EXTRACTION BATCH – A set of up to 20 Field Samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

3.7. FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

3.8. FIELD REAGENT BLANK (FRB) – An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.9. INTERNAL STANDARD (IS) – A pure chemical added to an extract or standard solution in a known amount(s) and used to measure the relative response of other method analytes and surrogates that are components of the same solution. The internal standard must be a chemical that is structurally similar to the method analytes, has no potential to be present in water samples, and is not a method analyte.

3.10. LABORATORY FORTIFIED BLANK (LFB) – A volume of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.11. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate sample extraction and the measured values in the LFSM corrected for background concentrations.

3.12. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, extracted, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the occurrence of method analytes is low.

3.13. LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, internal standard, and surrogates that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.14. LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50 and 150% recovery.1

3.15. MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can only be used if acceptable QC criteria for this standard are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.6.

3.16. PRECURSOR ION – For the purpose of this method, the precursor ion is the deprotonated molecule ([M-H]-) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller m/z.

3.17. PRIMARY DILUTION STANDARD (PDS) SOLUTION – A solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.18. PRODUCT ION – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisionally activated dissociation of the precursor ion.

3.19. QUALITATIVE STANDARD – A qualitative standard is a standard for which either the concentration is estimated or method analyte impurities exist at a concentration >1/3 of the MRL in the highest concentration calibration standard. For the purposes of this method, qualitative standards are used to identify retention times of branched isomers of method analytes and are not used for quantitation purposes.

3.20. QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. The second source SSS is used to fortify the QCS at a known concentration. The QCS is used to check calibration standard integrity.

3.21. QUANTITATIVE STANDARD – A quantitative standard is a standard of known concentration and purity. The quantitative standard must not contain any of the method analytes as impurities at concentrations >1/3 of the MRL in the highest concentration calibration standard.

3.22. SAFETY DATA SHEET (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.23. STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.24. SURROGATE ANALYTE (SUR) – A pure chemical which chemically resembles method analytes and is extremely unlikely to be found in any sample. This chemical is added to a sample aliquot in known amount(s) before processing and is measured with the same procedures used to measure other method analytes. The purpose of the SUR is to monitor method performance with each sample.

4. INTERFERENCES

4.1. All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by a reagent water rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 h or solvent rinsed. Volumetric glassware should be solvent rinsed and not be heated in an oven above 120 °C. Store clean glassware inverted or capped. **Do not cover with aluminum foil because PFAS can be potentially transferred from the aluminum foil to the glassware.**

NOTE: Samples and extracts should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces. PFAS analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers.

4.2. Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, SPE sample transfer lines, etc.[3] All items such as these must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3.1. **Subtracting blank values from sample results is not permitted.**

4.3. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent.[4-5] Total organic carbon (TOC) is a good indicator of humic content of the sample. Under the LC conditions used during method development, matrix effects due to total organic carbon (TOC) were not observed.

4.4. Relatively large quantities of the preservative (Sect. 8.1.2) are added to sample bottles. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks (Sect. 9.3.1), particularly when new lots of reagents are acquired.

4.5. SPE cartridges can be a source of interferences. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of SDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[6-8]

5.2. PFOA has been described as likely to be carcinogenic to humans.[9] Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES

(Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.) Due to potential adsorption of analytes onto glass, polypropylene containers were used for all standard, sample and extraction preparations. Other plastic materials (e.g., polyethylene) which meet the QC requirements of Section 9 may be substituted.

6.1. SAMPLE CONTAINERS – 250-mL polypropylene bottles fitted with polypropylene screw caps.

6.2. POLYPROPYLENE BOTTLES – 4-mL narrow-mouth polypropylene bottles (VWR Cat. No.: 16066-960 or equivalent).

6.3. CENTRIFUGE TUBES – 15-mL conical polypropylene tubes with polypropylene screw caps for storing standard solutions and for collection of the extracts (Thomas Scientific Cat. No.: 2602A10 or equivalent).

6.4. AUTOSAMPLER VIALS – Polypropylene 0.4-mL autosampler vials (ThermoFisher Cat. No.: C4000-11) with polypropylene caps (ThermoFisher Cat. No.: C5000-50 or equivalent).

NOTE: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, so evaporation occurs after injection. Thus, multiple injections from the same vial are not possible.

6.5. POLYPROPYLENE GRADUATED CYLINDERS – Suggested sizes include 25, 50, 100 and 1000-mL cylinders.

6.6. MICRO SYRINGES – Suggested sizes include 5, 10, 25, 50, 100, 250, 500 and 1000-µL syringes.

6.7. PLASTIC PIPETS – Polypropylene or polyethylene disposable pipets (Fisher Cat. No.: 13-711-7 or equivalent).

6.8. ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.9. SOLID PHASE EXTRACTION (SPE) APPARATUS FOR USING CARTRIDGES

6.9.1. SPE CARTRIDGES – 0.5 g, 6-mL SPE cartridges containing styrenedivinyl-benzene (SDVB) polymeric sorbent phase (Agilent Cat. No.: 1225-5021 or equivalent). The sorbent may not be modified with monomers other than SDVB.

6.9.2. VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold with Visiprep™ large volume sampler (Supelco Cat. No. 57030 and 57275 or equivalent) for cartridge extractions, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements discussed in Section 9 are met. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. Care must be taken with automated SPE systems to ensure the PTFE commonly used in these systems does not contribute to unacceptable analyte concentrations in the LRB (Sect. 9.3.1).

6.9.3. SAMPLE DELIVERY SYSTEM – Use of a polypropylene transfer tube system, which transfers the sample directly from the sample container to the SPE cartridge, is recommended, but not mandatory. Standard extraction manifolds come equipped with PTFE transfer tube systems. These can be replaced with 1/8" O.D. x 1/16" I.D. polypropylene or polyethylene tubing (Hudson Extrusions LLDPE or equivalent) cut to an appropriate length to ensure no sample contamination from the sample transfer lines. Other types of non-PTFE tubing may be used provided it meets the LRB (Sect. 9.3.1) and LFB (Sect. 9.3.3) QC requirements. The PTFE transfer tubes may be used, but an LRB must be run on each PTFE transfer tube and the QC requirements in Section 9.3.1 must be met. In the case of automated SPE, the removal of PTFE lines may not be feasible; therefore, LRBs will need to be rotated among the ports and must meet the QC requirements of Sections 9.2.2 and 9.3.1.

6.10. EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by evaporation with nitrogen using a water bath set no higher than 65 °C (Meyer N-Evap, Model 111, Organomation Associates, Inc. or equivalent).

6.11. LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 10 to 15 inches of mercury for extraction cartridges.

6.12. LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER (MS/MS) WITH DATA SYSTEM

6.12.1. LC SYSTEM – Instrument capable of reproducibly injecting up to 10-µL aliquots and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.3 mL/min). The usage of a column heater is optional.

NOTE: During the course of method development, it was discovered that while idle for more than one day, PFAS built up in the PTFE solvent transfer lines. To prevent long delays in purging high levels of PFAS from the LC solvent lines, they were replaced with PEEK™ tubing and the PTFE solvent frits were replaced with stainless steel frits. It is not possible to remove all PFAS background contamination, but these measures help to minimize their background levels.

6.12.2. LC/TANDEM MASS SPECTROMETER – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.3 mL/min. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.18) for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision. Data are demonstrated in Tables 5-9 using a triple quadrupole mass spectrometer (Waters XEVO TQMS). See the Note in Sect. 10.2.3 pertaining to potential limitations of some MS/MS instrumentation in achieving the required MS/MS transitions.

6.12.3. DATA SYSTEM – An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.12.4. ANALYTICAL COLUMN – An LC C18 column (2.1 x 150 mm) packed with 5 µm dp C18 solid phase particles (Waters #: 186001301 or equivalent) was used. Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

7. REAGENTS AND STANDARDS

7.1. GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1. REAGENT WATER – Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than 1/3 the MRL for each method analyte of interest. Prior to daily use, at least 3 L of reagent water should be flushed from the purification system to rinse out any build-up of analytes in the system's tubing.

7.1.2. METHANOL (CH3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher LC/MS grade or equivalent).

7.1.3. AMMONIUM ACETATE (NH4C2H3O2, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade or equivalent).

7.1.4. 20 mM AMMONIUM ACETATE/REAGENT WATER – To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water. This solution is volatile and must be replaced at least once a week. More frequent replacement may be necessary if unexplained loss in sensitivity or retention time shifts are encountered and attributed to loss of the ammonium acetate.

7.1.5. TRIZMA® PRESET CRYSTALS, pH 7.0 (Sigma cat# T-7193 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]. Alternatively, a mix of the two components with a weight ratio of 15.5/1 Tris HCL/Tris may be used. This blend is targeted to produce a pH near 7.0 at 25 °C in reagent water. Trizma® functions as a buffer and removes free chlorine in chlorinated finished waters (Sect. 8.1.2).

7.1.6. NITROGEN – Used for the following purposes:

7.1.6.1. Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. The nitrogen used should meet or exceed instrument manufacturer's specifications.

7.1.6.2. Nitrogen is used to concentrate sample extracts (Ultra High Purity or equivalent).

7.1.7. ARGON – Used as collision gas in MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen gas may be used as the collision gas provided sufficient sensitivity (product ion formation) is achieved.

7.2. STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. PFAS analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers. Solution concentrations listed in this Section were used to develop this method and are included as an example. Alternate concentrations may be used as necessary depending on instrument sensitivity and the calibration range used. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. PDS and calibration standards were found to be stable for, at least, one month during method development. Laboratories should use standard QC practices to determine when standards need to be replaced. The target analyte manufacturer's guidelines may be helpful when making the determination.

NOTE: Stock standards (Sect. 7.2.1.1, 7.2.2.1 and 7.2.3.1) were stored at ≤4 °C. Primary dilution standards (Sect.7.2.1.2, 7.2.2.2 and 7.2.3.2) were stored at room temperature to prevent adsorption of the method analytes onto the container surfaces that may occur when refrigerated. Storing the standards at room temperature will also minimize daily imprecision due to the potential of inadequate room temperature stabilization. However, standards may be stored cold provided the standards are allowed to come to room temperature and vortexed well prior to use.

7.2.1. INTERNAL (IS) STOCK STANDARD SOLUTIONS – This method uses three IS compounds listed in the table below. These isotopically labeled IS(s) were carefully chosen during method development because they encompass all the functional groups of the method analytes. Although alternate IS standards may be used provided they are isotopically labeled compounds with similar functional groups as the method analytes, the analyst must have documented reasons for using alternate IS(s). Alternate IS(s) must meet the QC requirements in Section 9.3.4. Note that different isotopic labels of the same IS(s) are acceptable (e.g., $^{13}C_2$-PFOA and $^{13}C_4$-PFOA) but will require modification of the MS/MS precursor and product ions.

Internal Standards	**Acronym**
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA

7.2.1.1. IS STOCK STANDARD SOLUTIONS (IS SSS) – These IS stocks can be obtained as individual certified stock standard solutions. The ISs can also be purchased as PDSs, making the preparation of individual SSSs unnecessary. Analysis of the IS(s) is less complicated if the IS(s) purchased contains only the linear isomer.

7.2.1.2. INTERNAL STANDARD PRIMARY DILUTION (IS PDS) STANDARD (1-4 ng/µL) – Prepare, or purchase commercially, the IS PDS at a suggested concentration of 1-4 ng/µL. The IS PDS (in methanol with 4 molar equivalents of sodium hydroxide) was purchased from Wellington Labs. Alternatively, the IS PDS can be prepared in methanol containing 4% reagent water. Use 10 µL of this 1-4 ng/µL solution to fortify the final 1-mL extracts (Sect. 11.5). This will yield a concentration of 10-40 ng/mL of each IS in the 1-mL extracts.

IS	Final Conc. of IS PDS (ng/µL)
$^{13}C_2$-PFOA	1.0
$^{13}C_4$-PFOS	3.0
d_3-NMeFOSAA	4.0

7.2.2. SURROGATE (SUR) STANDARD SOLUTIONS – The four SUR(s) listed in the table below were purchased from Wellington Labs as linear only isomers. These isotopically labeled SUR standards were carefully chosen during method development because they encompass most of the functional groups, as well as the water solubility range of the method analytes. Although alternate SUR standards may be used provided they are isotopically labeled compounds with similar functional groups as the method analytes, the analyst must have documented reasons for using alternate SUR standards. The alternate SUR standards chosen must still span the water solubility range of the method analytes. In addition, alternate SUR standards must meet the QC requirements in Section 9.3.5.

Surrogates	Acronym
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA

7.2.2.1. SUR STOCK STANDARD SOLUTIONS (SUR SSS) – These SUR stocks can be obtained as individual certified stock standard solutions. The SURs can also be purchased as PDSs, making the preparation of individual SSSs

unnecessary. Analysis of the SUR(s) is less complicated if the SUR(s) purchased contains only the linear isomer.

7.2.2.2. SURROGATE PRIMARY DILUTION STANDARD (SUR PDS) (1-4 ng/µL) – Prepare, or purchase commercially, the SUR PDS at a suggested concentration of 1-4 ng/µL. The SUR PDS (in methanol with 4 molar equivalents of sodium hydroxide) was purchased from Wellington Labs. Alternatively, the SUR PDS can be prepared in methanol containing 4% reagent water. Use 10 µL of this 1-4 ng/µL solution to fortify all QC and Field Samples. (Sect. 11.5). This will yield SUR concentrations of 40-160 ng/L in the 250 mL aqueous samples.

SUR	Final Conc. of SUR PDS (ng/µL)
$^{13}C_2$-PFHxA	1.0
$^{13}C_2$-PFDA	1.0
d_5-NEtFOSAA	4.0
$^{13}C_3$-HFPO-DA	1.0

7.2.3. ANALYTE STANDARD SOLUTIONS – Analyte standards may be purchased commercially as ampoulized solutions or prepared from neat materials. If commercially available, the method analytes must be purchased as technical grade (linear and branched isomers) standards or neat materials. Standards or neat materials that contain only the linear isomer can be substituted only if technical grade (linear and branched isomers) standards or neat material cannot be purchased as quantitative standards (see note below regarding PFOA). At the time of this method development, PFHxS, PFOS, NEtFOSAA and NMeFOSAA are available as technical grade (containing branched and linear isomers) and therefore must be purchased as technical grade.

A qualitative standard (Sect. 3.19) is available for PFOA that contains the linear and branched isomers (Wellington Labs, Cat. No. T-PFOA, or equivalent). This qualitative PFOA standard must be purchased and used to identify the retention times of the branched PFOA isomers, but the linear only PFOA standard must be used for quantitation (Sect. 12.2) until a quantitative PFOA standard containing the branched and linear isomers becomes commercially available.

PFHxS, PFOS, ADONA, 9Cl-PF3ONS and 11Cl-PF3OUdS may not be available as the acids listed in Section 1.1, but rather as their corresponding salts, such as NH_4^+, Na^+ and K^+. These salts are acceptable starting materials for the stock standards provided the measured mass is corrected for the salt content according to the equation below. Prepare the Analyte Stock and Primary Dilutions Standards as described below.

$$Mass_{acid} = MeasuredMass_{salt} \times \frac{MW_{acid}}{MW_{salt}}$$

where:

MW_{acid} = the molecular weight of PFAS
MW_{salt} = the molecular weight of purchased salt

7.2.3.1. ANALYTE STOCK STANDARD SOLUTION (SSS) – Analyte standards may be purchased commercially as ampoulized solutions prepared from neat materials. Commercially prepared SSSs are available for all method analytes. During method development, mixes or individual stocks were obtained from Accustandard, Absolute, Wellington Labs and Synquest. When using these stock standards to prepare a PDS, care must be taken to ensure that these standards are at room temperature and adequately vortexed.

7.2.3.2. ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION (0.5-2.5 ng/µL) – The analyte PDS contains all the method analytes of interest at various concentrations in methanol containing 4% water (or in methanol containing 4 molar equivalents of sodium hydroxide). The ESI and MS/MS response varies by compound; therefore, a mix of concentrations may be needed in the analyte PDS. See Tables 5-9 in Section 17 for suggested concentrations for each analyte. During method development, the analyte PDS was prepared such that approximately the same instrument response was obtained for all the analytes. The analyte PDS is prepared by dilution of the combined Analyte Stock Standard Solutions and is used to prepare the CAL standards, and fortify the LFBs, LFSMs, and LFSMDs with the method analytes. If the PDS is stored cold, care must be taken to ensure that these standards are at room temperature and adequately vortexed before usage.

7.2.4. CALIBRATION STANDARDS (CAL) – At least five calibration concentrations are required to prepare the initial calibration curve spanning a 20-fold concentration range (Sect. 10.2). Larger concentration ranges will require more calibration points. Prepare the CAL standards over the concentration range of interest from dilutions of the analyte PDS in methanol containing 4% reagent water. The suggested analyte concentrations found in Tables 5-9 can be used as a starting point for determining the calibration range. The IS and SUR are added to the CAL standards at a constant concentration. During method development, the concentrations of the SUR(s) were 10-40 pg/µL in the standard (40-160 ng/L in the sample) and the IS(s) were 10-40 ng/mL. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. The CAL standards may also be used as CCCs (Sect. 9.3.2).

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. SAMPLE BOTTLE PREPARATION

8.1.1. Samples must be collected in a 250-mL polypropylene bottle fitted with a polypropylene screw-cap.

8.1.2. The preservation reagent, listed in the table below, is added to each sample bottle as a solid prior to shipment to the field (or prior to sample collection).

Compound	Amount	Purpose
Trizma® (Sect. 7.1.5)	5.0 g/L	buffering reagent and removes free chlorine

8.2. SAMPLE COLLECTION

8.2.1. The sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. PFAS contamination during sampling can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves will aid in minimizing this type of accidental contamination of the samples.

8.2.1. Open the tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min). Collect samples from the flowing system.

8.2.2. Fill sample bottles, taking care not to flush out the sample preservation reagent. Samples do not need to be collected headspace free.

8.2.3. After collecting the sample, cap the bottle and agitate by hand until preservative is dissolved. Keep the sample sealed from time of collection until extraction.

8.3. FIELD REAGENT BLANKS (FRB)

8.3.1. An FRB must be handled along with each sample set. The sample set is composed of samples collected from the same sample site and at the same time. At the laboratory, fill the field blank sample bottle with reagent water, then seal, and ship to the sampling site along with the sample bottles. For each FRB shipped, a second FRB bottle containing only the preservative must also be shipped. At the sampling site, the sampler must open the shipped FRB and pour the preserved reagent water into the empty shipped sample bottle, seal and label this bottle as the FRB. The FRB is shipped back to the laboratory along with the samples and analyzed to ensure that PFAS were not introduced into the sample during sample collection/handling.

8.3.2. The same batch of preservative must be used for the FRBs as for the field samples.

8.3.3. The reagent water used for the FRBs must be initially analyzed for method analytes as a LRB (using the same lot of sample bottles as the field samples) and must meet the LRB criteria in Section 9.3.1 prior to use. This requirement will ensure samples are not being discarded due to contaminated reagent water or sample bottles rather than contamination during sampling.

8.4. SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10 °C when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction but must not be frozen.

NOTE: Samples that are significantly above 10° C, at the time of collection, may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice to meet the above requirements.

8.5. SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Table 10) indicated that all compounds listed in this method have adequate stability for 14 days when collected, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.4. Therefore, water samples should be extracted as soon as possible but must be extracted within 14 days. Extracts must be stored at room temperature and analyzed within 28 days after extraction. The extract storage stability study data are presented in Table 11.

9. QUALITY CONTROL

9.1. QC requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements that must be met when preparing and analyzing Field Samples. This Section describes the QC parameters, their required frequencies, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Table 12 and Table 13. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1. METHOD MODIFICATIONS – The analyst is permitted to modify LC columns, LC conditions, evaporation techniques, internal standards or surrogate standards, and MS and MS/MS conditions. Each time such method modifications are made, the analyst must repeat the procedures of the IDC. **Modifications to LC conditions should still produce conditions such that co-elution of the method analytes is minimized to reduce the probability of suppression/enhancement effects.**

9.2. INITIAL DEMONSTRATION OF CAPABILITY – The IDC must be successfully performed prior to analyzing any Field Samples. Prior to conducting the IDC, the

analyst must first generate an acceptable Initial Calibration following the procedure outlined in Section 10.2.

9.2.1. INITIAL DEMONSTRATION OF BRANCHED vs LINEAR ISOMER PROFILE for PFOA IN A QUALITATIVE STANDARD – Prepare and analyze a qualitative standard used for identifying retention times of branch isomers of PFOA. Identify the retention times of branched isomers of PFOA in the purchased technical grade PFOA standard. This qualitative PFOA standard is not used for quantitation (see Section 12.2). This branched isomer identification check must be repeated any time changes occur that affect the analyte retention times.

9.2.2. INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of SPE cartridges, solvents, centrifuge tubes, disposable pipets, and autosampler vials are used, it must be demonstrated that an LRB is reasonably free of contamination and that the criteria in Section 9.3.1 are met. If an automated extraction system is used, an LRB should be extracted on each port to ensure that all the valves and tubing are free from potential PFAS contamination.

9.2.3. INITIAL DEMONSTRATION OF PRECISION (IDP) – Prepare, extract, and analyze four to seven replicate LFBs fortified near the midrange of the initial calibration curve according to the procedure described in Section 11.4. Sample preservatives as described in Section 8.1.2 must be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.4. INITIAL DEMONSTRATION OF ACCURACY (IDA) – Using the same set of replicate data generated for Section 9.2.3, calculate average recovery. The average recovery of the replicate values must be within ± 30% of the true value.

9.2.5. INITIAL DEMONSTRATION OF PEAK ASYMMETRY FACTOR – Peak asymmetry factors must be calculated using the equation in Section 9.3.9 for the first two eluting peaks (if only two analytes are being analyzed, both must be evaluated) in a mid-level CAL standard. The peak asymmetry factors must fall in the range of 0.8 to 1.5. See guidance in Section 10.2.4.1 if the calculated peak asymmetry factors do not meet the criteria.

9.2.6. MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The MRL may be established by a laboratory for their specific purpose or may be set by a regulatory agency. Establish an Initial Calibration following the procedure outlined in Section 10.2. The lowest CAL standard used to establish the Initial Calibration (as well as the low-level CCC, Section 10.3) must be at or below the concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm the MRL following the procedure outlined below.

9.2.6.1. Fortify, extract, and analyze seven replicate LFBs at the proposed MRL concentration. These LFBs must contain all method preservatives described in Section 8.1.2. Calculate the mean measured concentration (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963s$$

where

s = the standard deviation
3.963 = a constant value for seven replicates.[1]

9.2.6.2. Confirm that the upper and lower limits for the Prediction Interval of Result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\,Concentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\,Concentration} \times 100\% \geq 50\%$$

9.2.6.3. The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above (Sect. 9.2.6.2). If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

9.2.7. CALIBRATION CONFIRMATION – Analyze a QCS as described in Section 9.3.10 to confirm the accuracy of the standards/calibration curve.

9.2.8. DETECTION LIMIT DETERMINATION *(optional) – While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

9.2.8.1. Replicate analyses for this procedure should be done over at least three days (i.e., both the sample extraction and the LC/MS/MS analyses should be done over at least three days). Prepare at least seven replicate LFBs at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. The DLs in Table 5 were calculated from LFBs fortified at various concentrations as

indicated in the table. The appropriate fortification concentrations will be dependent upon the sensitivity of the LC/MS/MS system used. All preservation reagents listed in Section 8.1.2 must also be added to these samples. Analyze the seven replicates through all steps of Section 11.

NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the *DL* using the following equation

$$DL = s \times t_{(n-1,\,1-\alpha=0.99)}$$

where

s = standard deviation of replicate analyses
$t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates.

NOTE: Do not subtract blank values when performing DL calculations. The DL is a statistical determination of precision only.[2] If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs. Therefore, no precision and accuracy criteria are specified.

9.2.8.2. If a laboratory is establishing their own MRL, the calculated DLs should not be used as the MRL for analytes that commonly occur as background contaminants. Method analytes that are seen in the background should be reported as present in Field Samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentrations + 3σ or 3 times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as an MRL in order to avoid reporting false positive results.

9.3. ONGOING QC REQUIREMENTS – This Section summarizes the ongoing QC criteria that must be followed when processing and analyzing Field Samples.

9.3.1. LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.6) to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. If more than 20 Field Samples are included in a batch, analyze an LRB for every 20 samples. If the LRB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before

proceeding. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. Blank contamination is estimated by extrapolation, if the concentration is below the lowest CAL standard. This extrapolation procedure is not allowed for sample results as it may not meet data quality objectives. If the method analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. Because background contamination is a significant problem for several method analytes, maintaining a historical record of LRB data is highly recommended.

9.3.2. CONTINUING CALIBRATION CHECK (CCC) – CCC Standards are analyzed at the beginning of each analysis batch, after every 10 Field Samples, and at the end of the analysis batch. See Section 10.3 for concentration requirements and acceptance criteria.

9.3.3. LABORATORY FORTIFIED BLANK (LFB) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The low concentration LFB must be as near as practical to, but no more than two times, the MRL. Similarly, the high concentration LFB should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the low-level LFB analyses must be 50-150% of the true value. Results of the medium and high-level LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.3.4. INTERNAL STANDARDS (IS) – The analyst must monitor the peak areas of the IS(s) in all injections during each analysis day. The IS responses (peak areas) in any chromatographic run must be within 70-140% of the response in the most recent CCC and must not deviate by more than 50% from the average area measured during initial analyte calibration. If the IS areas in a chromatographic run do not meet these criteria, inject a second aliquot of that extract aliquotted into a new capped autosampler vial. Random evaporation losses have been observed with the polypropylene caps causing high IS(s) areas.

9.3.4.1. If the reinjected aliquot produces an acceptable IS response, report results for that aliquot.

9.3.4.2. If the reinjected extract fails again, the analyst should check the calibration by reanalyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL standard is acceptable, extraction of the sample may need to be repeated provided the sample is still within the holding time.

Otherwise, report results obtained from the reinjected extract, but annotate as suspect. Alternatively, collect a new sample and re-analyze.

9.3.5. SURROGATE RECOVERY – The SUR standard is fortified into all samples, CCCs, LRBs, LFBs, LFSMs, LFSMDs, FD, and FRB prior to extraction. It is also added to the CAL standards. The SUR is a means of assessing method performance from extraction to final chromatographic measurement. Calculate the recovery (%R) for the SUR using the following equation

$$\%R = \left(\frac{A}{B}\right) \times 100$$

where

A = calculated SUR concentration for the QC or Field Sample
B = fortified concentration of the SUR.

9.3.5.1. SUR recovery must be in the range of 70-130%. When SUR recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and reanalyze the extract.

9.3.5.2. If the extract reanalysis meets the SUR recovery criterion, report only data for the reanalyzed extract.

9.3.5.3. If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by injecting the last CAL standard that passed. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the re-extracted sample also fails the recovery criterion, report all data for that sample as suspect/SUR recovery to inform the data user that the results are suspect due to SUR recovery. Alternatively, collect a new sample and re-analyze.

9.3.6. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (FD) (Sect. 9.3.7); however, infrequent occurrence of method analytes would hinder this assessment. If the occurrence of method analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFSMD, must be prepared, extracted, and analyzed from a duplicate of the Field Sample. Extraction batches that contain LFSMDs will not require the extraction of a FD. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be

established for each. Over time, LFSM data should be documented by the laboratory for all routine sample sources.

9.3.6.1. Within each extraction batch (Sect. 3.6), a minimum of one Field Sample is fortified as an LFSM for every 20 Field Samples analyzed. The LFSM is prepared by spiking a sample with an appropriate amount of the Analyte PDS (Sect. 7.2.3.2). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Use historical data and rotate through the low, mid and high concentrations when selecting a fortifying concentration.

9.3.6.2. Calculate the percent recovery (%*R*) for each analyte using the equation

$$\%R = \frac{(A - B)}{C} \times 100$$

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.3.6.3. Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70-130%, except for low-level fortification near or at the MRL (within a factor of 2-times the MRL concentration) where 50-150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7. FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or LFSMD) – Within each extraction batch (not to exceed 20 Field Samples, Sect. 3.6), a minimum of one FD or LFSMD must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If method analytes are not routinely observed in Field Samples, an LFSMD should be analyzed rather than an FD.

9.3.7.1. Calculate the relative percent difference (*RPD*) for duplicate measurements (*FD1* and *FD2*) using the equation

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \times 100$$

9.3.7.2. RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of 2 of the MRL. At these concentrations, FDs should have RPDs that are ≤50%. If the RPD of

any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7.3. If an LFSMD is analyzed instead of a FD, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

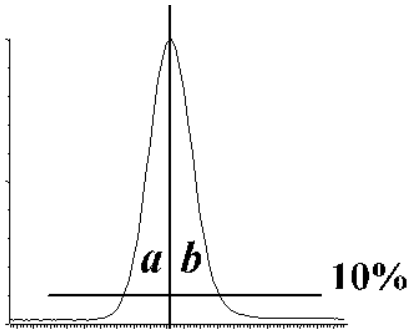
$$RPD = \frac{|LFSM - LFSMD|}{(LFSM + LFSMD)/2} \times 100$$

9.3.7.4. RPDs for duplicate LFSMs must be ≤30% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are fortified at analyte concentrations that are within a factor of 2 of the MRL. LFSMs fortified at these concentrations must have RPDs that are ≤50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.8. FIELD REAGENT BLANK (FRB) – The purpose of the FRB is to ensure that PFAS measured in the Field Samples were not inadvertently introduced into the sample during sample collection/handling. Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample. If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.

9.3.9. PEAK ASYMMETRY FACTOR – A peak asymmetry factor must be calculated using the equation below during the IDC and every time chromatographic changes are made that may affect peak shape. The peak asymmetry factor for the first two eluting peaks in a mid-level CAL standard (if only two analytes are being analyzed, both must be evaluated) must fall in the range of 0.8 to 1.5. Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted. See guidance in Section 10.2.4.1 if the calculated peak asymmetry factors do not meet the criteria.

$$A_s = \frac{b}{a}$$



where:

A_s = peak asymmetry factor

B = width of the back half of the peak measured (at 10% peak height) from the trailing edge of the peak to a line dropped perpendicularly from the peak apex

a = the width of the front half of the peak measured (at 10% peak height) from the leading edge of the peak to a line dropped perpendicularly from the apex.

9.3.10. QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new Analyte PDS (Sect. 7.2.3.2) is prepared, and at least quarterly, analyze a QCS sample from a source different from the source of the CAL standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared at a mid-level concentration and analyzed just like a CCC. Acceptance criteria for the QCS are identical to the CCCs; the calculated amount for each analyte must be ± 30% of the expected value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

10. CALIBRATION AND STANDARDIZATION

10.1. Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a CCC is required at the beginning and end of each period in which analyses are performed, and after every tenth Field Sample.

10.2. INITIAL CALIBRATION

10.2.1. ESI-MS/MS TUNE

10.2.1.1. Calibrate the mass scale of the MS with the calibration compounds and procedures prescribed by the manufacturer.

10.2.1.2. Optimize the $[M-H]^-$ or $[M-CO_2]^-$ for each method analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow

rate (approximately 0.3 mL/min). This tune can be done on a mix of the method analytes. The MS parameters (voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. The method analytes may have different optima requiring some compromise between the optima. See Table 2 for ESI-MS conditions used in method development.

10.2.1.3. Optimize the product ion (Sect. 3.18) for each analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). This tune can be done on a mix of the method analytes. The MS/MS parameters (collision gas pressure, collision energy, etc.) are varied until optimal analyte responses are determined. Typically, the carboxylic acids have very similar MS/MS conditions and the sulfonic acids have similar MS/MS conditions. See Table 4 for MS/MS conditions used in method development.

10.2.2. Establish LC operating parameters that optimize resolution and peak shape. Suggested LC conditions can be found in Table 1. The LC conditions listed in Table 1 may not be optimum for all LC systems and may need to be optimized by the analyst (See Sect. 10.2.4.1). Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

Cautions: LC system components, as well as the mobile phase constituents, contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, prior to daily use, flush the column with 100% methanol for at least 20 min before initiating a sequence. It may be necessary on some systems to flush other LC components such as wash syringes, sample needles or any other system components before daily use.

Mobile phase modifiers other than 20 mM ammonium acetate may be used at the discretion of the analyst, provided that the retention time stability criteria in Sect. 11.7.2 can be met over a period of two weeks. During method development, retention times shifted to shorter and shorter times as days progressed when mobile phases with less than 20 mM ammonium acetate were used.

10.2.3. Inject a mid-level CAL standard under LC/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into retention time windows each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$; Sect. 3.16) for the analytes in each window and choose the most abundant product

ion. The product ions (also the quantitation ions) chosen during method development are in Table 4, although these will be instrument dependent. For maximum sensitivity, small mass windows of ±0.5 daltons around the product ion mass were used for quantitation.

NOTE: There have been reports[10] that not all product ions in the linear PFOS are produced in all branched PFOS isomers. (This phenomenon may exist for many of the PFAS.) Thus, to reduce PFOS, PFBS and PFHxS bias, it is required that the precursor *m/z* → *m/z* 80 transition be used as the quantitation transition. Some MS/MS instruments, may not be able to scan a product ion with such a wide mass difference from the precursor ion; therefore, if the MS/MS cannot measure the precursor *m/z* → *m/z* 80 transition they may not be used for this method if PFOS, PFBS, or PFHxS analysis is to be conducted.

10.2.4. Inject a mid-level CAL standard under optimized LC/MS/MS conditions to ensure that each method analyte is observed in its MS/MS window and that there are at least 10 scans across the peak for optimum precision.

NOTE: Ensure that the retention time window used to collect data for each analyte is sufficient to detect earlier eluting branched isomers.

10.2.4.1. If broad, split or fronting peaks are observed for the first two eluting chromatographic peaks (if only two analytes are being analyzed, both must be evaluated), change the initial mobile phase conditions to higher aqueous content until the peak asymmetry ratio for each peak is 0.8 – 1.5. The peak asymmetry factor is calculated as described in Section 9.3.9 on a mid-level CAL standard. The peak asymmetry factor must meet the above criteria for the first two eluting peaks during the IDC and every time a new calibration curve is generated. Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

10.2.4.2. Most PFAS are produced by two different processes. One process gives rise to linear PFAS only while the other process produces both linear and branched isomers. Thus, both branched and linear PFAS can potentially be found in the environment. Refer to Section 12.2 for guidance on integration and quantitation of PFAS.

10.2.5. Prepare a set of at least five CAL standards as described in Section 7.2.4. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the CAL standards are at a concentration greater than or equal to the MRL.

10.2.6. The LC/MS/MS system is calibrated using the IS technique. Use the LC/MS/MS data system software to generate a linear regression or quadratic calibration curve

for each of the analytes. This curve **must always** be forced through zero and may be concentration weighted, if necessary. Forcing zero allows for a better estimate of the background levels of method analytes.

10.2.7. CALIBRATION ACCEPTANCE CRITERIA – Validate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. For calibration levels that are ≤ MRL, the result for each analyte must be within ± 50% of the true value. All other calibration points must calculate to be within ± 30% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. It is recommended that corrective action is taken to reanalyze the CAL standards, restrict the range of calibration, or select an alternate method of calibration (forcing the curve through zero is still required).

CAUTION: When acquiring MS/MS data, LC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the segment time window.

10.3. CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. In this context, a "sample" is considered to be a Field Sample. LRBs, CCCs, LFBs, LFSMs, FDs FRBs and LFSMDs are not counted as samples. The beginning CCC of each analysis batch must be at or below the MRL to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL standards to meet this requirement. Alternatively, the analyte concentrations in the analyte PDS may be customized to meet these criteria. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

10.3.1. Inject an aliquot of the appropriate concentration CAL standard and analyze with the same conditions used during the initial calibration.

10.3.2. Determine that the absolute areas of the quantitation ions of the IS(s) are within 70-140% of the areas measured in the most recent continuing calibration check, and within 50-150% from the average areas measured during initial calibration. If any of the IS areas has changed by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may include cleaning of the MS ion source, or other maintenance as indicated in Section 10.3.4. Major instrument maintenance requires recalibration (Sect. 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect. 10.3). Control charts are useful aids in documenting system sensitivity changes.

10.3.3. Calculate the concentration of each analyte and SUR in the CCC. The calculated amount for each analyte and SUR for medium and high level CCCs must be within ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50% and the SUR must be within ± 30% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken (Sect. 10.3.4) which may require recalibration. Any Field or QC Samples that have been analyzed since the last acceptable calibration verification that are still within holding time must be reanalyzed after adequate calibration has been restored, with the following exception. **If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular method analyte, and Field Sample extracts show no detection for that method analyte, non-detects may be reported without re-analysis.**

10.3.4. REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance, such as cleaning the electrospray probe, atmospheric pressure ionization source, cleaning the mass analyzer, replacing the LC column, etc., requires recalibration (Sect. 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect. 10.3)

11. PROCEDURE

11.1. This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. The data presented in Tables 5-11 demonstrate data collected by manual extraction. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the LRBs should be rotated among the ports to ensure that all the valves and tubing meet the LRB requirements (Sect. 9.3.1).

11.2. Some of the PFAS adsorb to surfaces, including polypropylene. Therefore, the aqueous sample bottles must be rinsed with the elution solvent (Sect. 11.4.4) whether extractions are performed manually or by automation. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected (Sect. 11.4.4).

NOTE: The SPE cartridges and sample bottles described in this Section are designed as single use items and must be discarded after use. They may not be refurbished for reuse in subsequent analyses.

11.3. SAMPLE PREPARATION

11.3.1. Samples are preserved, collected and stored as presented in Section 8. All Field and QC Samples, including the LRB, LFB and FRB, must contain the dechlorinating agent listed in Section 8.1.2. Before extraction, verify that the sample pH is 7 ± 0.5. Determine sample volume. An indirect measurement may

be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 1 g. After extraction, proceed to Section 11.6 for final volume determination. Some of the PFAS adsorb to surfaces, thus the sample volume may **NOT** be transferred to a graduated cylinder for volume measurement. The LRB, LFB and FRB may be prepared by measuring 250 mL of reagent water with a polypropylene graduated cylinder or filling a 250-mL sample bottle to near the top.

11.3.2. Add an aliquot of the SUR PDS (Sect. 7.2.2.2) to each sample, cap and invert to mix. During method development, a 10-µL aliquot of the 1-4 ng/µL SUR PDS (Sect. 7.2.2.2) was added to 250 mL of sample for a final concentration of 40 ng/L for $^{13}C_2$-PFHxA, $^{13}C_3$-HFPO-DA, and $^{13}C_2$-PFDA and 160 ng/L for d_5-NEtFOSAA.

11.3.3. In addition to the SUR(s) and dechlorination agent, if the sample is an LFB, LFSM, or LFSMD, add the necessary amount of analyte PDS (Sect. 7.2.3.2). Cap and invert each sample to mix.

11.4. CARTRIDGE SPE PROCEDURE

11.4.1. CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 15 mL of methanol. Next, rinse each cartridge with 18 mL of reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Add 2-3 mL of reagent water to each cartridge, attach the sample transfer tubes (Sect. 6.9.3), turn on the vacuum, and begin adding sample to the cartridge.

NOTE: If low recoveries are observed for PFBS and PFHxA during the IDC, recoveries may be improved by allowing a one- or two-minute soak time after each addition of the methanol and water used in the clean-up and conditioning step.

11.4.2. SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is 10-15 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

11.4.3. SAMPLE BOTTLE AND CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the sample bottles with two 7.5-mL aliquots of reagent water and draw each aliquot through the sample transfer tubes and the cartridges. Draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

NOTE: If empty plastic reservoirs are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs

must be treated like the transfer tubes. After the entire sample has passed through the cartridge, the reservoirs must be rinsed to waste with reagent water.

11.4.4. SAMPLE BOTTLE AND CARTRIDGE ELUTION – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Rinse the sample bottles with 4 mL of methanol and elute the analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 4-mL aliquot of methanol.

NOTE: If low recoveries are observed for PFBS and PFHxA during the IDC, recoveries may be improved by allowing a one or two-minute soak time after each four mL addition of the methanol and water used in the clean-up and conditioning step.

NOTE: If empty plastic reservoirs are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the transfer tubes. After the reservoirs have been rinsed in Section 11.4.3, the elution solvent used to rinse the sample bottles must be swirled down the sides of the reservoirs while eluting the cartridge to ensure that any method analytes on the surface of the reservoirs are transferred to the extract.

11.5. EXTRACT CONCENTRATION – Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (60-65 °C) to remove all the water/methanol mix. Add the appropriate amount of 96:4% (vol/vol) methanol:water solution and the IS PDS (Sect. 7.2.1.2) to the collection vial to bring the volume to 1 mL and vortex. (10 µL of the 1-4 ng/µL IS PDS for extract concentrations of 10-40 ng/mL were used for method development). Transfer a small aliquot with a plastic pipet (Sect. 6.7) to a polypropylene autosampler vial.

NOTE: It is recommended that the entire 1-mL aliquot not be transferred to the autosampler vial because the polypropylene autosampler caps do not reseal after injection. Therefore, do not store the extracts in the autosampler vials as evaporation losses occur in these autosampler vials. Extracts can be stored in 15-mL centrifuge tubes (Sect. 6.3).

11.6. SAMPLE VOLUME DETERMINATION – If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 2 mL. If using weight to determine volume, weigh the empty bottle to the nearest 1 g and determine the sample weight by subtraction of the empty bottle weight from the original sample weight (Sect. 11.3.1). Assume a sample density of 1.0 g/mL.

In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 12.3).

11.7. EXTRACT ANALYSIS

11.7.1. Establish operating conditions equivalent to those summarized in Tables 1-4 of Section 17. Instrument conditions and columns should be optimized prior to the initiation of the IDC.

11.7.2. Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in CAL standard solutions analyzed on the LC over the course of time. A value of plus or minus three times the standard deviation of the retention time obtained for each method analyte while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

11.7.3. Calibrate the system by either the analysis of a calibration curve (Sect. 10.2) or by confirming the initial calibration is still valid by analyzing a CCC as described in Section 10.3. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2.

11.7.4. Begin analyzing Field Samples, including QC samples, at their appropriate frequency by injecting the same size aliquots (10 µL was used in method development), under the same conditions used to analyze the CAL standards.

11.7.5. At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard. Comparison of the MS/MS mass spectra is not particularly useful given the limited ±0.5 dalton mass range around a single product ion for each method analyte.

11.7.6. The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with 96%:4% (vol/vol) methanol:water solution and the appropriate amount of IS added to match the original concentration. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable SUR performance (Sect. 9.3.5.1) should be determined from the undiluted sample extract. The resulting data must be documented as a dilution and MRLs adjusted accordingly.

12. DATA ANALYSIS AND CALCULATION

12.1. Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Table 4. Other ions may be selected at the discretion of the analyst.

12.2. Because environmental samples may contain both branched and linear isomers for method analytes, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes, integration and quantitation of the PFAS is dependent on type of standard available for each PFAS. It is recognized that some of the procedures described below for integration of standards, QC samples and Field Samples may cause a small amount of unavoidable bias in the quantitation of the method analytes due to the current state of the commercially available standards.

12.2.1. During method development, multiple chromatographic peaks were observed for standards of PFHxS, PFOS, NMeFOSAA, and NEtFOSAA using the LC conditions in Table 1 due to chromatographic resolution of the linear and branched isomers of these compounds. For PFHxS, PFOS, NMeFOSAA and NEtFOSAA, all the chromatographic peaks observed in the standard must be integrated and the areas summed. Chromatographic peaks in all Field Samples and QC samples must be integrated in the same way as the CAL standard for analytes with quantitative standards containing the branched and linear isomers.

12.2.2. For PFOA, identify the branched isomers by analyzing a qualitative standard that includes both linear and branched isomers and compare retention times and tandem mass spectrometry transitions. Quantitate Field Samples and QC samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration with a linear-isomer quantitative PFOA standard.

12.2.3. If standards containing the branched and linear isomers cannot be purchased (i.e., only linear isomer is available), only the linear isomer can be identified and quantitated in Field Samples and QC samples using the linear standard because the retention time of the branched isomers cannot be confirmed.

12.3. Calculate analyte and SUR concentrations using the multipoint calibration as described in Section 10.2. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.6.

12.4. Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

12.5. Calculations must utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

NOTE: Some data in Section 17 of this method are reported with more than two significant figures. This is done to better illustrate the method performance.

13. METHOD PERFORMANCE

13.1. PRECISION, ACCURACY, AND MINIMUM REPORTING LEVELS – Tables for these data are presented in Section 17. LCMRLs and DLs for each method analyte are presented in Table 5. Precision and accuracy are presented for four water matrices: reagent water (Table 6); chlorinated (finished) ground water (Table 7); chlorinated (finished) surface water (Table 8); and private well water (Table 9).

13.2. SAMPLE STORAGE STABILITY STUDIES – An analyte storage stability study was conducted by fortifying the analytes into chlorinated surface water samples that were collected, preserved, and stored as described in Section 8. The precision and mean recovery (n=4) of analyses, conducted on Days 0, 8, and 14 are presented in Table 10.

13.3. EXTRACT STORAGE STABILITY STUDIES – Extract storage stability studies were conducted on extracts obtained from a chlorinated surface water fortified with the method analytes. The precision and mean recovery (n=4) of injections conducted on Days 0, 8, 14, 22, and 28 are reported in Table 11.

13.4. MULTI-LABORATORY DEMONSTRATION – The performance of this method was demonstrated by multiple laboratories, with results similar to those reported in Section 17. The authors wish to acknowledge the work of 1) EPA Region 2 in Edison, NJ., 2) Eurofins Eaton Analytical, LLC in Monrovia, CA, and 3) New Jersey Department of Health in Ewing, NJ.

14. POLLUTION PREVENTION

14.1. This method utilizes SPE to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2. For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036 or on-line at http://membership.acs.org/c/ccs/pub_9.htm (accessed August 2008).

15. WASTE MANAGEMENT

The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Table 1. LC Method Conditions

Time (min)	% 20 mM ammonium acetate	% Methanol
Initial	60.0	40.0
1.0	60.0	40.0
25.0	10.0	90.0
32.0	10.0	90.0
32.1	60.0	40.0
37.0	60.0	40.0
Waters Atlantis® dC_{18} 2.1 x 150 mm packed with 5.0 µm C_{18} stationary phase Flow rate of 0.3 mL/min 10 µL injection into a 50 µL loop		

Table 2. ESI-MS Method Conditions

ESI Conditions	
Polarity	Negative ion
Capillary needle voltage	-3 kV
Cone gas flow	50 L/hr
Nitrogen desolvation gas	800 L/hr
Desolvation gas temp.	350°C

Table 3. Method Analytes, Retention Times (RT) and Suggested IS References

Analyte	Peak # (Fig. 1)	RT (min)	IS# Ref
PFBS	1	7.62	2
PFHxA	2	10.42	1
HFPO-DA	4	11.38	1
PFHpA	6	13.40	1
PFHxS	7	13.58	2
ADONA	8	13.73	1
PFOA	9	15.85	1
PFOS	11	17.91	2
PFNA	13	17.92	1
9Cl-PF3ONS	14	18.91	2
PFDA	15	19.69	1
NMeFOSAA	17	20.50	3
PFUnA	19	21.21	1
NEtFOSAA	20	21.26	3
11Cl-PF3OUdS	22	21.84	2
PFDoA	23	22.52	1
PFTrDA	24	23.66	1
PFTA	25	24.64	1
$^{13}C_2$-PFHxA	3	10.42	1
$^{13}C_3$-HFPO-DA	5	11.40	1
$^{13}C_2$-PFDA	16	19.69	1
d_5-NEtFOSAA	21	21.24	3
$^{13}C_2$-PFOA– IS#1	10	15.85	-
$^{13}C_4$-PFOS– IS#2	12	17.91	-
d_3-NMeFOSAA–IS#3	18	20.49	-

Table 4. MS/MS Method Conditions[a]

Segment[b]	Analyte	Precursor Ion[c] (*m/z*)	Product Ion[c,d] (*m/z*)	Cone Voltage (v)	Collision Energy[e] (v)
1	PFBS[g]	299	80	42	30
1	PFHxA	313	269	14	10
1	HFPO-DA	285[f]	169	12	8
2	PFHpA	363	319	12	10
2	PFHxS[g,h]	399	80	46	32
2	ADONA	377	251	14	12
3	PFOA	413	369	14	10
3	PFOS[g,h]	499	80	52	42
3	PFNA	463	419	16	12
4	9Cl-PF3ONS	531	351	34	24
4	PFDA	513	469	14	10
4	NMeFOSAA[g]	570	419	30	20
4	PFUnA	563	519	12	10
4	NEtFOSAA[g]	584	419	30	20
4	11Cl-PF3OUdS	631	451	40	24
4	PFDoA	613	569	18	10
5	PFTrDA	663	619	14	14
5	PFTA	713	669	14	12
1	$^{13}C_2$-PFHxA	315	270	16	10
1	$^{13}C_3$-HFPO-DA	287	169	10	6
4	$^{13}C_2$-PFDA	515	470	18	10
4	d_5-NEtFOSAA	589	419	28	22
3	$^{13}C_2$-PFOA	415	370	16	10
3	$^{13}C_4$-PFOS	503	80	58	42
4	d_3-NMeFOSAA	573	419	28	14

[a] An LC/MS/MS chromatogram of the analytes is shown in Figure 1.

[b] Segments are time durations in which single or multiple scan events occur.

[c] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak place (e.g., *m/z* 498.9→79.9 for PFOS). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

[d] Ions used for quantitation purposes.

[e] Argon used as collision gas at a flow rate of 0.15 mL/min.

[f] HFPO-DA is not stable in the ESI source and the $[M-H]^-$ is not observed under typical ESI conditions. The precursor ion used during method development was $[M-CO_2]^-$.

[g] Analyte has multiple resolved chromatographic peaks due to linear and branched isomers. All peaks summed for quantitation purposes.

[h] To reduce bias regarding detection of branch and linear isomers, the *m/z* 80 product ion must be used for this analyte.

Table 5. DLs and LCMRLs in Reagent Water

Analyte	Fortified Conc. (ng/L)[a]	DL[b] (ng/L)	LCMRL[c] (ng/L)
PFBS	4.0	1.8	6.3
PFHxA	4.0	1.0	1.7
HFPO-DA	4.0	1.9	4.3
PFHpA	4.0	0.71	0.63
PFHxS	4.0	1.4	2.4
ADONA	4.0	0.88	0.55
PFOA	4.0	0.53	0.82
PFOS	4.0	1.1	2.7
PFNA	4.0	0.70	0.83
9Cl-PF3ONS	4.0	1.4	1.8
PFDA	4.0	1.6	3.3
NMeFOSAA	4.0	2.4	4.3
PFUnA	4.0	1.6	5.2
NEtFOSAA	4.0	2.8	4.8
11Cl-PF3OUdS	4.0	1.5	1.5
PFDoA	4.0	1.2	1.3
PFTrDA	4.0	0.72	0.53
PFTA	4.0	1.1	1.2

[a] Spiking concentration used to determine DL.

[b] Detection limits were determined by analyzing seven replicates over three days according to Section 9.2.8.

[c] LCMRLs were calculated according to the procedure in reference 1.

Table 6. Precision and Accuracy (n=8) of PFAS in Fortified Reagent Water

18. Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	90.8	6.8	80.0	85.1	6.7
PFHxA	16.0	101	8.0	80.0	96.5	4.6
HFPO-DA	16.0	97.8	1.8	80.0	96.8	5.1
PFHpA	16.0	105	3.3	80.0	104	2.7
PFHxS	16.0	109	6.7	80.0	107	4.4
ADONA	16.0	108	1.3	80.0	106	3.6
PFOA	16.0	106	1.8	80.0	104	3.1
PFOS	16.0	111	4.7	80.0	107	4.8
PFNA	16.0	110	2.6	80.0	104	3.6
9Cl-PF3ONS	16.0	108	8.8	80.0	101	3.8
PFDA	16.0	111	2.4	80.0	107	3.6
NMeFOSAA	16.0	104	5.2	80.0	102	5.4
PFUnA	16.0	107	2.8	80.0	101	1.3
NEtFOSAA	16.0	97.7	6.8	80.0	101	2.5
11Cl-PF3OUdS	16.0	109	3.4	80.0	103	6.1
PFDoA	16.0	101	7.2	80.0	107	3.7
PFTrDA	16.0	108	2.6	80.0	99.1	3.6
PFTA	16.0	110	0.9	80.0	97.2	3.6
$^{13}C_2$-PFHxA	40.0	88.5	6.4	40.0	97.0	4.9
$^{13}C_3$-HFPO-DA	40.0	94.5	3.2	40.0	101	9.9
$^{13}C_2$-PFDA	40.0	99.1	3.4	40.0	106	2.7
d_5-NEtFOSAA	160	90.0	2.6	160	99.5	4.8

Table 7. Precision and Accuracy (n=4) of PFAS in Tap Water[a] from a Ground Water Source

19. Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	104	3.1	80.0	90.2	2.1
PFHxA	16.0	105	3.5	80.0	91.6	3.9
HFPO-DA	16.0	99.6	4.0	80.0	90.6	2.9
PFHpA	16.0	101	3.4	80.0	91.2	4.2
PFHxS	16.0	110.0	3.3	80.0	93.5	4.8
ADONA	16.0	104	3.9	80.0	92.2	4.7
PFOA	16.0	105	2.7	80.0	91.1	4.8
PFOS	16.0	108	3.3	80.0	93.9	3.8
PFNA	16.0	105	2.4	80.0	92.4	6.9
9Cl-PF3ONS	16.0	101	8.1	80.0	92.4	4.9
PFDA	16.0	102	4.5	80.0	92.5	7.7
NMeFOSAA	16.0	92.6	7.4	80.0	87.1	9.4
PFUnA	16.0	104	4.8	80.0	92.8	5.6
NEtFOSAA	16.0	108	18.4	80.0	94.1	6.7
11Cl-PF3OUdS	16.0	103	3.4	80.0	95.4	5.4
PFDoA	16.0	99.4	4.6	80.0	92.0	5.0
PFTrDA	16.0	98.8	4.1	80.0	93.1	5.9
PFTA	16.0	102	3.7	80.0	93.9	5.0
$^{13}C_2$-PFHxA	40.0	97.7	3.4	40.0	87.0	6.2
$^{13}C_3$-HFPO-DA	40.0	97.2	3.9	40.0	88.8	6.2
$^{13}C_2$-PFDA	40.0	97.5	5.3	40.0	86.0	10
d_5-NEtFOSAA	160	94.7	8.8	160	80.8	10

[a] TOC = 0.53 mg/L and hardness = 377 mg/L measured as calcium carbonate.

Table 8. Precision and Accuracy (n=4) Of PFAS in Tap Water[a] from a Surface Water Source

20. Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	91.6	3.8	80.0	91.9	7.1
PFHxA	16.0	92.0	5.5	80.0	99.3	4.0
HFPO-DA	16.0	88.6	1.3	80.0	102	2.2
PFHpA	16.0	95.5	3.6	80.0	101	3.3
PFHxS	16.0	99.1	2.5	80.0	102	0.9
ADONA	16.0	95.5	2.9	80.0	102	3.5
PFOA	16.0	97.9	5.2	80.0	98.8	3.9
PFOS	16.0	93.5	5.9	80.0	101	2.4
PFNA	16.0	96.4	3.4	80.0	101	2.8
9Cl-PF3ONS	16.0	93.1	4.6	80.0	102	3.3
PFDA	16.0	95.3	1.7	80.0	99.2	3.3
NMeFOSAA	16.0	99.3	7.2	80.0	94.9	4.5
PFUnA	16.0	99.8	1.7	80.0	100	4.1
NEtFOSAA	16.0	93.3	8.0	80.0	90.5	3.9
11Cl-PF3OUdS	16.0	97.6	6.7	80.0	97.5	3.1
PFDoA	16.0	88.0	1.8	80.0	97.0	2.7
PFTrDA	16.0	94.7	2.5	80.0	95.5	1.8
PFTA	16.0	94.1	5.9	80.0	97.8	3.3
$^{13}C_2$-PFHxA	40.0	86.3	2.8	40.0	90.6	4.1
$^{13}C_3$-HFPO-DA	40.0	92.9	2.4	40.0	101	1.8
$^{13}C_2$-PFDA	40.0	89.3	4.3	40.0	95.8	2.2
d_5-NEtFOSAA	160	86.5	5.4	160	83.1	4.4

[a] TOC = 2.4 mg/L and hardness = 103 mg/L measured as calcium carbonate.

Table 9. Precision and Accuracy (n=4) Of PFAS in Tap Water[a] from a Private Well

21. Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	80.0	99.7	3.1
PFHxA	80.0	96.3	2.7
HFPO-DA	80.0	94.2	4.3
PFHpA	80.0	97.4	1.9
PFHxS	80.0	99.4	4.0
ADONA	80.0	98.7	2.8
PFOA	80.0	97.2	1.5
PFOS	80.0	100	1.9
PFNA	80.0	99.4	1.3
9Cl-PF3ONS	80.0	101	2.2
PFDA	80.0	98.7	2.3
NMeFOSAA	80.0	93.2	4.6
PFUnA	80.0	98.8	1.7
NEtFOSAA	80.0	94.4	0.6
11Cl-PF3OUdS	80.0	99.8	2.5
PFDoA	80.0	99.3	1.9
PFTrDA	80.0	96.2	1.3
PFTA	80.0	97.9	1.2
$^{13}C_2$-PFHxA	40.0	89.9	2.7
$^{13}C_3$-HFPO-DA	40.0	95.7	5.3
$^{13}C_2$-PFDA	40.0	92.3	1.8
d_5-NEtFOSAA	160	86.3	4.5

[a] TOC = 0.56 mg/L and hardness = 394 mg/L measured as calcium carbonate.

Table 10. Aqueous Sample Holding Time Data for Tap Water Samples from a Surface Water Source[a], Fortified with Method Analytes and Preserved and Stored According to Section 8 (n=4)

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Recovery	Day 0 % RSD	Day 8 Mean % Recovery	Day 8 % RSD	Day 14 Mean % Recovery	Day 14 % RSD
PFBS	80.0	91.9	7.1	99.4	4.2	93.4	11
PFHxA	80.0	99.3	4.0	101	5.4	93.4	7.9
HFPO-DA	80.0	102	2.2	101	5.3	100	11
PFHpA	80.0	101	3.3	99.2	2.2	101	3.6
PFHxS	80.0	102	0.9	103	4.0	107	4.5
ADONA	80.0	102	3.5	102	4.7	101	4.4
PFOA	80.0	98.8	3.9	99.8	0.63	100	3.5
PFOS	80.0	101	2.4	101	3.6	106	6.8
PFNA	80.0	101	2.8	101	0.87	105	4.8
9Cl-PF3ONS	80.0	102	3.3	100	2.2	102	4.4
PFDA	80.0	99.2	3.3	99.6	1.6	102	5.5
NMeFOSAA	80.0	94.9	4.5	98.0	3.5	95.4	7.3
PFUnA	80.0	100	4.1	101	4.4	100	6.2
NEtFOSAA	80.0	90.5	3.9	102	5.3	96.5	7.7
11Cl-PF3OUdS	80.0	97.5	3.1	101	4.5	102	5.5
PFDoA	80.0	97.0	2.7	98.4	3.5	103	3.8
PFTrDA	80.0	95.5	1.8	99.5	3.2	99.4	3.8
PFTA	80.0	97.8	3.3	102	3.2	96.2	2.1
$^{13}C_2$-PFHxA	40.0	90.6	4.1	93.6	5.5	93.0	8.8
$^{13}C_3$-HFPO-DA	40.0	101	1.8	101	3.1	91.5	12
$^{13}C_2$-PFDA	40.0	95.8	2.2	92.6	6.8	104	2.8
d_5-NEtFOSAA	160	83.1	4.4	87.6	2.6	95.2	4.3

[a] TOC = 2.4 mg/L and hardness = 103 mg/L measured as calcium carbonate.

Table 11. Extract Holding Time Data for Tap Water Samples from a Surface Water Source, Fortified with Method Analytes and Preserved and Stored According to Section 8 (n=4)

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Recovery	Day 0 % RSD	Day 8 Mean % Recovery	Day 8 % RSD	Day 14 Mean % Recovery	Day 14 % RSD	Day 28 Mean % Recovery	Day 28 % RSD
PFBS	80.0	91.9	7.1	96.9	5.1	90.6	10	99.4	5.3
PFHxA	80.0	99.3	4.0	10	1.3	94.1	2.9	105	2.6
HFPO-DA	80.0	102	2.2	103	1.4	98.7	2.6	103	1.1
PFHpA	80.0	101	3.3	102	2.9	98.3	1.0	104	3.5
PFHxS	80.0	102	0.9	105	2.9	99.7	1.8	107	2.5
ADONA	80.0	102	3.5	104	3.7	98.6	2.5	106	2.5
PFOA	80.0	98.8	3.9	106	3.7	101	1.8	106	2.8
PFOS	80.0	101	2.4	102	1.1	103	1.8	109	2.2
PFNA	80.0	101	2.8	105	1.8	103	2.3	107	2.4
9Cl-PF3ONS	80.0	102	3.3	99.4	3.1	97.6	2.9	107	2.2
PFDA	80.0	99.2	3.3	104	1.9	101.2	0.9	107	3.4
NMeFOSAA	80.0	94.9	4.5	101	3.9	90.5	5.2	105	6.8
PFUnA	80.0	100	4.1	104	5.5	102	4.2	106	3.0
NEtFOSAA	80.0	90.5	3.9	104	3.1	93.6	7.7	102	2.9
11Cl-PF3OUdS	80.0	97.5	3.1	103	1.9	97.3	1.6	108	2.7
PFDoA	80.0	97.0	2.7	102	3.7	99.8	3.3	106	2.6
PFTrDA	80.0	95.5	1.8	102	3.0	97.2	1.6	104	3.1
PFTA	80.0	97.8	3.3	105	4.2	98.8	2.1	108	2.5
$^{13}C_2$-PFHxA	40.0	90.6	4.1	101	1.2	101	2.6	114	2.1
$^{13}C_3$-HFPO-DA	40.0	101	1.8	95.5	3.2	96.5	2.7	111	2.5
$^{13}C_2$-PFDA	40.0	95.8	2.2	100	2.7	109	1.9	124	4.4
d_5-NEtFOSAA	160	83.1	4.4	94.7	1.6	91.4	4.8	113	9.1

Table 12. Initial Demonstration of Capability Quality Control Requirements

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.2	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all method analytes are below 1/3 the MRL and that possible interferences from extraction media do not prevent the identification and quantification of method analytes.
Sect. 9.2.3	Initial Demonstration of Precision (IDP)	Analyze four to seven replicate LFBs fortified near the midrange calibration concentration.	%RSD must be <20%
Sect. 9.2.4	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP.	Mean recovery ± 30% of true value
Sect. 9.2.5	Initial Demonstration of Peak Asymmetry Factor	Calculate the peak asymmetry factor using the equation in Section 9.3.9 for the first two eluting chromatographic peaks in a mid-level CAL standard.	Peak asymmetry factor of 0.8 - 1.5
Sect. 9.2.6	Minimum Reporting Limit (MRL) Confirmation	Fortify, extract and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the Mean and the Half Range (HR). Confirm that the upper and lower limits for the Prediction Interval of Result (Upper PIR, and Lower PIR, Sect. 9.2.6.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Sect. 9.2.7 and 9.3.10	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of IDC.	Results must be within 70-130% of true value.
Sect. 9.2.8	Detection Limit (DL) Determination (optional)	Over a period of three days, prepare a minimum of seven replicate LFBs fortified at a concentration estimated to be near the DL. Analyze the replicates through all steps of the analysis. Calculate the DL using the equation in Sect. 9.2.8.1.	Data from DL replicates are not required to meet method precision and accuracy criteria. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

NOTE: Table 12 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Section 9 supersedes any missing or conflicting information in this table.

Table 13. Ongoing Quality Control Requirements (Summary)

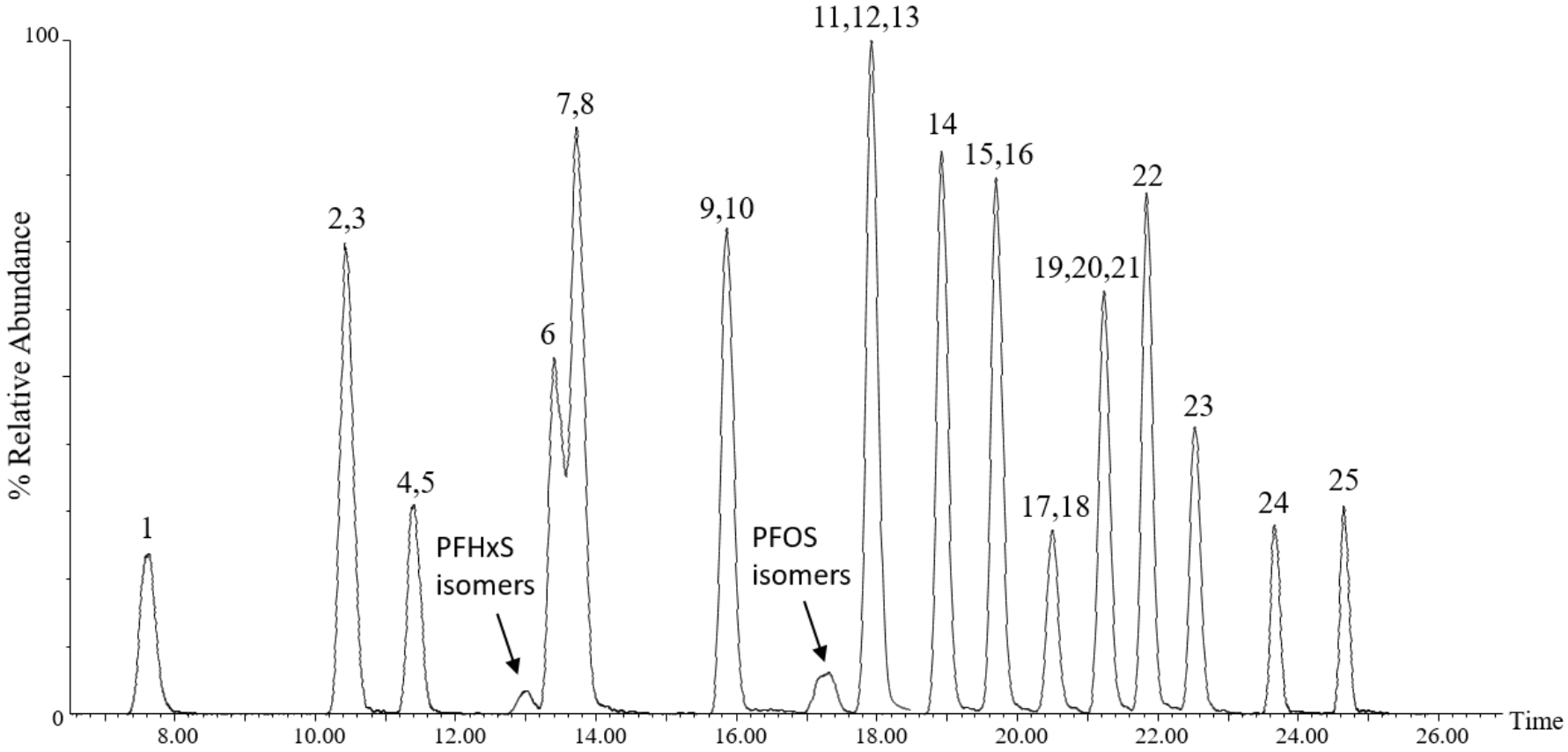
Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.5	Sample Holding Time	14 days with appropriate preservation and storage as described in Sections 8.1-8.5.	Sample results are valid only if samples are extracted within the sample holding time.
Sect. 8.5	Extract Holding Time	28 days when stored at room temperature in polypropylene centrifuge tubes.	Extract results are valid only if extracts are analyzed within the extract holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each extraction batch of up to 20 samples.	Demonstrate that all method analytes are below 1/3 the MRL and confirm that possible interferences do not prevent quantification of method analytes. If targets exceed 1/3 the MRL or if interferences are present, results for these subject analytes in the extraction batch are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	One LFB is required for each extraction batch of up to 20 Field Samples. Rotate the fortified concentrations between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each method analyte for all fortified concentrations except the lowest CAL point. Results of the LFBs corresponding to the lowest CAL point for each method analyte must be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standards, $^{13}C_2$-PFOA (IS#1), $^{13}C_4$-PFOS (IS#2), and d_3-NMeFOSAA (IS#3), are added to all standards and sample extracts, including QC samples. Compare IS areas to the average IS area in the initial calibration and to the most recent CCC.	Peak area counts for all ISs in all injections must be within ± 50% of the average peak area calculated during the initial calibration and 70-140% from the most recent CCC. If ISs do not meet this criterion, corresponding target results are invalid.
Sect. 9.3.5	Surrogate Standards (SUR)	Surrogate standards, $^{13}C_2$-PFHxA, $^{13}C_3$-HFPO-DA, $^{13}C_2$-PFDA, and d_5-NEtFOSAA, are added to all CAL standards and samples, including QC samples. Calculate SUR recoveries.	SUR recoveries must be 70-130% of the true value. If a SUR fails this criterion, report all results for sample as suspect/SUR recovery.

Table 13. (Continued)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.6	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration, if known. Calculate LFSM recoveries.	Recoveries at mid and high levels must be within 70-130% and within 50-150% at the low-level fortified amount (near the MRL). If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Extract and analyze at least one FD or LFSMD with each extraction batch (20 samples or less). A LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	Method analyte RPDs for the LFMD or FD must be ≤30% at mid and high levels of fortification and ≤50% near the MRL. If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.8	Field Reagent Blank (FRB)	Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample.	If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.
Sect. 9.3.9	Peak Asymmetry Factor	Calculate the peak asymmetry factor for the first two eluting chromatographic peaks in a mid-level CAL standard during IDC and when chromatographic changes are made that affect peak shape.	Peak asymmetry factor of 0.8 - 1.5
Sect. 9.3.10	Quality Control Sample (QCS)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Results must be within 70-130% of true value.
Sect. 10.2 and Sect. 9.3.2	Initial Calibration	Use IS calibration technique to generate a first or second order calibration curve forced through zero. Use at least five standard concentrations. Check the calibration curve as described in Sect. 10.2.4.4.	When each CAL standard is calculated as an unknown using the calibration curve, the analyte and SUR results must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value. Recalibration is recommended if these criteria are not met.
Sect. 9.3.2 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte and SUR must be within 70-130% of the true value for all but the lowest level of calibration. Recovery for each analyte in the lowest CAL level CCC must be within 50-150% of the true value and the SUR must be within 70-130% of the true value.

NOTE: Table 13 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Sections 8-10 supersedes any missing or conflicting information in this table.

Figure 1. Example Chromatogram for Reagent Water Fortified with Method 537.1 Analytes at 80 ng/L. Numbered Peaks are Identified in Table 3



Attachment 11

Designation: D7979 – 20

Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D7979; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This procedure covers the determination of selected per- and polyfluoroalkyl substances (PFASs) in a water matrix using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). These analytes are qualitatively and quantitatively determined by this test method. This test method adheres to a technique known as selected reaction monitoring (SRM) or sometimes referred to as multiple reaction monitoring (MRM). This is not a drinking water method; performance of this test method has not been evaluated on drinking water matrices.

1.2 The method detection limit (MDL)[2] and reporting range[3] for the target analytes are listed in Table 1. The target concentration for the reporting limit for this test method was 10 ng/L for most of the target analytes at the time of development.

1.2.1 The reporting limit in this test method is the minimum value below which data are documented as non-detects. The reporting limit may be lowered providing your lab meets the minimum performance requirements of this test method at the lower concentrations, this test method is performance based and modifications are allowed to improve performance. Analyte detections between the method detection limit and the reporting limit are estimated concentrations and are not reported following this test method. In most cases, the reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for the PFASs after taking into account the 50 % dilution with methanol. It is above the Level 1 calibration concentration for FHEA and FOEA, these compounds can be identified at the Level 1 concentration but the standard deviation among replicates at this lower spike level resulted in a higher reporting limit.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents
D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] The MDL is determined following the Code of Federal Regulations (CFR) , 40 CFR Part 136, Appendix B utilizing dilution and filtration. Five-mL sample of water was utilized. A detailed process determining the MDL is explained in the reference and is beyond the scope of this test method to be explained here.

[3] Reporting range concentration is calculated from Table 4 concentrations assuming a 30-µL injection of the Level 1 calibration standard for PFASs, and the highest level calibration standard with a 10-mL final extract volume of a 5-mL water sample. Volume variations will change the reporting limit and ranges.

[4] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

TABLE 1 Method Detection Limit and Reporting Range

Analyte[A]	MDL (ng/L)	Reporting Ranges (ng/L)
PFTreA[B]	1.2	10 – 400
PFTriA[B]	0.7	10 – 400
PFDoA[B]	1.2	10 – 400
PFUnA[B]	1.2	10 – 400
PFDA[B]	1.4	10 – 400
PFOS[B]	2.2	10 – 400
PFNA[B]	1.1	10 – 400
PFecHS[B]	1.9	10 – 400
PFOA[B]	1.7	10 – 400
PFHxS[B]	1.2	10 – 400
PFHpA[B]	1.0	10 – 400
PFHxA[B]	2.0	10 – 400
PFBS[B]	0.8	10 – 400
PFPeA[B]	4.6	50 – 2000
PFBA[B]	4.6	50 – 2000
FHEA	92.9	300 – 8000
FOEA	106.8	300 – 8000
FDEA	47.2	200 – 8000
FOUEA	2.3	10 – 400
FHpPA	3.3	10 – 400
FHUEA	1.5	10 – 400

[A] Acronyms are defined in 3.3.
[B] New MDL study was reported in August 2016, which resulted in a reporting limit and range update.

2.2 *Other Standards:*[5]
EPA Publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods
Code of Federal Regulations 40 CFR Part 136, Appendix B

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *per- and polyfluoroalkyl substances, n—in this test method*, 11 perfluoroalkyl carboxylic acids, 3 perfluoroalkylsulfonates, Decafluoro-4-(pentafluoroethyl)cyclohexanesulfonate and 6 fluorotelomer acids listed in Table 1 collectively (not including any mass labeled surrogates).

3.2.2 *reporting limit, n*—the minimum concentration below which data are documented as non-detects.

3.3 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *FTAs and FTUAs, n*—Fluorotelomer and Unsaturated Fluorotelomer Acids

3.3.2.1 *FDEA, n*—2-perfluorodecyl ethanoic acid

3.3.2.2 *FHEA, n*—2-perfluorohexyl ethanoic acid

3.3.2.3 *FHpPA, n*—3-perfluoroheptyl propanoic acid

3.3.2.4 *FHUEA, n*—2H-perfluoro-2-octenoic acid

3.3.2.5 *FOEA, n*—2-perfluorooctyl ethanoic acid

3.3.2.6 *FOUEA, n*—2H-perfluoro-2-decenoic acid

3.3.3 *IC, n*—Initial Calibration

3.3.4 *LC, n*—Liquid Chromatography

3.3.5 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.6 *MDL, n*—Method Detection Limit

3.3.7 *MeOH, n*—Methanol

3.3.8 *mM, n*—millimolar, 1×10^{-3} moles/L

3.3.9 *MPFAC, n*—Isotopically labeled Perfluoroalkylcarboxylates

3.3.9.1 *MPFBA, n*—$^{13}C_4$-Perfluorobutanoate

3.3.9.2 *MPFDA, n*—$^{13}C_2$-Perfluorodecanoate

3.3.9.3 *MPFDoA, n*—$^{13}C_2$-Perfluorododecanoate

3.3.9.4 *MPFHxA, n*—$^{13}C_2$-Perfluorohexanoate

3.3.9.5 *MPFNA, n*—$^{13}C_5$-Perfluorononanoate

3.3.9.6 *MPFOA, n*—$^{13}C_4$-Perfluorooctanoate

3.3.9.7 *MPFUnA, n*—$^{13}C_2$-Perfluoroundecanoate

3.3.10 *MPFAlS, n*—Isotopically labeled Perfluoroalkylsulfonates

3.3.10.1 *MPFHxS, n*—$^{18}O_2$-Perfluorohexylsulfonate

3.3.10.2 *MPFOS, n*—$^{13}C_4$-Perfluorooctylsulfonate

3.3.11 *MRM, n*—Multiple Reaction Monitoring

3.3.12 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.13 *NA, adj*—Not Available

3.3.14 *ND, n*—non-detect

3.3.15 *P&A, n*—Precision and Accuracy

3.3.16 *PFAC, n*—Perfluoroalkyl Carboxylic Acid

3.3.16.1 *PFBA, n*—Perfluorobutanoate

3.3.16.2 *PFDA, n*—Perfluorodecanoate

3.3.16.3 *PFDoA, n*—Perfluorododecanoate

3.3.16.4 *PFHpA, n*—Perfluoroheptanoate

3.3.16.5 *PFHxA, n*—Perfluorohexanoate

3.3.16.6 *PFNA, n*—Perfluorononanoate

3.3.16.7 *PFOA, n*—Perfluorooctanoate

3.3.16.8 *PFPeA, n*—Perfluoropentanoate

3.3.16.9 *PFTreA, n*—Perfluorotetradecanoate

3.3.16.10 *PFTriA, n*—Perfluorotridecanoate

3.3.16.11 *PFUnA, n*—Perfluoroundecanoate

3.3.17 *PFAlS, n*—Perfluoroalkylsulfonate

3.3.17.1 *PFBS, n*—Perfluorobutylsulfonate

3.3.17.2 *PFecHS, n*—Decafluoro-4-(pentafluoroethyl) cyclohexanesulfonate

3.3.17.3 *PFHxS, n*—Perfluorohexylsulfonate

3.3.17.4 *PFOS, n*—Perfluorooctylsulfonate

3.3.18 *PFASs, n*—Per- and Polyfluoroalkyl Substances

3.3.19 *ppt, n*—parts per trillion, ng/L

3.3.20 *QA, adj*—Quality-Assurance

3.3.21 *QC, adj*—Quality-Control

[5] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

3.3.22 *RL, n*—Reporting Limit

3.3.23 *RLCS, n*—Reporting Limit Check Sample

3.3.24 *RSD, n*—Relative Standard Deviation

3.3.25 *RT, n*—Retention Time

3.3.26 *SRM, n*—Selected Reaction Monitoring

3.3.27 *SS, n*—Surrogate Standard

3.3.28 *TC, n*—Target Compound

4. Summary of Test Method

4.1 The operating conditions presented in this test method have been successfully used in the determination of PFASs in water; however, this test method is intended to be performance based and alternative operating conditions can be used to perform this test method provided data quality objectives are attained.

4.2 For PFASs analysis, samples are shipped to the lab at a temperature between 0°C and 6°C and analyzed within 28 days of collection. A sample (5 mL) is collected in a polypropylene tube in the field and that total sample is processed in order to limit target analyte loss due to sample manipulation and losses to surfaces, spiked with surrogates (all samples) and target PFASs (laboratory control and matrix spike samples) and hand shaken for 2 minutes after adding 5 mL of methanol. The samples are then filtered through a polypropylene filter unit. Acetic acid (~10 µL) is added to all the samples to adjust to pH ~3 and analyzed by LC/MS/MS. For 5-mL sludge samples; 5 mL methanol is added, adjusted to pH ~9 (adding ~20 µL of ammonium hydroxide), hand shaken, filtered, acidified to pH ~3 (~50 µL acetic acid), and then analyzed by LC/MS/MS.

NOTE 1—Sludge in this test method is defined as sewage sample containing between 0.1 and 2 % solids based upon a sample by weight.

NOTE 2—Since surface binding of target compounds may bias data, it is best to collect a 5.0-mL sample in a graduated 15-mL polypropylene BD Falcon tube in the field so that the whole sample is processed in the lab (NO ALIQUOTING). Once this 5.0-mL sample is spiked according to this test method and methanol is added, it is then thoroughly shaken and transferred to a new 15-mL polypropylene tube during filtration. In order to have accurate volumes, the weight of the 15-mL polypropylene BD Falcon tube may be taken before and after sampling in order to obtain an exact volume. The density of water is assumed to be 1.0 g/mL unless the exact density of the water sample is known, then that conversion should be used.

4.3 Most of the PFASs are identified by comparing the SRM transition and its confirmatory SRM transition if correlated to the known standard SRM transition (Table 3) and quantitated utilizing an external calibration. The surrogates and some PFASs (PFPeA, PFBA, FOUEA, and FHUEA) only utilize one SRM transition due to a less sensitive or non-existent secondary SRM transition. As an additional quality-control measure, isotopically labeled PFASs surrogates (listed in 12.4) recoveries are monitored. There is no correction to the data based upon surrogate recoveries. The final report issued for each sample lists the concentration of PFASs, if detected, or as a non-detect at the RL, if not detected, in ng/L and the surrogate recoveries.

5. Significance and Use

5.1 PFASs are widely used in various industrial and commercial products; they are persistent, bio-accumulative, and ubiquitous in the environment. PFASs have been reported to exhibit developmental toxicity, hepatotoxicity, immunotoxicity, and hormone disturbance. A draft Toxicological Profile for Perfluoroalkyls from the U.S. Department of Health and Human Services is available.[6] PFASs have been detected in soils, sludges, surface, and drinking waters. Hence, there is a need for quick, easy, and robust method to determine these compounds at trace levels in water matrices for understanding of the sources and pathways of exposure.

5.2 This test method has been investigated for use with reagent, surface, sludge and wastewaters for selected PFASs. This test method has not been evaluated on drinking water matrices.

6. Interferences

6.1 All glassware is washed in hot water (typically >45ºC) with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven (typically at 105ºC) for 15 to 30 minutes. All glassware is subsequently rinsed with methanol or acetonitrile.

6.2 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems. The use of PFASs containing caps shall be avoided.

6.3 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably depending on variations of the sample matrices.

6.4 Contaminants have been found in reagents, glassware, tubing, glass disposable pipettes, filters, degassers, and other apparatus that release PFASs. All of these materials and supplies are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples. If found, measures should be taken to remove the contamination or data should be qualified, background subtraction of blank contamination is not allowed.

6.5 The LC system used should consist, as much as practical, of sample solution or eluent contacting components free of PFASs of interest.

6.6 Polyethylene LC vial caps or any other target analyte free vial caps should be used.

6.7 Polyethylene disposable pipettes or target analyte free pipettes should be used. All disposable pipettes should be checked for release of target analytes of interest.

6.8 Degassers are important to continuous LC operation and most commonly are made of fluorinated polymers. To enable use, an isolator column should be placed after the degasser and prior to the sample injection valve to separate the PFASs in the sample from the PFASs in the LC system.

[6] A Draft Toxicological Profile for Perfluoroalkyls can be found at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237 (2014).

7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography System*[7]—A complete LC system is required in order to analyze samples, this should include a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. This test method used a ternary pumping system. At a minimum, a binary pumping system may be used but the LC conditions in Table 2 must be adjusted to account for a binary system. A LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be used.

7.1.2 *Analytical Column*[8]—A reverse phase Charged Surface Hybrid Phenyl-Hexyl particle column was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and needs to be monitored.

7.1.3 *Isolator Column*[9]—A reverse phase C18 column was used in this test method to separate the target analytes in the LC system and solvents from the target analytes in the analytical sample. This column was placed between the solvent mixing chamber and the injector sample loop.

7.2 *Tandem Mass Spectrometer System*[10]—A MS/MS system capable of multiple reaction monitoring (MRM) analysis or any system that is capable of performing at the requirements in this test method shall be used.

7.3 *Filtration Device:*

7.3.1 *Hypodermic Syringe*—A luer-lock tip glass syringe capable of holding a syringe driven filter unit.

7.3.2 A 10-mL Lock Tip Glass Syringe size is recommended in this test method.

7.3.3 *Filter Unit*[11]—Polypropylene filter units were used to filter the samples.

8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[12] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D1193. It shall be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 *Vials*—2-mL amber glass autosampler vials or equivalent.

8.5 *Polyethylene autosampler vial caps*, or equivalent.

8.6 *Syringe*—10 or 25-mL filter-adaptable glass syringe with luer lock.

8.7 *Polypropylene Tubes*—15 and 50 mL.

8.8 *pH Paper* (pH range 1–14).

8.9 *Class A Volumetric Glassware.*

8.10 *Pipette tips*—Polypropylene pipette tips free of release agents or low retention coating of various sizes.

8.11 *Polyethylene Disposable Pipettes.*

8.12 *Acetonitrile* (CAS #75-05-8).

8.13 *Methanol* (CAS #67-56-1).

8.14 *Ammonium Acetate* (CAS #631-61-8).

8.15 *Acetic Acid* (CAS #64-19-7).

8.16 *2-Propanol* (isopropyl alcohol, CAS #67-63-0).

8.17 *Ammonium hydroxide* (CAS #1336-21-6).

8.18 *PFASs Standards:*[13]

8.18.1 *Perfluorobutylsulfonate* (PFBS, CAS #29420-49-3).

8.18.2 *Perfluorohexylsulfonate* (PFHxS, CAS #3871-99-6).

8.18.3 *Perfluorooctylsulfonate* (PFOS, CAS #1763-23-1).

8.18.4 *Perfluorobutanoate* (PFBA, CAS #375-22-4).

[7] A Waters Acquity UPLC H-Class System, or equivalent, has been found suitable for use.

[8] A Waters Acquity UPLC CSH Phenyl-Hexyl, 2.1 × 100 mm and 1.7 µm particle size column, or equivalent, has been found suitable for use. It was used to develop this test method and generate the precision and bias data presented in Section 16.

[9] A Waters Acquity UPLC BEH C18, 2.1 × 50 mm and 1.7 µm particle size column, or equivalent, has been found suitable for use. Note: If back pressure is high, a larger particle size may be used (3–3.5 µm).

[10] A Waters Xevo TQ-S triple quadrupole mass spectrometer, or equivalent, has been found suitable for use.

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (mL/min)	95 % Water: 5 % Acetonitrile %	Acetonitrile %	95 % Water: 5 % Acetonitrile, 400 mM Ammonium Acetate %
0	0.3	95	0	5
1	0.3	75	20	5
6	0.3	50	45	5
13	0.3	15	80	5
14	0.4	0	95	5
17	0.4	0	95	5
18	0.4	95	0	5
21	0.4	95	0	5

[11] An Acrodisc GxF/0.2 µm GHP membrane syringe driven filter unit, or equivalent, has been found suitable for use.

[12] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[13] PFASs standards may be difficult to find, some sources of PFASs standards that have been found suitable for use were from Aldrich Chemical Company, Wellington Laboratories Inc., and Wako Laboratory. Standards from other vendors may be used.

TABLE 3 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Chemical	Primary/ Confirmatory	Retention Times (min)	Cone (V)	Collision (eV)	MRM Transition	Primary/ Confirmatory SRM Area Ratio
PFTreA	Primary	10.63	20	13	712.9→668.9	7.4
	Confirmatory		20	30	712.9→169	
PFTriA	Primary	10.17	25	12	662.9→618.9	7.4
	Confirmatory		25	28	662.9→169	
PFDoA	Primary	9.61	10	12	612.9→568.9	8.2
	Confirmatory		10	25	612.9→169	
PFUnA	Primary	9.05	15	10	562.9→519	7.2
	Confirmatory		15	18	562.9→269	
PFDA	Primary	8.45	20	10	512.9→468.9	6.5
	Confirmatory		20	16	512.9→219	
PFOS	Primary	8.78	10	42	498.9→80.1	1.3
	Confirmatory		10	40	498.9→99.1	
PFNA	Primary	7.78	20	10	462.9→418.9	4.9
	Confirmatory		20	16	462.9→219	
PFecHS	Primary	8.1	10	25	460.9→381	2.2
	Confirmatory		10	25	460.9→99.1	
PFOA	Primary	7.11	20	10	412.9→369	3.6
	Confirmatory		20	16	412.9→169	
PFHxS	Primary	7.39	15	32	398.9→80.1	1
	Confirmatory		15	32	398.9→99.1	
PFHpA	Primary	6.35	15	10	362.9→319	4.1
	Confirmatory		15	15	362.9→169	
PFHxA	Primary	5.54	15	8	312.9→269	24.1
	Confirmatory		15	18	312.9→119.1	
PFBS	Primary	5.66	10	30	298.9→80.1	1.6
	Confirmatory		10	25	298.9→99.1	
PFPeA	Primary	4.68	10	8	263→219	NA
PFBA	Primary	3.67	10	8	212.9→169	NA
FHEA	Primary	6.14	15	20	376.9→293	3.6
	Confirmatory		15	6	376.9→313	
FOEA	Primary	7.54	15	18	476.9→393	4.3
	Confirmatory		15	12	476.9→413	
FDEA	Primary	8.83	15	8	576.8→493	3.2
	Confirmatory		15	15	576.8→513	
FOUEA	Primary	7.54	20	12	456.9→392.9	NA
FHpPA	Primary	7.54	15	12	440.9→337	1.1
	Confirmatory		15	20	440.9→317	
FHUEA	Primary	6.08	10	12	357→293	NA
MPFBA	Primary	3.67	10	7	217→172.1	NA
MPFHxA	Primary	5.54	15	8	315→270	NA
MPFHxS	Primary	7.39	15	34	402.9→84.1	NA
MPFOA	Primary	7.11	15	10	417→372	NA
MPFNA	Primary	7.81	15	9	467.9→423	NA
MPFOS	Primary	8.78	15	40	502.9→80.1	NA
MPFDA	Primary	8.45	15	10	514.9→470	NA
MPFUnA	Primary	9.05	15	10	564.9→519.9	NA
MPFDoA	Primary	9.61	15	12	614.9→569.9	NA

TABLE 4 Concentrations of Calibration Standards (ng/L)

Analyte/Surrogate	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8	LV9
PFPeA, PFBA	25	50	100	200	300	400	500	750	1000
PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, MPFBS, MPFHxA, MPFUnA, MPFOA, MPFDA, MPFOS, MPFNA, MPFHxS, MPFBA	5	10	20	40	60	80	100	150	200
FHEA, FOEA, FDEA	100	200	400	800	1200	1600	2000	3000	4000

8.18.5 *Perfluoropentanoate* (PFPeA, CAS #2706-90-3).
8.18.6 *Perfluorohexanoate* (PFHxA, CAS #307-24-4).
8.18.7 *Perfluoroheptanoate* (PFHpA, CAS #375-85-9).
8.18.8 *Perfluorooctanoate* (PFOA, CAS #335-67-1).
8.18.9 *Perfluorononanoate* (PFNA, CAS #375-95-1).
8.18.10 *Perfluorodecanoate* (PFDA, CAS #335-76-2).
8.18.11 *Perfluoroundecanoate* (PFUnA, CAS #2058-94-8).
8.18.12 *Perfluorododecanoate* (PFDoA, CAS #307-55-1).
8.18.13 *Perfluorotridecanoate* (PFTriA, CAS #72629-94-8).

8.18.14 *Perfluorotetradecanoate* (PFTreA, CAS #376-06-7).

8.18.15 *Decafluoro-4-(pentafluoroethyl)cyclohexanesulfonate* (PFecHS, CAS #67584-42-3).

8.18.16 *3-perfluoropheptyl propanoic acid* (FHpPA, CAS #812-70-4).

8.18.17 *2H-perfluoro-2-decenoic acid* (FOUEA, CAS #70887-84-2).

8.18.18 *2-perfluorodecyl ethanoic acid* (FDEA, CAS # not available).

8.18.19 *2-perfluorooctyl ethanoic acid* (FOEA, CAS #27854-31-5).

8.18.20 *2H-perfluoro-2-octenoic acid* (FHUEA, CAS # not available).

8.18.21 *2-perfluorohexyl ethanoic acid* (FHEA, CAS #53826-12-3).

8.19 *PFAS Surrogates:*[14]

8.19.1 $^{18}O_2$*-Perfluorohexylsulfonate* (MPFHxS).

8.19.2 $^{13}C_4$*-Perfluorooctylsulfonate* (MPFOS).

8.19.3 $^{13}C_4$*-Perfluorobutanoate* (MPFBA).

8.19.4 $^{13}C_2$*-Perfluorohexanoate* (MPFHxA).

8.19.5 $^{13}C_4$*-Perfluorooctanoate* (MPFOA).

8.19.6 $^{13}C_5$*-Perfluorononanoate* (MPFNA).

8.19.7 $^{13}C_2$*-Perfluorodecanoate* (MPFDA).

8.19.8 $^{13}C_2$*-Perfluoroundecanoate* (MPFUnA).

8.19.9 $^{13}C_2$*-Perfluorododecanoate* (MPFDoA).

9. Hazards

9.1 Normal laboratory safety applies to this test method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Safety Data Sheets (SDS) for all reagents used in this test method.

10. Sampling

10.1 *Sampling and Preservation*—Grab samples are collected in polypropylene containers. Sample containers and contact surfaces with PTFE shall be avoided. As part of the overall quality-assurance program for this test method, field blanks exposed to the same field conditions as samples are collected and analyzed according to this test method to assess the potential for field contamination. Surface binding may bias data. This test method is based on a 5-mL sample size per analysis. If different sample sizes are used, spiking solution amounts may need to be modified. Conventional sampling practices should be followed with the caution that PFASs containing products may be present in sampling equipment. All sampling equipment and supplies shall be PFASs free in order to prevent contamination of the samples. EPA Publication SW-846, Guide D3856, and Practices D3694 may be used as guides. Samples shall be shipped on ice with a trip blank. Once received the sample temperature is taken and should be less than 6°C. If the receiving temperature is greater than 6°C, the sample temperature is noted in the case narrative accompanying the data. Samples should be stored refrigerated between 0°C and 6°C from the time of collection until analysis. Analyze the sample within 28 days of collection. No in-depth holding time study has been done on the different water matrices tested in this test method. A holding time study was done on sewage treatment plant influent over 31 days and showed all concentrations over the time period to be within the performance of the test method. This study used the complete sample, NO ALIQUOTING. Another study, where aliquots of sample were taken, resulted in large losses for many of the target analytes. Holding time may vary depending on the matrix and individual laboratories should determine the holding time in their matrix.[15]

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*

11.1.1 Injections of all standards and samples are made at a 30-µL volume. Other injection volumes may be used to optimize conditions. Standards and samples shall be in a 50:50 methanol:water solution containing 0.1 % acetic acid. In the case of extreme concentration differences amongst samples, it is wise to analyze a blank after a concentrated sample and before a dilute sample to eliminate carryover of analytes from sample injection to sample injection. The gradient conditions for LC are shown in Table 2.

11.2 *LC Sample Manager Conditions:*

11.2.1 *Needle Wash Solvent*—60 % acetonitrile/40 % 2-propanol. Eight second wash time before and after injection. Instrument manufacturer's specifications should be followed in order to eliminate sample carry-over.

11.2.2 *Temperatures*—Column, 35°C; Sample compartment, 15°C.

11.2.3 *Seal Wash*—Solvent: 60 % acetonitrile/40 % 2-propanol; Time: 5 minutes.

11.3 *Mass Spectrometer Parameters:*

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This test method contains nine surrogates, which are select isotopically labeled PFASs, and 21 PFASs which were split up into eighteen MRM acquisition functions to optimize sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this test method are listed below:

The instrument is set in the Electrospray negative source setting.
Capillary Voltage: 0.75 kV
Cone: Variable depending on analyte
Source Temperature: 150°C
Desolvation Gas Temperature: 450°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 200 L/hr
Collision Gas Flow: 0.15 mL/min
Low Mass Resolution 1: 2.6
High Mass Resolution 1: 14
Ion Energy 1: 1
Entrance Energy: 1
Collision Energy: Variable depending on analyte

[14] PFAS surrogates from Wellington Laboratories Inc. or equivalent, have been found suitable for use.

[15] Guides to help determine holding times can be found at: http://www.epa.gov/esd/cmb/research/bs_033cmb06.pdf (2014) and Practice D4841.

Exit Energy: 1
Low Mass Resolution 2: 2.5
High Mass Resolution 2: 14
Ion Energy 2: 3
Gain: 1.0
Multiplier: 511.1
Inter-Scan Delay: 0.004 seconds

12. Calibration and Standardization

12.1 The mass spectrometer shall be calibrated as in accordance with manufacturer's specifications before analysis. Analytical values satisfying test method criteria have been achieved using the following procedures. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze nine calibration standards containing the PFASs and surrogates prior to analysis as shown in Table 4. Calibration stock standard solution is prepared from the target and surrogate spike solutions directly to ensure consistency. Stock standard Solution A containing the PFASs and surrogates is prepared at Level 9 concentration and aliquots of that solution are diluted to prepare Levels 1 through 8. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations. At a minimum, five calibration levels are required when using a linear calibration curve and six calibration levels are required when using a quadratic calibration curve. An initial nine-point curve may be used to allow for the dropping of the lower calibration points if the individual laboratory's instrument can't achieve low detection limits on certain PFASs. This should allow for at least a five or six-point calibration curve to be obtained. No problems were encountered while using the nine-point calibration curve in developing this test method.

12.2.1 Calibration Stock Standard Solution A (Level 9, Table 4) is prepared from the target and surrogate spike solutions directly to ensure consistency. 500 µL of the surrogate spike (20 µg/L), 500 µL of PFASs Target Spike I and 500 µL of PFASs Target Spike II (refer to Table 6) is added to a 50-mL volumetric flask and diluted to 50-mL volume with 50:50 methanol:water containing 0.1 % acetic acid. The preparation of the Level 9 standard can be accomplished using appropriate volumes and concentrations of stock solutions as in accordance with a particular laboratory's standard procedure. It is critical to ensure that the analytes are solubilized in the Level 9 standard.

12.2.2 Aliquots of Solution A are then diluted with 50:50 methanol:water containing 0.1 % acetic acid to prepare the desired calibration levels in 2-mL amber glass LC vials. The calibration vials shall be used within 24 hours to ensure optimum results. The end calibration check shall be prepared in a separate LC vial near the mid-level. All calibration standards

TABLE 5 QC Acceptance Criteria

NOTE 1—Table 5 data is preliminary until a multi-lab validation study is completed.

Analyte/Surrogate	Spike Conc. ng/L	Initial Demonstration of Performance			Laboratory Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Control Limit (LCL) %	Upper Control Limit (UCL) %
PFTreA	160	70	130	30	70	130
PFTriA	160	70	130	30	70	130
PFDoA	160	70	130	30	70	130
PFUnA	160	70	130	30	70	130
PFDA	160	70	130	30	70	130
PFOS	160	70	130	30	70	130
PFNA	160	70	130	30	70	130
PFecHS	160	70	130	30	70	130
PFOA	160	70	130	30	70	130
PFHxS	160	70	130	30	70	130
PFHpA	160	50	130	30	50	130
PFHxA	160	50	130	30	50	130
PFBS	160	70	130	30	70	130
PFPeA	800	70	130	30	70	130
PFBA	800	50	130	30	50	130
FHEA	3200	70	130	30	70	130
FOEA	3200	70	130	30	70	130
FDEA	3200	70	130	30	70	130
FOUEA	160	70	130	30	70	130
FHpPA	160	70	130	30	70	130
FHUEA	160	70	130	30	70	130
MPFBA	160	70	130	30	70	130
MPFHxA	160	70	130	30	70	130
MPFHxS	160	70	130	30	70	130
MPFOA	160	70	130	30	70	130
MPFNA	160	70	130	30	70	130
MPFOS	160	70	130	30	70	130
MPFDA	160	70	130	30	70	130
MPFUnA	160	70	130	30	70	130
MPFDoA	160	70	130	30	70	130

TABLE 6 PFASs Target Spike Solutions (PPB)

Analyte	Concentration of Analyte in PFASs Target Spike Solutions		
	PFASs High Target Spike Solutions		PFASs Reporting Limit Spike Solution
	Target Spike I	Target Spike II	
PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS	20 µg/L	–	2 µg/L
PFBA, PFPeA	100 µg/L	–	10 µg/L
FOUEA, FHUEA, FHpPA	–	20 µg/L	2 µg/L
FHEA, FOEA, FDEA	–	400 µg/L	40 µg/L

should only be used once. The analyte concentration in the vial may change after the vial cap is pierced because the vial caps do not reseal after puncture. Changing the caps immediately after the injection should alleviate this problem. Calibration standards are not filtered.

12.2.3 A second source verification standard should be incorporated into this test method at the discretion of the laboratory or project requirements. A second source standard should be analyzed near the midpoint of the calibration range to determine if the standards used are within ±30 % of the second source concentration. If they are not within ±30 %, the data shall be qualified stating in the narrative that the two different sources of standards did not match the acceptance criteria. Currently, a second source from a different vendor may not be readily available for all twenty-four target analytes. In this case, a second lot number from the same vendor may be used. If a second source for any target analyte is not used it should be clearly stated in a narrative accompanying the data package so that the end user of the data is aware that a second source check standard was not used. At a minimum, a second source for PFOA and PFOS is strongly suggested when using this test method.

12.2.4 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the primary and confirmatory SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the primary SRM transition. The ratios of the primary/confirmatory SRM transition area counts are given in Table 3 and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio shall be within 35 % of the individual labs' accepted primary/confirmatory SRM transition area ratio. The primary SRM transition of each analyte is used for quantitation and the confirmatory SRM transition for confirmation. This gives added confirmation by isolating the parent ion, forming two product ions by means of fragmentation, and relating it to the retention time in the calibration standard.

NOTE 3—Isotope dilution may be used instead of external standard calibration for the native analytes that have a labeled isotope only. Acceptance criteria must still be met. If a dilution is required, the isotope correction may not be applicable.

12.2.5 Depending on sensitivity and matrix interference issues dependent on sample type, the confirmatory SRM transition can be used as the primary SRM transition for quantitation during analysis. This shall be explained in a narrative accompanying the generated data. A new primary/confirmatory ion ratio will then be determined if switching the SRM transitions used to quantitate and confirm. The primary/confirmatory SRM transition area ratio shall be required to be within 35 % of the individual labs' new primary/confirmatory SRM transition area ratio.

12.2.6 The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin (X = 0, Y = 0) is not recommended.

12.2.7 Linear calibration may be used if the coefficient of determination, r^2, is ≥0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point shall be re-injected or a new calibration curve shall be regenerated. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve. If the low or high point(s), or both, are excluded, minimally a five-point curve is acceptable but the reporting range shall be modified to reflect this change.

12.2.8 Quadratic calibration may be used if the coefficient of determination, r^2, is ≥0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point shall be re-injected or a new calibration curve shall be regenerated. If the low or high point(s), or both, are excluded, minimally a six-point curve is acceptable but the reporting range shall be modified to reflect this change. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve.

12.2.9 The retention time window of the SRM transitions shall be within 5 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.10 A midpoint calibration check standard shall be analyzed at the end of each batch of 30 samples or within 24 hours after the initial calibration curve was generated, the criteria in the individual labs' quality system may be more restrictive pertaining to the number of samples. This end calibration check, in a new not pierced sealed vial, should come from the same calibration standard solution that was used to generate the initial curve. The results from the end calibration check standard shall have a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria,

corrective action including re-occurrence minimization is performed and either all samples in the batch are re-analyzed against a new calibration curve or the affected results are qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standard and notices that the sample evaporated affecting the concentration or other anomaly, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates, the results may be reported unqualified.

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., an instrument qualification study including method detection limit (MDL), calibration range determination and precision and bias determination shall be performed to demonstrate laboratory capability.

12.3.1 Analyze at least four replicates of a spiked water sample containing the PFASs and surrogates at a prepared sample concentration in the calibration range of Levels 4–7. The Level 6 concentration of the nine-point calibration curve was used to set the QC acceptance criteria in this test method. The matrix and chemistry should be similar to the matrix used in this test method. Each replicate shall be taken through the complete analytical test method including any sample manipulation and pretreatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 were generated from the single-laboratory data shown in the Precision and Bias, Section 16. Data from reagent, surface, and wastewater matrices are shown in the Precision and Bias, Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meet or exceed the criteria in this test method. References on how to generate QC acceptance criteria are Practices D2777, D5847, and E2554, or Method 8000 in EPA Publication SW-846.

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate spiking solution containing nine isotopically labeled PFASs – MPFBA, MPFHxA, MPFHxS, MPFDA, MPFOA, MPFOS, MPFNA, MPFUnA, and MPFDoA are added to all samples; including method blanks, duplicates, laboratory control samples, matrix spikes, and reporting limit checks. A stock surrogate spiking solution is prepared at 20 µg/L in 95 % acetonitrile: 5 % water. Spiking 40 µL of this spiking solution into a 5-mL water sample results in a concentration of 160 ng/L of the surrogate in the sample. The results obtained for the surrogate recoveries shall fall within the limits of Table 5. If the limits are not met, the affected results shall be qualified with an indication that they do not fall within the performance criteria of the test method.

12.4.1.1 The surrogate spiking solution was prepared by adding 500 µL of a 2-mg/L Surrogate Mix[16] in a 50-mL volumetric and diluted to 50 mL with 95 % acetonitrile: 5 % water. Surrogate spiking solutions are routinely replaced every year if not previously discarded for quality-control failure.

12.5 *Method Blank:*

12.5.1 At least two method blanks for every 30 samples are prepared in water to investigate for contamination during sample preparation and extraction. The concentration of target analytes in either/both blank(s) shall be less than half the reporting limit or the data shall be qualified as having a blank issue and the reporting limit for the affected samples shall be raised to at least 3 times above the blank contamination concentration. PFASs are common in the environment and laboratories requiring continual evaluation to ensure that quality data is produced.

12.6 *Reporting Limit Check Sample (RLCS):*

12.6.1 Each batch or within the 24 hour analysis window, a reporting limit check sample shall be analyzed. The reporting limit check sample is processed like a Laboratory Control Sample just spiked at or near the reporting limit. The concentration of the RLCS may be reported below the reporting limit since the spike is at or near the reporting limit. This sample is to check if the analytes were present at the reporting limit, they would be identified. The recovery limits for the RLCS are 35 to 150 %, if any analytes are outside of these limits the QC failure is explained in a narrative accompanying the data.

12.6.2 Five mL of ASTM Type I water is added to a 15-mL polypropylene centrifuge tube. The sample is spiked with 40 µL of surrogate spiking solution and 25 µL of PFASs Reporting Limit Check solution (Table 6) and then taken through the sample preparation and analyzed.

12.7 *Laboratory Control Sample (LCS):*

12.7.1 To ensure that the test method is in control, analyze at least one LCS with the PFASs at a mid-level concentration. A prepared sample, at the Level 6 calibration concentration, was used in this test method, any mid-level (Levels 4–7) concentration may be chosen using this test method. The LCS is prepared following the analytical method and analyzed with each batch of 30 samples or less. Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the 21 PFASs at concentrations listed in Table 6. Spike 40 µL each of Target Spike I and Target Spike II into 5 mL of water to yield a concentration of 800 ng/L (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA), and 160 ng/L of remaining 16 PFASs (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) in the sample. The result obtained for the LCS shall fall within the limits in Table 5. Spiking solutions are routinely replaced every year if not previously discarded for quality-control failure.

[16] Surrogate Mix from Wellington Laboratories Inc. has been found suitable for use.

12.7.2 If the result is not within these limits, sample analysis is halted until corrective action resolving the problem has been performed. Impacted samples in the batch are ether re-analyzed, or the results are flagged with a qualifier stating that they do not fall within the performance criteria of this test method.

12.8 *Matrix Spike (MS):*

12.8.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 30 or fewer samples by spiking the sample with a known concentration of PFASs and following the analytical method. Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the 21 PFASs at concentrations listed in Table 6. Spike 40 µL of these stock solutions into 5 mL of the site water sample to yield a concentration of 800 ng/L (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA), and 160 ng/L of remaining 16 PFASs (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) in the sample.

12.8.2 If the spiked concentration plus the background concentration exceeds that of the Level 9 calibration standard, the sample shall be diluted (using 50 % methanol/50 % water with 0.1 % acetic acid) to a level near the midpoint of the calibration curve.

12.8.3 Calculate the percent recovery of the spike (P) using Eq 1:

$$P = 100\frac{|A(V_s + V) - BV_s|}{CV} \quad (1)$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = percent recovery.

12.8.4 The percent recovery of the spike shall fall within the limits in Table 7. If the percent recovery is not within these limits, a matrix interference may be present. Under these circumstances either all samples in the batch may be analyzed by a test method not affected by the matrix interference, or the results shall be qualified indicating that they do not fall within the performance criteria of the test method. It has been found that in some cases the matrix spike concentration may be minimal compared to the concentration in the native sample. If this is the case, the sample may be spiked at a higher level or the generated data may be reported explaining in the narrative accompanying the data that the spike was negligible compared to the native concentration found in the sample.

12.8.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 7 were generated by a single-laboratory study using the data in the Precision and Bias, Section 16. The limits in Table 7 are preliminary until a multi-lab validation study is completed. The matrix variation between different waters may have a tendency to generate significantly wider control limits than those generated for this test method. It is recommended that each laboratory determine in-house QC acceptance criteria meeting or exceeding the criteria stated in this test method.

TABLE 7 MS/MSD QC Acceptance Criteria

NOTE 1—Table 7 data is preliminary until a multi-lab validation study is completed.

Analyte	Spike Conc. ng/L	MS/MSD Recovery (%)		Precision RPD (%)
		Lower Limit	Upper Limit	
PFTreA	160	70	130	30
PFTriA	160	70	130	30
PFDoA	160	70	130	30
PFUnA	160	70	130	30
PFDA	160	70	130	30
PFOS	160	70	130	30
PFNA	160	70	130	30
PFecHS	160	70	130	30
PFOA	160	70	130	30
PFHxS	160	70	130	30
PFHpA	160	50	130	30
PFHxA	160	50	130	30
PFBS	160	70	130	30
PFPeA	800	70	130	30
PFBA	800	50	130	30
FHEA	3200	70	130	30
FOEA	3200	70	130	30
FDEA	3200	70	130	30
FOUEA	160	70	130	30
FHpPA	160	70	130	30
FHUEA	160	70	130	30
MPFBA	160	70	130	30
MPFHxA	160	70	130	30
MPFHxS	160	70	130	30
MPFOA	160	70	130	30
MPFNA	160	70	130	30
MPFOS	160	70	130	30
MPFDA	160	70	130	30
MPFUnA	160	70	130	30
MPFDoA	160	70	130	30

12.8.5.1 The laboratory should generate its own in-house QC acceptance criteria after the analysis of 15–20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria are Practices D5847, D2777, and E2554, or Method 8000 in EPA Publication SW-846.

12.9 *Duplicate:*

12.9.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 30 or fewer samples. If the sample contains the analyte at a level greater than 5 times the reporting limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, a matrix spike/matrix spike duplicate should be used.

12.9.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. Compare to the RPD limit in Table 7.

$$RPD = \frac{|MSR - MSDR|}{(MSR + MSDR)\div 2} \times 100 \quad (2)$$

where:

RPD = relative percent difference,
MSR = matrix spike recovery, and
$MSDR$ = matrix spike duplicate recovery.

NOTE 4—If using duplicates to calculate RPD, MSR is the sample concentration and MSDR is the duplicates concentration.

12.9.3 If the result exceeds the precision limit (Table 7 RPD %), the batch shall be re-analyzed or the results shall be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 5-mL sample size per analysis. The samples shall be analyzed within 28 days of collection. If the samples are received or stored above 6°C, or are not analyzed within 28 days of collection, it is noted in the case narrative that accompanies the data.

13.2 Each batch of samples (30 or less) shall contain at least two method blanks, laboratory control sample, matrix spike, duplicate, and a reporting limit check sample at a minimum.

13.3 The entire collected 5.0-mL sample shall be used without transferring to another sample container. In order to have accurate volumes, the weight of the 15-mL polypropylene BD Falcon tube may be taken before and after sampling in order to obtain an exact volume. The density of water is assumed to be 1.0 g/mL unless the exact density of the water sample is known, then that conversion should be used. Some of these target analytes adhere to the surfaces of the sampling container over time. The entire sample shall be processed in the original container it was collected, otherwise biased low data will result. The entire collected sample is appropriately spiked.

13.4 To all samples, 5 mL of methanol is added and hand shaken/vortexed for ~2 minute (refer to 13.5 for additional steps for sludge samples).

13.5 After vortexing, pH of the sludge sample is adjusted to pH ~9 with ammonium hydroxide (~20 µL) and hand shaken/vortexed again for ~2 minute. This step is not required for water, wastewater, influent, and effluent unless high percent solids (≥0.1 %) are present or low recoveries were observed historically when no base was added.

13.6 All the samples are filtered through the filter unit using a lock tip glass syringe (refer to 13.7 and 13.8 before use) to remove particulates in the samples. Acetic acid (~10 µL for water samples and ~50 µL for ammonium hydroxide prepared samples) is added to all samples to adjust the pH ~3 after filtration. An aliquot of the solution is transferred to a LC vial and a polyethylene cap is applied. The final volume of the solution is estimated to be 10 mL for quantitation purposes since 5 mL of methanol was added to 5 mL of sample.

13.7 The filters shall be washed with two 10-mL volumes of acetonitrile followed by two 10-mL volumes of methanol prior to use to ensure removal of possible PFASs.

NOTE 5—If the filter units were manufactured in a facility that produces or uses PFASs containing products there is a good chance they may be contaminated with PFASs that need to be removed by rinsing.

13.8 The syringe shall be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of acetonitrile, then 3 volumes of methanol, and a final rinse with water.

13.9 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be method blank(s), reporting limit check, laboratory control sample(s), sample(s), duplicate(s), and matrix spike sample(s) followed by an end calibration check standard.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the PFASs and surrogates, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. The target compounds are identified by comparing the sample primary SRM transition and its confirmatory SRM transition if correlated to the known standard SRM transition. Confirmatory transitions are available for most of the target analytes (Table 3). The primary/confirmatory SRM ion ratio shall meet the criteria set in the quantitation method by ±35 %. The primary/confirmatory SRM ion ratio is the average of the individual levels primary /confirmatory SRM ion ratios in the calibration curve on the day of analysis. This ratio will vary depending on the instrumental acquisition parameters and shall be checked for every sample batch. External calibration curves are used to calculate the amounts of PFASs and surrogates. Calculate the concentration in ng/L (ppt) for each analyte. The individual PFASs may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with a solution of 50 % Water/ 50 % MeOH containing 0.1 % acetic acid to obtain a concentration near the mid-point of the calibration range and re-analyzed. This test method uses nine surrogates, MPFBA, MPFHxA, MPFHxS, MPFDA, MPFOA, MPFOS, MPFNA, MPFUnA, and MPFDoA, to monitor performance. The surrogate recoveries are provided with all data generated from this test method.

14.2 If there is no confirmatory transition for the analyte (refer to Table 3), and the presence of the analyte in the sample can't be confirmed with the primary transition and retention time, the analyte is listed as a non-detect or as having a matrix interference present.

14.3 *Example Calculation of Sample Concentration Reported*—The concentration of sample is calculated using Eq 3.

$$\frac{V_f}{V_i}(C_u) = C_f \tag{3}$$

where:

V_f = final volume,
V_i = initial volume,
C_u = uncorrected concentration, and
C_f = final concentration (corrected for dilution).

14.4 There are nine labeled surrogates for this analysis. The labeled analyte represents the unlabeled native analytes. PFTreA and PFTriA are represented by MPFDoA. PFHpA is represented by MPFHxA, PFecHS, and PFBS are represented by MPFHxS and PFPeA is represented by MPFBA. The six fluorotelomer acids do not have associated labeled surrogates. The recoveries of the nearest labeled surrogate should be monitored but does not represent the native compound. No qualifications based on surrogate recovery will be made for the

six fluorotelomer acids. It is a user's judgment to qualify data based upon no-representative surrogates.

14.5 Some of the analytes are comprised of isomeric mixtures, this is the case for PFOS, PFecHS, and PFHxS in this test method. The entire isomeric group shall be quantitated. This is one reason why a secondary transition is required and allows easier determinations to be made by the analyst by comparing the two transitions. If there are parts of the isomeric mixture in the sample that do not match the retention times of the standard they may not be included in the integration and this shall be explained in the narrative accompanying the data.

14.6 The confirmatory ion ratios in "weathered samples" may not match the ion ratios in the calibration standards for the target analytes that may contain isomeric mixtures. Figs. X1.1-X1.4 in Appendix X1 are examples of this for PFHxS and PFOS, these differences in isomer mixtures may be observed with analytes that have the possibility of containing isomeric mixtures. These differences for PFHxS and PFOS were found in groundwater samples and may either be the cause of different compositions used, weathering or degradation or the affinity of the branched isomers to be more soluble than the linear in water and may leach into the water from the soil at a higher rate than the linear. If the ion ratios do not match the ion ratio criteria, document in the case narrative and the affected data should be qualified and explained in the narrative accompanying the data.

15. Report

15.1 Determine the results in units of ng/L (ppt) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method shall be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted by U.S. EPA Region 5 Chicago Regional Laboratory (CRL) and generated applicable data to determine the precision and bias as described in Practice D2777 for a single laboratory validation study.

16.2 This test method was tested by CRL on reagent water. The samples were spiked with the PFASs to obtain a 800 ng/L (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA) and 160 ng/L of the remaining (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) and a

TABLE 8 Single-Laboratory Recovery Data in Reagent Water

	Measured ng/L from ASTM Type I Water — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
P&A 1	134.3	138.6	140.7	144.9	147.2	148.6	144.5	98.8	92.0	695.7	550.5
P&A 2	146.5	148.3	148.5	147.8	150.3	151.3	145.2	119.8	93.8	708.2	574.2
P&A 3	152.9	147.7	148.1	149.3	150.8	148.6	145.4	100.5	89.7	692.7	559.6
P&A 4	144.1	147.8	151.7	150.4	150.3	152.0	149.4	102.0	94.1	719.2	562.2
P&A 5	157.5	149.4	149.1	151.5	151.1	153.3	146.4	103.3	92.9	708.6	573.7
P&A 6	146.8	148.3	146.6	148.2	149.9	152.7	143.3	101.0	94.3	703.5	557.4
Average Recovery (ng/L)	147.0	146.7	147.5	148.7	149.9	151.1	145.7	104.2	92.8	704.6	562.9
Average % Recovery	91.9	91.7	92.2	92.9	93.7	94.4	91.1	65.2	58.0	88.1	70.4
Standard Deviation	7.9	4.0	3.7	2.3	1.4	2.0	2.1	7.8	1.7	9.6	9.4
RSD (%)	8.6	4.4	4.0	2.5	1.5	2.2	2.3	7.4	1.9	1.4	1.7
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	150.0	151.0	151.0	143.3	150.0	150.7	145.5	3252.1	3037.1	3183.8	
P&A 2	216.2	150.1	155.6	147.2	148.7	136.1	143.0	3069.8	2776.8	3048.4	
P&A 3	146.2	151.2	153.3	145.0	148.0	144.8	147.0	3161.0	3055.6	3346.8	
P&A 4	149.6	144.5	152.3	145.8	147.5	147.0	151.6	3235.8	3007.3	3243.3	
P&A 5	145.7	147.4	147.1	146.9	146.2	146.9	145.8	3099.8	2853.9	3233.2	
P&A 6	142.8	139.7	146.4	145.6	145.9	133.1	142.4	2977.0	3197.8	3152.9	
Average Recovery (ng/L)	158.4	147.3	150.9	145.6	147.7	143.1	145.9	3132.6	2988.1	3201.4	
Average % Recovery	99.0	92.1	94.3	91.0	92.3	89.4	91.2	97.9	93.4	100.0	
Standard Deviation	28.4	4.5	3.6	1.4	1.5	6.9	3.3	104.8	151.0	100.0	
RSD (%)	17.9	3.1	3.8	1.6	1.0	4.8	2.3	3.3	5.1	3.1	

160 ng/L of surrogates as described in Section 12. Table 8 contains the recoveries and standard deviation (SD) for the target compounds and Table 9 the surrogate recoveries.

16.3 This test method was tested by CRL on Chicago River water. The samples were spiked with target compounds and surrogates as described in Section 12. Table 10 and Table 11 contain the recoveries for the target compounds and surrogates respectively.

16.4 This test method was tested by CRL on waste water from three representative sewage treatment plants. The samples were spiked with target compounds and surrogates as described in Section 12. Tables 12-19 contain the recoveries for the surrogates and target compounds in samples from different treatment plants.

17. Quality Control

17.1 A crucial part of a test method is quality control. A laboratory should follow their in-house QA/QC procedures and should meet or exceed the criteria given in this test method. The quality-control criteria are given in the various test method sections. Section 10 contains the sampling and preservation requirements and Section 12 contains the majority of quality-control requirements when following this test method. Section 12 includes requirements for calibration, second source verification, precision and bias study to demonstrate laboratory capability, initial demonstration of performance, surrogate, method blank, reporting limit check, laboratory control, matrix spike, and duplicate sample requirements.

18. Keywords

18.1 liquid chromatography; mass spectrometry; per- and polyfluoroalkyl substances; water

TABLE 9 Single-Laboratory Surrogate Recovery Data in Reagent Water

	Measured ng/L from ASTM Type I Water — 160 ng/L spike								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	145.2	157.1	153.8	160.4	155.4	159.4	159.3	156.8	151.4
Unspiked 2	147.8	158.6	156.8	162.6	155.4	154.0	154.2	154.7	153.8
P&A 1	144.7	158.0	165.2	161.8	164.4	154.1	157.2	158.1	152.3
P&A 2	147.2	154.9	156.9	156.5	157.2	155.0	156.4	157.0	155.7
P&A 3	142.9	154.6	150.1	154.8	153.0	155.4	154.4	154.0	153.2
P&A 4	140.0	157.1	153.9	162.0	159.2	156.5	154.5	156.7	153.6
P&A 5	150.6	161.2	154.1	161.7	160.9	155.3	160.8	157.6	157.7
P&A 6	148.1	155.7	156.8	162.2	160.4	155.0	159.2	156.4	153.1
Average Recovery (ng/L)	145.8	157.1	155.9	160.3	158.2	155.6	157.0	156.4	153.8
Average % Recovery	91.1	98.2	97.5	100.2	98.9	97.2	98.1	97.8	96.2
Standard Deviation	3.3	2.2	4.4	2.9	3.7	1.7	2.5	1.4	2.0
RSD (%)	2.3	1.4	2.8	1.8	2.3	1.1	1.6	0.9	1.3

TABLE 10 Single-Laboratory Target Compound Recovery Data in Chicago River Water

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Measured ng/L from Chicago River Water — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	11.44	<RL	9.54[A]	<RL	<RL
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	11.4	<RL	9.5[A]	<RL	<RL
P&A 1	142.0	146.4	149.1	145.7	142.5	146.9	145.4	96.5	89.5	691.1	517.4
P&A 2	143.8	149.7	157.5	150.6	153.0	154.9	147.5	100.5	91.4	708.4	530.4
P&A 3	138.0	144.6	147.9	147.8	150.0	152.8	136.4	99.1	90.7	697.7	551.9
P&A 4	147.7	143.4	152.6	149.3	150.9	147.5	137.4	98.7	89.4	681.7	535.4
P&A 5	160.7	160.7	153.7	149.1	145.7	153.2	145.8	102.3	90.7	706.3	547.8
P&A 6	150.6	144.7	148.5	142.2	139.5	144.4	135.4	97.1	87.8	695.1	529.1
Average Recovery (ng/L)	147.1	148.3	151.6	147.5	146.9	150.0	141.3	99.0	89.9	696.7	535.3
Average % Recovery	92.0	92.7	94.7	92.2	91.8	93.7	88.3	61.9	56.2	87.1	66.9
Standard Deviation	8.0	6.5	3.7	3.1	5.3	4.2	5.5	2.2	1.3	9.9	12.7
RSD (%)	5.4	4.4	2.5	2.1	3.6	2.8	3.9	2.2	1.4	1.4	2.4
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	<RL	<RL	10.34[A]	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	<RL	<RL	11.9[A]	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	142.2	147.9	146.1	145.1	146.9	166.1	151.2	2985.2	3412.6	3386.7	
P&A 2	146.0	150.7	137.1	154.2	148.4	158.3	144.8	2905.5	3330.0	3438.3	
P&A 3	138.2	147.8	132.6	144.7	149.9	158.0	153.0	2891.0	3256.4	3462.6	
P&A 4	138.3	139.5	143.6	141.2	149.2	155.3	155.0	2878.3	2921.8	3197.9	
P&A 5	140.0	146.6	145.4	141.0	151.4	160.7	157.0	2813.9	3369.1	3628.1	
P&A 6	153.7	145.0	136.4	149.1	148.0	154.0	149.2	2644.8	3192.2	3440.9	
Average Recovery (ng/L)	143.1	146.3	140.2	145.9	149.0	158.7	151.7	2853.1	3247.0	3425.7	
Average % Recovery	89.4	91.4	87.6	91.2	93.1	99.2	94.8	89.2	101.5	107.1	
Standard Deviation	6	3.8	5.6	5.0	1.6	4.3	4.4	115.9	177.8	138.7	
RSD (%)	4.2	2.6	4.0	3.5	1.1	2.7	2.9	4.1	5.5	4.0	

[A] Slightly below reporting limit.

TABLE 11 Single-Laboratory Surrogate Recovery Data in Chicago River Water

	Measured ng/L from Chicago River Water — 160 ng/L spike								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	148.0	167.4	169.9	166.7	160.0	169.8	164.6	167.2	171.0
Unspiked 2	148.2	159.3	164.2	152.1	156.4	159.8	157.6	158.8	162.0
P&A 1	148.5	169.1	173.1	164.2	166.3	172.3	167.8	167.2	170.9
P&A 2	148.6	158.3	155.1	157.6	156.7	152.6	157.8	165.3	160.7
P&A 3	144.0	154.7	154.2	152.3	156.3	149.5	159.6	159.3	154.7
P&A 4	134.4	157.4	154.6	153.7	147.5	150.4	152.6	154.2	156.4
P&A 5	144.7	159.5	158.3	156.0	159.8	156.7	153.6	162.3	158.4
P&A 6	151.6	167.4	168.7	165.0	163.2	165.8	164.6	168.6	168.2
Average Recovery (ng/L)	146.0	161.6	162.3	158.5	158.3	159.6	159.8	162.9	162.8
Average % Recovery	91.3	101.0	101.4	99.0	98.9	99.8	99.9	101.8	101.7
Standard Deviation	5.3	5.5	7.7	6.0	5.6	8.8	5.5	5.1	6.5
RSD (%)	3.6	3.4	4.7	3.8	3.5	5.5	3.4	3.1	4.0

TABLE 12 Single-Laboratory Target Compound Recovery Data in Sewage Treatment Plant I (Effluent Samples)

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Measured ng/L from Treatment Plant I — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	<RL	11.62	<RL	<RL	<RL	<RL	11.48	<RL	<RL	<RL	<RL
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	11.16	<RL	<RL	<RL	<RL
P&A 1	175.7	139.5	147.7	145.4	143.8	142.5	139.4	98.8	96.7	673.3	510.8
P&A 2	177.6	143.5	149.1	145.4	144.7	138.6	136.0	99.8	99.6	680.8	549.8
P&A 3	169.2	141.9	142.1	143.2	138.0	138.8	134.3	98.4	96.4	672.5	517.3
P&A 4	158.1	137.3	147.4	141.8	138.9	134.9	134.7	97.7	95.4	668.0	501.0
Average Recovery (ng/L)	170.2	140.5	146.6	143.9	141.3	138.7	136.1	98.7	97.0	673.6	519.7
Average % Recovery	106.4	87.8	91.6	90.0	88.3	86.7	85.1	61.7	60.6	84.2	65.0
Standard Deviation	8.8	2.7	3.1	1.8	3.4	3.1	2.3	0.9	1.8	5.3	21.1
RSD (%)	5.2	1.9	2.1	1.2	2.4	2.3	1.7	0.9	1.8	0.8	4.1
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	10.94	<RL	14[A]	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	8.96[A]	<RL	11.86[A]	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	140.1	146.3	144.2	147.8	151.8	166.0	145.4	2989.8	3018.0	4751.9	
P&A 2	142.0	156.5	140.9	146.9	152.9	165.5	152.3	3060.9	3452.7	4808.4	
P&A 3	130.8	145.2	136.7	143.4	139.7	153.8	149.7	2949.8	3209.3	4835.9	
P&A 4	142.5	148.7	139.3	146.9	143.3	163.5	147.6	2691.4	2953.7	4630.2	
Average Recovery (ng/L)	138.8	149.2	140.3	146.3	146.9	162.2	148.8	2923.0	3158.4	4756.6	
Average % Recovery	86.8	93.2	87.7	91.4	92.4	102.0	93.5	91.3	98.7	148.6	
Standard Deviation	5.4	5.1	3.1	1.9	6.4	5.7	3.0	161.1	224.2	91.3	
RSD (%)	3.9	3.4	2.2	1.3	4.4	3.5	2.0	5.5	7.1	1.9	

[A] Slightly below reporting limit.

TABLE 13 Single-Laboratory Surrogate Recovery Data in Sewage Treatment Plant I (Effluent Samples)

	Measured ng/L from Treatment Plant I (Effluent Sample — 160 ng/L spike)								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	151.6	152.7	154.8	150.6	147.5	158.1	158.1	157.2	161.9
Unspiked 2	139.0	147.4	145.7	143.4	148.8	152.3	149.3	154.5	157.0
P&A 1	133.9	152.4	155.1	148.8	150.2	151.4	151.2	154.7	155.2
P&A 2	142.8	152.4	152.5	144.7	153.7	149.4	153.8	151.5	158.1
P&A 3	136.3	149.2	149.5	147.0	149.4	148.5	145.5	151.4	153.6
P&A 4	137.4	149.9	152.9	146.4	149.4	149.1	150.6	155.6	155.3
Average Recovery (ng/L)	140.1	150.7	151.8	146.8	149.8	151.5	151.4	154.2	156.8
Average % Recovery	87.6	94.2	94.8	91.8	93.7	94.7	94.6	96.4	98.0
Standard Deviation	6.3	2.2	3.6	2.6	2.1	3.6	4.3	2.3	2.9
RSD (%)	4.5	1.4	2.4	1.8	1.4	2.4	2.8	1.5	1.9

TABLE 14 Single-Laboratory Target Compound Recovery Data in Sewage Treatment Plant I (Influent Sample)

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Measured ng/L from Treatment Plant I — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
P&A 1	180.4	156.4	146.6	146.7	144.0	143.3	144.6	98.0	93.9	648.6	511.2
P&A 2	167.2	153.4	140.8	141.0	141.8	139.0	142.2	96.8	91.4	629.6	497.7
P&A 3	186.3	157.3	148.7	146.6	143.1	143.6	144.8	97.4	93.8	639.9	509.4
P&A 4	166.9	156.5	145.3	148.6	144.4	146.8	144.9	97.7	93.7	651.1	516.8
Average Recovery (ng/L)	175.2	155.9	145.4	145.7	143.3	143.2	144.1	97.5	93.2	642.3	508.8
Average % Recovery	109.5	97.4	90.8	91.1	89.6	89.5	90.1	60.9	58.3	80.3	63.6
Standard Deviation	9.7	1.7	3.3	3.3	1.2	3.2	1.3	0.5	1.2	9.8	8.0
RSD (%)	5.5	1.1	2.3	2.2	0.8	2.2	0.9	0.5	1.3	1.5	1.6
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	<RL	<RL	124.4	18.2	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	<RL	<RL	145.9	32.8	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	146.7	152.4	53.8	127.4	160.7	176.6	150.9	3033.2	3054.1	5104.7	
P&A 2	144.6	157.3	45.3	124.0	160.8	167.6	152.5	3094.3	3343.2	5185.4	
P&A 3	139.9	145.9	44.7	124.6	161.9	175.9	150.6	2915.4	3346.1	5120.9	
P&A 4	147.5	157.6	57.1	142.7	162.8	164.5	151.0	2972.2	3422.9	5066.5	
Average Recovery (ng/L)	144.7	153.3	50.2	129.7	161.6	171.2	151.3	3003.8	3291.6	5119.4	
Average % Recovery	90.4	95.8	31.4	81.1	101.0	107.0	94.5	93.9	102.9	160.0	
Standard Deviation	3.4	5.5	6.2	8.8	1.0	6.0	0.9	77.2	162.5	49.6	
RSD (%)	2.4	3.6	12.3	6.8	0.6	3.5	0.6	2.6	4.9	1.0	

TABLE 15 Single-Laboratory Surrogate Recovery Data in Sewage Treatment Plant I (Influent Sample)

	Measured ng/L from Treatment Plant I (Influent Sample — 160 ng/L spike)								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	131.1	146.6	151.4	148.2	147.8	153.9	148.2	148.1	151.2
Unspiked 2	134.5	144.8	149.6	143.0	148.1	151.6	149.8	147.1	149.3
P&A 1	138.5	150.3	151.2	150.4	152.0	157.1	150.8	151.1	151.2
P&A 2	141.5	143.8	150.0	150.4	152.7	154.6	152.1	150.3	151.0
P&A 3	142.6	150.8	153.1	148.1	147.4	160.4	154.0	153.2	157.0
P&A 4	140.5	149.9	158.3	153.5	151.1	158.8	154.8	154.6	155.8
Average Recovery (ng/L)	138.1	147.7	152.3	148.9	149.8	156.1	151.6	150.7	152.6
Average % Recovery	86.3	92.3	95.2	93.1	93.7	97.5	94.8	94.2	95.4
Standard Deviation	4.5	3.0	3.2	3.5	2.4	3.3	2.5	2.9	3.1
RSD (%)	3.2	2.1	2.1	2.4	1.6	2.1	1.7	1.9	2.0

TABLE 16 Single-Laboratory Target Compound Recovery Data in Sewage Treatment Plant II (Effluent with Supplemental Sewage)

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Measured ng/L from Treatment Plant II — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	13.1	9.7[A]	40.1	26.6	60.6	26.2	30.8	<RL	9.3[A] †	<RL	<RL
Unspiked 2	12.8	10.6	39.7	24.8	55.9	27.7	33.5	<RL	10.6	<RL	<RL
P&A 1	181.9	160.0	165.5	156.3	140.2	139.3	136.1	98.7	85.0	668.9	552.8
P&A 2	171.8	154.3	165.8	149.1	132.3	139.1	136.5	100.8	88.2	657.7	537.3
P&A 3	155.0	149.3	153.0	144.8	137.1	136.9	133.9	95.6	85.1	646.3	543.9
P&A 4	144.1	147.8	154.0	144.0	143.0	138.1	136.2	98.4	85.7	644.0	540.1
P&A 5	153.7	146.0	150.9	142.8	133.0	134.4	123.2	94.2	82.3	623.4	533.9
P&A 6	160.6	164.8	171.2	161.8	157.0	151.7	149.2	107.6	94.2	695.5	579.4
Average Recovery (ng/L)	161.2	153.7	160.1	149.8	140.4	139.9	135.8	99.2	86.7	656.0	547.9
Average % Recovery	100.7	96.1	100.0	93.6	87.8	87.5	84.9	62.0	54.2	82.0	68.5
Standard Deviation	13.7	7.4	8.5	7.7	9.1	6.0	8.3	4.7	4.1	24.6	16.7
RSD (%)	8.5	4.8	5.3	5.1	6.5	4.3	6.1	4.8	4.7	3.8	3.1
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	<RL	<RL	162.7	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	9.6[A]	<RL	156.1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	143.8	147.6	151.9	150.2	146.9	166.1	151.2	2985.2	3412.6	3386.7	
P&A 2	140.6	154.9	136.3	148.1	148.4	158.3	144.8	2905.5	3330.0	3438.3	
P&A 3	137.0	151.0	131.3	147.2	149.9	158.0	153.0	2891.0	3256.4	3462.6	
P&A 4	145.7	147.0	255.0	145.4	149.2	155.3	155.0	2878.3	2921.8	3197.9	
P&A 5	132.1	145.2	116.9	142.7	151.4	160.7	157.0	2813.9	3369.1	3628.1	
P&A 6	152.1	162.2	161.7	159.2	148.0	154.0	149.2	2644.8	3192.2	3440.9	
Average Recovery (ng/L)	141.9	151.3	158.8	148.8	149.0	158.7	151.7	2853.1	3247.0	3425.7	
Average % Recovery	88.7	94.6	99.3	93.0	93.1	99.2	94.8	89.2	101.5	107.1	
Standard Deviation	7.0	6.3	49.7	5.7	1.6	4.3	4.4	115.9	177.8	138.7	
RSD (%)	4.9	4.2	31.3	3.8	1.1	2.7	2.9	4.1	5.5	4.0	

[A] Slightly below reporting limit.

TABLE 17 Single-Laboratory Surrogate Recovery Data in Sewage Treatment Plant II (Effluent with Supplemental Sewage)

	Measured ng/L from Treatment Plant II (Effluent with Supplemental Sewage — 160 ng/L spike)								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	143.0	148.9	153.1	149.5	156.7	161.5	157.2	165.8	176.2
Unspiked 2	149.5	152.8	160.7	159.1	161.0	163.7	160.6	166.2	175.0
P&A 1	138.7	145.5	150.3	148.6	151.1	158.8	151.2	158.3	175.2
P&A 2	132.4	142.3	151.9	144.4	152.0	152.1	148.7	156.4	167.7
P&A 3	127.3	137.4	150.4	141.6	144.8	146.5	150.4	150.1	163.5
P&A 4	131.1	142.3	143.6	139.1	145.3	147.2	144.3	155.0	167.0
P&A 5	128.4	136.3	148.5	140.7	143.7	145.5	144.9	152.7	165.2
P&A 6	141.5	145.4	157.8	150.5	158.2	159.8	155.5	166.5	170.8
Average Recovery (ng/L)	136.5	143.9	152.1	146.7	151.6	154.4	151.6	158.9	170.1
Average % Recovery	85.3	89.9	95.0	91.7	94.7	96.5	94.8	99.3	106.3
Standard Deviation	7.9	5.5	5.3	6.6	6.6	7.4	5.8	6.5	4.9
RSD (%)	5.8	3.8	3.5	4.5	4.4	4.8	3.8	4.1	2.9

TABLE 18 Single-Laboratory Target Compound Recovery Data in Sewage Treatment Plant III (Effluent with Supplemental Sewage)

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Measured ng/L from Treatment Plant III — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	13.1	19.7	104.6	44.1	162.4	23.0	61.0	<RL	19.2	61.8	6869.2
Unspiked 2	12.8	13.3	71.3	31.5	128.1	21.7	61.4	<RL	17.3	64.2	7069.8
P&A 1	136.0	138.6	144.9	141.0	127.9	135.5	138.3	94.0	82.4	633.4	572.5
P&A 2	134.3	140.6	155.9	146.0	149.5	135.3	140.4	99.4	86.2	628.8	1013.9
P&A 3	123.1	132.2	127.4	145.6	112.5	143.3	137.9	102.8	94.5	678.5	1363.5
P&A 4	142.8	142.0	153.8	144.7	142.8	145.2	137.1	102.0	86.6	636.2	874.3
P&A 5	151.8	141.3	149.3	151.7	144.6	150.5	151.6	104.3	95.2	675.2	1155.3
P&A 6	163.5	149.3	152.8	148.2	137.4	141.1	139.5	101.5	88.8	658.8	1243.5
Average Recovery (ng/L)	141.9	140.7	147.4	146.2	135.8	141.8	140.8	100.7	88.9	651.8	1037.2
Average % Recovery	88.7	87.9	92.1	91.4	84.9	88.6	88.0	62.9	55.6	81.5	129.6
Standard Deviation	14.2	5.5	10.5	3.6	13.6	5.9	5.4	3.6	5.0	22.0	285.0
RSD (%)	10.0	3.9	7.1	2.4	10.0	4.1	3.9	3.6	5.6	3.4	27.5
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	20.7	<RL	222.3	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	24.1	<RL	175.6	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	140.0	147.3	130.7	145.2	146.9	166.1	151.2	2985.2	3412.6	3386.7	
P&A 2	138.0	138.2	145.2	140.4	148.4	158.3	144.8	2905.5	3330.0	3438.3	
P&A 3	149.5	151.2	107.9	153.6	149.9	158.0	153.0	2891.0	3256.4	3462.6	
P&A 4	138.9	154.5	127.1	143.6	149.2	155.3	155.0	2878.3	2921.8	3197.9	
P&A 5	153.5	157.5	147.5	148.8	151.4	160.7	157.0	2813.9	3369.1	3628.1	
P&A 6	143.8	156.0	150.9	142.2	148.0	154.0	149.2	2644.8	3192.2	3440.9	
Average Recovery (ng/L)	143.9	150.8	134.9	145.7	149.0	158.7	151.7	2853.1	3247.0	3425.7	
Average % Recovery	90.0	94.2	84.3	91.0	93.1	99.2	94.8	89.2	101.5	107.1	
Standard Deviation	6.3	7.2	16.3	4.8	1.6	4.3	4.4	115.9	177.8	138.7	
RSD (%)	4.4	4.8	12.1	3.3	1.1	2.7	2.9	4.1	5.5	4.0	

TABLE 19 Single-Laboratory Surrogates Recovery Data in Sewage Treatment Plant III (Effluent with Supplemental Sewage)

	Measured ng/L from Treatment Plant III (Effluent with Supplemental Sewage — 160 ng/L spike)								
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA
Unspiked 1	136.1	144.9	147.7	149.4	151.2	152.6	150.8	162.4	170.5
Unspiked 2	142.5	146.6	150.2	144.6	150.1	155.9	149.1	150.6	163.7
P&A 1	134.3	141.7	143.1	147.5	147.1	150.2	143.9	156.4	162.8
P&A 2	134.2	144.5	143.7	147.3	146.3	147.7	147.9	152.6	165.3
P&A 3	146.5	149.8	152.7	151.4	158.6	155.8	155.3	164.0	162.1
P&A 4	134.9	146.9	150.2	141.7	147.7	151.5	147.9	157.9	164.3
P&A 5	148.9	152.7	159.3	155.4	163.8	163.4	155.4	164.6	170.6
P&A 6	142.2	145.3	147.6	147.4	150.0	155.3	148.6	157.9	166.4
Average Recovery (ng/L)	140.0	146.6	149.3	148.1	151.9	154.1	149.9	158.3	165.7
Average % Recovery	87.5	91.6	93.3	92.6	94.9	96.3	93.7	98.9	103.6
Standard Deviation	5.9	3.4	5.2	4.1	6.2	4.8	3.9	5.1	3.3
RSD (%)	4.2	2.3	3.5	2.8	4.1	3.1	2.6	3.2	2.0

APPENDIXES

(Nonmandatory Information)

X1. PRELIMINARY DATA SUGGESTS THAT OTHER PFASs MAY BE DETERMINED BY THIS TEST METHOD AND THIS APPENDIX INFORMATION MAY BE OF USEFUL INFORMATION TO THE USER

X1.1 Preliminary data for an additional ten fluorinated compounds and isotopically labelled surrogates show promise for their analysis in water using this test method. These analytes are listed in Table X1.1 with their MDL and reporting range. Table X1.2 lists their MRM Transitions, Cone and Collision Energies, Retention Times, and Ion Ratios. Table X1.3 lists their precision and accuracy in reagent water. Tables X1.4-X1.18 lists the precision and accuracy for the additional ten fluorinated compounds and fourteen of this test method's analytes and surrogates in various waters.

TABLE X1.1 List of Additional Analytes, Surrogates, MDLs, and Reporting Ranges

Analyte	Abbreviation	Chemical Abstract Number	MDL (ng/L)	Reporting Range (ng/L)
Perfluoro-1-decanesulfonate	PFDS	2806-15-7	2.2	10–400
Perfluoro-1-nonanesulfonate	PFNS	68259-12-1	1.4	10–400
Perfluoro-1-heptanesulfonate	PFHpS	375-92-8	2.5	10–400
Perfluoro-1-pentanesulfonate	PFPeS	2706-91-4	1.3	10–400
1H, 1H, 2H, 2H-perfluorohexane sulfonate	4:2 FTS	757124-72-4	1.5	10–400
1H, 1H, 2H, 2H-perfluorooctane sulfonate	6:2 FTS	27619-97-2	1.6	10–400
1H, 1H, 2H, 2H-perfluordecane sulfonate	8:2 FTS	39108-34-4	2.7	10–400
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	1.9	10–400
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	1.1	10–400
Perfluoro-1-octanesulfonamide	FOSA	754-91-6	1.6	10–400
Surrogates				
1H, 1H, 2H, 2H-perfluoro-(1,2 – $^{13}C_2$)hexane sulfonate	M 4:2 FTS	NA	NA	NA
1H, 1H, 2H, 2H-perfluoro-1(1,2 – $^{13}C_2$)octane sulfonate	M 6:2 FTS	NA	NA	NA
1H, 1H, 2H, 2H-perfluoro-1(1,2 – $^{13}C_2$)decane sulfonate	M 8:2 FTS	NA	NA	NA
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	M NMeFOSAA	NA	NA	NA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	M NEtFOSAA	NA	NA	NA

TABLE X1.2 Analyte MRM Transitions, Cone and Collision Energies, Retention Times, and Ion Ratios

Analyte	Primary/Confirmatory	MRM Transition	Cone (V)	Collision Energy (eV)	Retention Time Minutes	Primary/Confirmatory SRM Area Ratio
PFDS	Primary	598.9→79.9	15	45	9.8	1.2
	Confirmatory	598.9→98.9	15	45		
PFNS	Primary	548.9→79.9	15	42	9.2	1.2
	Confirmatory	548.9→98.9	15	42		
PFHpS	Primary	448.9→79.9	15	38	7.95	1.3
	Confirmatory	448.9→98.9	15	36		
PFPeS	Primary	348.9→79.9	15	34	6.4	1.4
	Confirmatory	348.9→98.9	15	30		
4:2 FTS	Primary	327→307	10	20	5.2	3.5
	Confirmatory	327→80.9	10	24		
6:2 FTS	Primary	427→406.9	10	22	6.7	4.3
	Confirmatory	427→80.9	10	30		
8:2 FTS	Primary	526.9→506.9	10	26	8	4.5
	Confirmatory	526.9→80.9	10	34		
N-MeFOSAA	Primary	569.9→419	15	20	8.4	1.8
	Confirmatory	569.9→482.9	15	16		
N-EtFOSAA	Primary	583.9→419	15	20	8.7	1.7
	Confirmatory	583.9→482.9	15	16		
FOSA	Primary	497.9→77.9	15	28	9.8	NA
M 4:2 FTS	Primary	329→309	20	10	5.2	NA
M 6:2 FTS	Primary	429→408.9	10	22	6.7	NA
M 8:2 FTS	Primary	528.9→508.9	10	26	8	NA
M NMeFOSAA	Primary	572.9→419	15	20	8.4	NA
M NEtFOSAA	Primary	588.9→419	15	20	8.7	NA

TABLE X1.3 Precision and Accuracy Data in Reagent Water

Analyte	Spike Amount (ng/L)	Average Recovery (%)	Standard Deviation (%)	# of Replicates (n)
PFDS	160	101.4	4.3	6
PFNS	160	99.8	3.2	6
PFHpS	160	98.8	4.5	6
PFPeS	160	93.5	2.7	6
FOSA	160	98.3	2.5	6
4:2 FTS	160	99.5	4.3	6
6:2 FTS	160	105.1	13.8	6
8:2 FTS	160	111.7	7.2	6
N-EtFOSAA	160	103.4	5.0	6
N-MeFOSAA	160	101.3	3.7	6
M 4:2 FTS	160	101.7	5.0	17
M 6:2 FTS	160	108.2	9.0	17
M 8:2 FTS	160	107.3	12.4	17
M NEtFOSAA	160	111.0	6.1	17
M NMeFOSAA	160	103.9	3.3	17

TABLE X1.4 Precision and Accuracy Study for Additional PFASs in Sewage Treatment Plant IV (Effluent Sample)

	Stickney, IL, POTW (Effluent Sample)										
	Measured ng/L from 160 ng/L Spike for All PFASs Except PFBA and PFPeA (800 ng/L spike)										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	137	146	151	146	147	149	155	141	141	673	416
Spiked 2	127	131	137	137	132	135	141	127	134	628	347
Spiked 3	130	144	145	141	143	145	149	137	137	638	331
Spiked 4	134	142	144	144	146	148	146	141	142	677	368
Spiked 5	125	141	143	146	144	147	148	140	140	673	436
Spiked 6	142	145	147	149	146	146	146	137	142	674	372
Average Recovery (ng/L)	132	142	145	144	143	145	148	137	139	661	378
% Average Recovery	82.7	88.5	90.3	89.8	89.4	90.7	92.2	85.8	87.0	82.6	47.3
Standard Deviation	6.29	5.36	4.85	4.08	5.72	5.02	4.46	5.34	3.22	21.7	40.2
RSD (%)	4.75	3.79	3.36	2.84	4.00	3.46	3.02	3.89	2.31	3.28	10.6

TABLE X1.5 Precision and Accuracy Study for Additional PFASs in Sewage Treatment Plant IV (Effluent Sample)

	Stickney, IL, POTW (Effluent Sample)												
	Measured ng/L from 160 ng/L Spike												
Sample	PFBS	PFHxS	PFOS	PFDS	PFNS	PFHpS	PFPeS	FOSA	4:2 FTS	6:2 FTS	8:2 FTS	N-Et FOSAA	N-MeFOSAA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	138	147	146	151	148	142	143	150	141	147	159	158	153
Spiked 2	127	134	140	144	146	142	139	146	133	141	148	150	143
Spiked 3	134	142	148	143	144	143	133	143	134	139	151	151	148
Spiked 4	140	143	150	151	153	148	144	152	146	149	155	159	151
Spiked 5	136	146	147	151	149	146	140	151	139	141	154	154	150
Spiked 6	140	142	149	149	151	151	141	148	140	145	167	148	158
Average Recovery (ng/L)	136	142	147	148	148	145	140	148	139	144	156	153	150
% Average Recovery	84.7	88.8	91.7	92.7	92.8	90.8	87.5	92.7	86.8	89.8	97.2	95.8	94.0
Standard Deviation	4.98	4.74	3.76	3.59	3.30	3.51	3.81	3.41	4.78	3.96	6.48	4.75	4.93
RSD (%)	3.67	3.33	2.56	2.42	2.22	2.42	2.72	2.30	3.44	2.76	4.17	3.10	3.28

TABLE X1.6 Precision and Accuracy Study for Additional PFASs in Sewage Treatment Plant IV (Influent Sample)

	Stickney, IL, POTW (Influent Sample)										
	Measured ng/L from 160 ng/L Spike for All PFASs Except PFBA and PFPeA (800 ng/L Spike)										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	133	143	146	142	150	146	158	141	150	648	378
Spiked 2	129	138	142	142	150	148	155	144	152	649	390
Spiked 3	125	142	147	149	157	153	164	148	161	666	410
Spiked 4	131	139	143	140	148	146	156	136	148	615	337
Spiked 5	134	150	152	158	162	162	171	153	165	678	413
Spiked 6	132	141	144	146	152	150	161	139	150	633	345
Average Recovery (ng/L)	131	142	146	146	153	151	161	144	154	648	379
% Average Recovery	81.7	88.9	91.1	91.3	95.8	94.3	101	89.8	96.5	81.0	47.3
Standard Deviation	3.19	4.12	3.61	6.63	5.19	6.11	5.98	6.25	6.94	22.4	32.0
RSD (%)	2.44	2.90	2.48	4.54	3.39	4.05	3.72	4.35	4.50	3.45	8.46

TABLE X1.7 Precision and Accuracy Study for Additional PFASs in Sewage Treatment Plant IV (Influent Sample)

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Stickney, IL, POTW (Influent Sample)												
	Measured ng/L from 160 ng/L Spike												
Sample	PFBS	PFHxS	PFOS	PFDS	PFNS	PFHpS	PFPeS	FOSA	4:2 FTS	6:2 FTS	8:2 FTS	N-Et FOSAA	N-MeFOSAA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.8	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	18.2	ND	ND	ND
Spiked 1	139	146	150	144	143	140	140	128	153	146	149	154	153
Spiked 2	144	141	152	143	150	142	143	128	151	145	162	153	157
Spiked 3	152	153	158	139	148	149	145	130	156	146	164	156	155
Spiked 4	138	143	141	146	142	145	136	127	150	143	155	145	155
Spiked 5	156	153	158	149	151	151	151	132	167	158	163	163	163
Spiked 6	140	148	154	146	147	145	143	126	148	142	151	152	159
Average Recovery (ng/L)	145	147	152	144	147	145	143	129	154	146	157	154	157
% Average Recovery	90.5	92.1	95.1	90.3	91.8	90.8	89.4	80.4	96.4	91.5	98.4	96.2	98.0
Standard Deviation	7.30	4.86	6.13	3.59	3.63	4.03	4.80	2.10	6.82	5.87	6.42	5.71	3.57
RSD (%)	5.05	3.30	4.03	2.48	2.47	2.78	3.35	1.63	4.42	4.01	4.08	3.71	2.28

TABLE X1.8 Precision and Accuracy Study for Additional PFASs in Chicago River Water II

NOTE 1—P&A concentration for each analyte are values after subtracting average unspiked concentration.

	Chicago River Water										
	Measured ng/L from 160 ng/L Spike for All PFASs Except PFBA and PFPeA (800 ng/L Spike)										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	10.4	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	10.1	ND	11.2	ND	ND
Spiked 1	149	161	155	151	147	147	138	147	145	764	538
Spiked 2	159	165	162	156	158	158	148	153	152	772	561
Spiked 3	165	172	162	160	155	162	147	157	154	751	575
Spiked 4	162	168	160	157	154	157	146	152	146	767	500
Spiked 5	160	164	160	152	158	156	139	154	146	752	542
Spiked 6	167	170	164	157	157	157	152	153	148	764	561
Average Recovery (ng/L)	160	167	161	155	155	156	145	153	149	762	546
% Average Recovery	100	104	100	97.1	96.8	97.6	90.6	95.5	92.9	95.2	68.3
Standard Deviation	6.49	4.28	3.11	3.36	4.36	4.95	5.11	3.31	3.70	8.27	26.3
RSD (%)	4.04	2.57	1.94	2.16	2.81	3.17	3.52	2.16	2.49	1.09	4.82

TABLE X1.9 Precision and Accuracy Study for Additional PFASs in Chicago River Water II

	Chicago River Water												
	Measured ng/L from 160 ng/L Spike												
Sample	PFBS	PFHxS	PFOS	PFDS	PFNS	PFHpS	PFPeS	FOSA	4:2 FTS	6:2 FTS	8:2 FTS	N-Et FOSAA	N-MeFOSAA
Unspiked 1	ND	ND	13.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	16.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	143	165	157	172	164	166	160	150	155	150	172	167	157
Spiked 2	152	170	162	177	176	167	162	150	158	170	172	179	163
Spiked 3	155	165	163	178	172	170	163	151	160	165	172	163	158
Spiked 4	150	167	169	175	172	170	165	148	153	160	170	163	156
Spiked 5	146	161	162	172	168	165	160	147	153	164	172	171	157
Spiked 6	150	163	169	176	170	165	161	153	154	167	175	173	157
Average Recovery (ng/L)	149	165	163	175	170	167	162	150	156	163	172	169	158
% Average Recovery	93.4	103	102	109	106	104	101	93.6	97.2	102	108	106	98.6
Standard Deviation	4.09	3.25	4.56	2.28	3.97	2.39	1.86	2.13	2.85	7.17	1.76	6.32	2.62
RSD (%)	2.74	1.97	2.79	1.30	2.33	1.43	1.15	1.42	1.83	4.40	1.02	3.73	1.66

TABLE X1.10 Precision and Accuracy Study for Additional PFASs in Lake Water

	Lake Michigan Water										
	Measured ng/L from 160 ng/L Spike for All PFASs Except PFBA and PFPeA (800 ng/L Spike)										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	160	164	162	154	156	158	155	152	155	728	566
Spiked 2	160	155	160	151	152	155	144	144	145	642	398
Spiked 3	159	160	154	155	149	157	153	148	151	701	521
Spiked 4	161	160	156	146	144	151	142	143	145	680	510
Spiked 5	152	155	158	147	148	155	146	145	145	698	540
Spiked 6	159	162	160	154	150	153	148	149	137	589	359
Average Recovery (ng/L)	159	159	158	151	150	155	148	147	146	673	482
% Average Recovery	99.1	99.6	98.8	94.4	93.6	96.8	92.5	91.8	91.5	84.1	60.3
Standard Deviation	3.14	3.69	3.05	3.64	3.91	2.68	4.82	3.57	6.07	50.1	83.4
RSD (%)	1.98	2.32	1.93	2.41	2.61	1.73	3.25	2.43	4.15	7.45	17.3

TABLE X1.11 Precision and Accuracy Study for Additional PFASs in Lake Water

	Lake Michigan Water												
	Measured ng/L from 160 ng/L Spike												
Sample	PFBS	PFHxS	PFOS	PFDS	PFNS	PFHpS	PFPeS	FOSA	4:2 FTS	6:2 FTS	8:2 FTS	N-Et FOSAA	N-MeFOSAA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	150	164	174	176	167	169	161	155	161	168	184	184	164
Spiked 2	136	159	164	172	172	168	148	153	150	167	172	170	159
Spiked 3	139	166	165	171	170	168	160	152	154	166	173	167	163
Spiked 4	137	158	158	172	170	162	157	153	155	166	168	173	157
Spiked 5	141	158	162	167	166	158	155	151	154	155	159	166	151
Spiked 6	134	158	166	172	173	163	151	148	147	157	179	178	157
Average Recovery (ng/L)	140	160	165	172	170	165	155	152	153	163	173	173	159
% Average Recovery	87.3	100	103	107	106	103	97.0	94.9	95.7	102	108	108	99.1
Standard Deviation	5.79	3.54	5.30	2.85	2.55	4.37	4.95	2.31	4.65	5.79	8.68	6.92	4.72
RSD (%)	4.15	2.21	3.22	1.66	1.50	2.66	3.19	1.52	3.04	3.56	5.03	4.00	2.98

TABLE X1.12 Precision and Accuracy Study for Additional PFASs in Ground Water

	Homer Glen, IL, Ground Water										
	Measured ng/L from 160 ng/L Spike for All PFASs Except PFBA and PFPeA (800 ng/L Spike)										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	155	165	161	156	158	157	149	151	160	772	543
Spiked 2	148	155	153	149	148	153	142	149	152	748	530
Spiked 3	146	153	154	148	147	155	149	148	152	737	537
Spiked 4	147	153	152	146	145	152	140	143	153	735	466
Spiked 5	149	154	156	149	150	154	148	148	153	742	531
Spiked 6	153	161	158	151	149	156	144	148	157	797	553
Average Recovery (ng/L)	150	157	156	150	149	155	145	148	154	755	527
% Average Recovery	93.6	98.0	97.3	93.8	93.3	96.6	90.8	92.5	96.5	94.4	65.8
Standard Deviation	3.46	4.96	3.29	3.53	4.63	1.93	3.80	2.50	3.45	24.7	30.7
RSD (%)	2.31	3.16	2.12	2.35	3.10	1.25	2.62	1.69	2.24	3.27	5.83

TABLE X1.13 Precision and Accuracy Study for Additional PFASs in Ground Water

	Homer Glen, IL, Ground Water												
	Measured ng/L from 160 ng/L Spike												
Sample	PFBS	PFHxS	PFOS	PFDS	PFNS	PFHpS	PFPeS	FOSA	4:2 FTS	6:2 FTS	8:2 FTS	N-Et FOSAA	N-MeFOSAA
Unspiked 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unspiked 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spiked 1	163	160	168	171	165	165	160	150	160	163	175	170	161
Spiked 2	157	161	159	166	168	161	158	148	150	162	163	170	158
Spiked 3	157	157	162	162	165	160	152	144	150	152	162	164	151
Spiked 4	156	160	164	156	162	158	149	149	149	147	163	158	151
Spiked 5	158	161	163	165	166	169	155	148	154	157	168	171	160
Spiked 6	159	164	163	168	164	164	152	146	149	153	163	167	154
Average Recovery (ng/L)	158	160	163	165	165	163	155	148	152	156	166	167	156
% Average Recovery	98.9	100	102	103	103	102	96.6	92.3	95.0	97.3	104	104	97.4
Standard Deviation	2.61	2.27	2.84	5.11	2.02	4.06	4.18	2.12	4.44	6.07	4.88	4.85	4.45
RSD (%)	1.65	1.42	1.74	3.10	1.22	2.49	2.70	1.43	2.92	3.90	2.95	2.91	2.85

TABLE X1.14 Surrogate Recoveries for Precision and Accuracy Study in Sewage Treatment Plant IV (Effluent)

	Surrogates – Stickney, IL, POTW (Effluent Sample – 160 ng/L Spike)													
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA	M4:2 FTS	M6:2 FTS	M8:2 FTS	MN-Et FOSAA	MN-MeFOSAA
Unspiked 1	81.2	147	154	149	149	149	149	152	151	135	148	151	163	157
Unspiked 2	85.9	144	149	148	145	145	142	142	142	128	136	147	154	145
Spiked 1	86.2	141	147	141	141	138	144	144	145	139	150	147	154	146
Spiked 2	86.2	140	146	143	138	141	141	143	142	132	148	142	151	148
Spiked 3	70.4	150	154	152	153	156	154	151	152	140	154	163	164	156
Spiked 4	76.9	143	147	147	147	145	144	144	145	134	147	160	155	149
Spiked 5	88.4	145	150	148	145	145	143	145	144	137	143	156	159	152
Spiked 6	78.6	146	147	149	141	144	148	145	147	136	150	163	151	151
Average Recovery (ng/L)	81.0	145	149	147	145	145	145	146	146	135	147	154	156	151
% Average Recovery	50.6	90.4	93.3	92.0	90.6	90.9	90.9	91.0	91.2	84.6	91.8	96.1	97.6	94.1
Standard Deviation	5.88	3.14	3.36	3.60	4.72	5.40	4.27	3.67	3.86	3.78	5.23	8.03	5.14	4.18
RSD (%)	7.25	2.17	2.25	2.44	3.26	3.72	2.94	2.52	2.64	2.79	3.56	5.23	3.29	2.78

TABLE X1.15 Surrogate Recoveries for Precision and Accuracy Study in Sewage Treatment Plant IV (Influent)

	Surrogates – Stickney, IL, POTW (Influent Sample – 160 ng/L spike)													
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA	M4:2 FTS	M6:2 FTS	M8:2 FTS	MN-Et FOSAA	MN-MeFOSAA
Unspiked 1	72.4	149	151	148	148	148	152	145	146	134	144	148	154	157
Unspiked 2	77.5	148	152	151	153	149	156	152	149	147	147	156	155	162
Spiked 1	70.3	147	145	146	147	138	151	142	147	145	146	153	152	153
Spiked 2	73.9	151	153	151	148	145	153	145	144	151	149	149	157	158
Spiked 3	74.6	152	153	154	152	150	153	148	146	154	156	157	156	158
Spiked 4	66.6	141	144	144	143	140	148	143	145	137	148	155	149	154
Spiked 5	82.7	165	161	163	157	151	161	159	154	158	161	169	170	167
Spiked 6	67.5	149	147	148	151	146	150	145	144	147	146	148	156	153
Average Recovery (ng/L)	73.2	150	151	151	150	146	153	147	147	147	150	154	156	158
% Average Recovery	45.7	94.0	94.3	94.1	93.7	91.2	95.6	92.1	91.8	91.6	93.5	96.5	97.7	98.7
Standard Deviation	5.29	6.59	5.54	6.03	4.32	4.65	4.09	5.54	3.34	8.19	5.91	7.02	6.16	4.62
RSD (%)	7.22	4.38	3.67	4.01	2.88	3.19	2.67	3.76	2.27	5.59	3.95	4.55	3.94	2.92

TABLE X1.16 Surrogate Recoveries for Precision and Accuracy Study in Ground Water

	Surrogates — Homer Glen, IL, Ground Water (Ground Water Sample – 160 ng/L Spike)													
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA	M4:2 FTS	M6:2 FTS	M8:2 FTS	MN-Et FOSAA	MN-MeFOSAA
Unspiked 1	119	156	163	155	156	171	154	159	155	156	153	160	176	166
Unspiked 2	113	155	164	154	154	170	160	158	158	158	164	172	171	170
Spiked 1	107	152	161	151	154	167	157	154	157	156	161	163	176	161
Spiked 2	116	152	167	154	153	172	157	158	158	162	161	185	177	167
Spiked 3	114	152	161	151	153	166	152	154	153	158	158	167	170	159
Spiked 4	104	147	158	151	147	164	150	151	151	156	153	167	165	155
Spiked 5	113	156	169	156	158	176	156	159	158	158	157	176	176	163
Spiked 6	113	154	159	158	156	173	156	154	157	160	158	170	180	166
Average Recovery (ng/L)	112	153	163	154	154	170	155	156	156	158	158	170	174	163
% Average Recovery	70.1	95.7	102	96.1	96.1	106	97.0	97.5	97.4	98.7	98.8	106	109	102
Standard Deviation	4.76	2.78	3.80	2.69	3.17	3.96	3.10	2.97	2.63	2.03	3.81	7.81	4.87	4.77
RSD (%)	4.24	1.82	2.33	1.75	2.06	2.33	2.00	1.90	1.69	1.28	2.41	4.59	2.80	2.92

TABLE X1.17 Surrogate Recoveries for Precision and Accuracy Study in Lake Water

	Surrogates — Lake Michigan Water (Lake Water Sample – 160 ng/L Spike)													
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA	M4:2 FTS	M6:2 FTS	M8:2 FTS	MN-Et FOSAA	MN-MeFOSAA
Unspiked 1	107	148	158	150	152	169	157	154	158	150	155	168	182	167
Unspiked 2	112	158	173	161	162	183	164	163	165	155	163	167	192	175
Spiked 1	112	154	163	155	159	172	157	161	160	166	168	178	173	168
Spiked 2	81.3	151	162	154	156	171	160	158	159	155	166	171	176	169
Spiked 3	102	152	164	155	155	171	156	155	158	160	162	180	173	167
Spiked 4	106	148	164	156	156	174	157	160	163	159	164	179	179	164
Spiked 5	112	153	163	156	152	169	154	154	156	160	163	172	168	162
Spiked 6	71.1	137	155	149	148	167	145	150	155	148	154	164	182	165
Average Recovery (ng/L)	100	150	163	155	155	172	156	157	159	157	162	172	178	167
% Average Recovery	62.8	93.8	102	96.6	96.9	107	97.7	97.9	99.5	97.8	101	108	111	104
Standard Deviation	15.6	6.19	5.04	3.73	4.31	4.96	5.44	4.41	3.27	5.80	4.77	6.05	7.33	4.04
RSD (%)	15.6	4.13	3.10	2.41	2.78	2.89	3.48	2.82	2.05	3.71	2.95	3.51	4.12	2.42

TABLE X1.18 Surrogate Recoveries for Precision and Accuracy Study in River Water

	Surrogates — Chicago River Water (River Water Sample – 160 ng/L Spike)													
Sample	MPFBA	MPFHxA	MPFHxS	MPFOA	MPFNA	MPFOS	MPFDA	MPFUnA	MPFDoA	M4:2 FTS	M6:2 FTS	M8:2 FTS	MN-Et FOSAA	MN-MeFOSAA
Unspiked 1	121	153	164	161	158	174	157	157	161	160	156	167	175	167
Unspiked 2	115	157	167	155	157	177	161	158	157	158	152	168	173	168
Spiked 1	117	149	165	153	153	171	153	154	158	157	155	167	167	165
Spiked 2	121	154	169	157	160	177	156	161	159	165	172	178	177	162
Spiked 3	118	153	164	156	155	169	155	157	159	163	166	181	171	161
Spiked 4	107	152	158	154	149	168	155	160	157	154	156	173	166	164
Spiked 5	116	153	163	156	153	170	158	161	163	157	156	168	171	162
Spiked 6	119	156	165	154	155	173	162	160	161	164	159	182	173	169
Average Recovery (ng/L)	117	153	164	156	155	172	157	158	160	160	159	173	172	165
% Average Recovery	72.9	95.8	103	97.3	96.9	108	98.2	99.0	99.7	99.8	99.4	108	107	103
Standard Deviation	4.59	2.38	3.15	2.65	3.32	3.38	3.32	2.38	2.21	4.18	6.72	6.31	3.50	3.01
RSD (%)	3.93	1.56	1.91	1.70	2.14	1.96	2.11	1.50	1.38	2.62	4.22	3.65	2.04	1.83

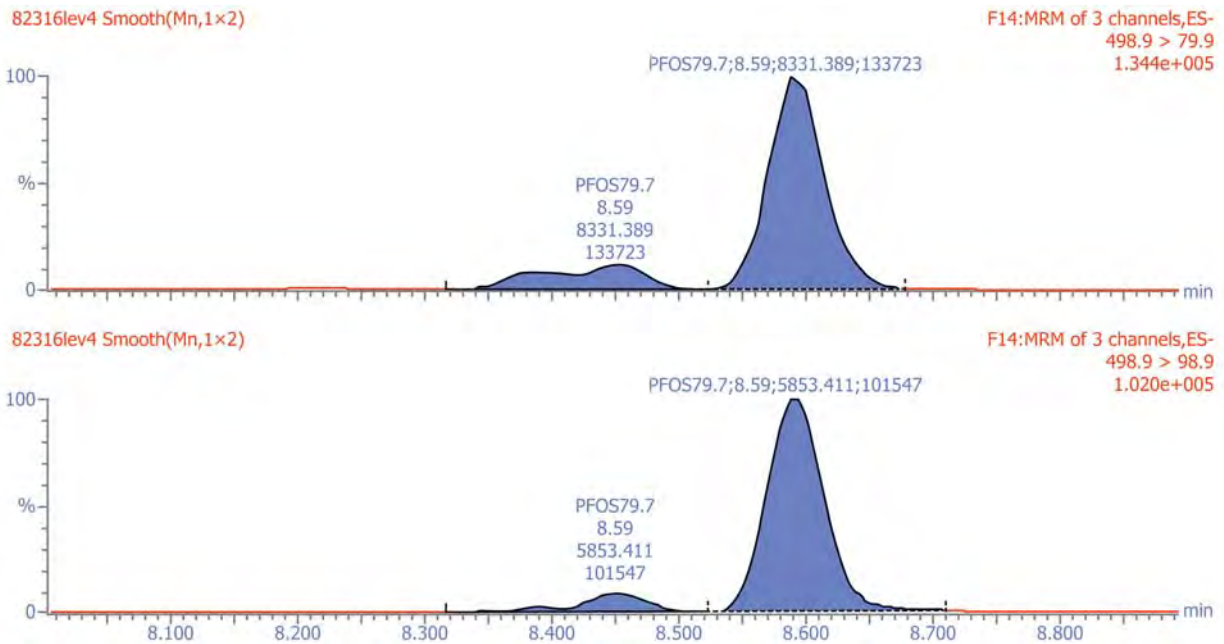
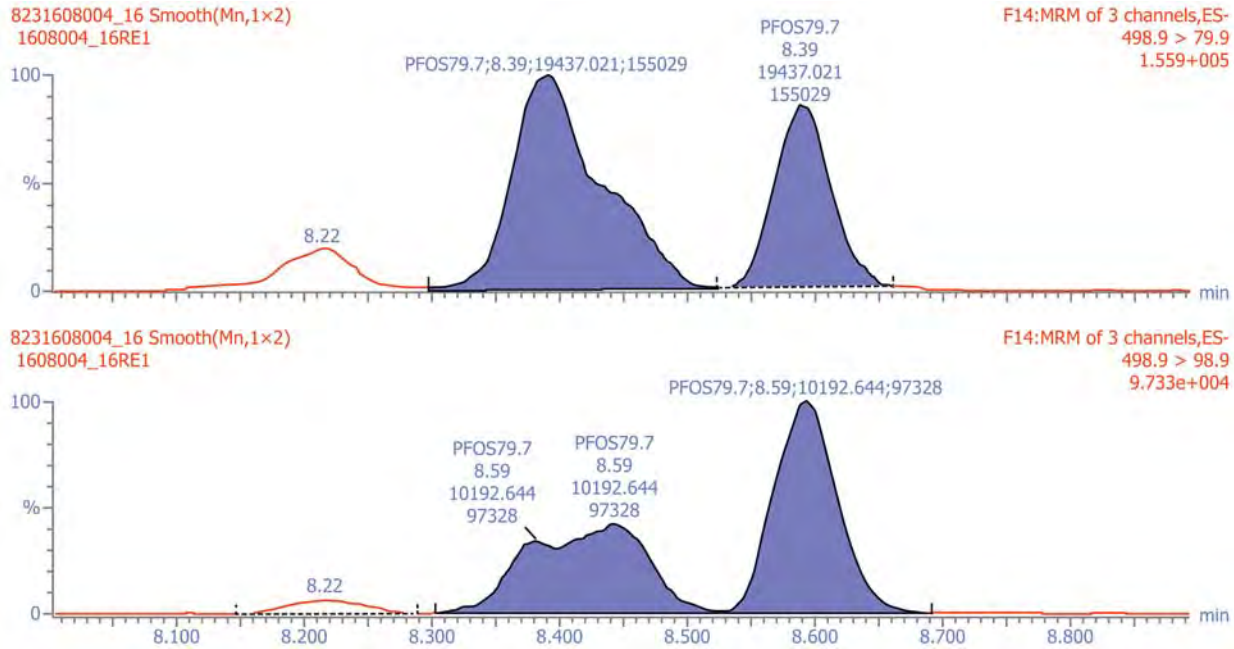


FIG. X1.1 PFOS in Calibration Standard



NOTE 1—The peak at 8.22 minutes is probably another isomer group of PFOS, but it is not included in the calibration standard so it cannot be included here for quantitation.

FIG. X1.2 PFOS in Groundwater Sample

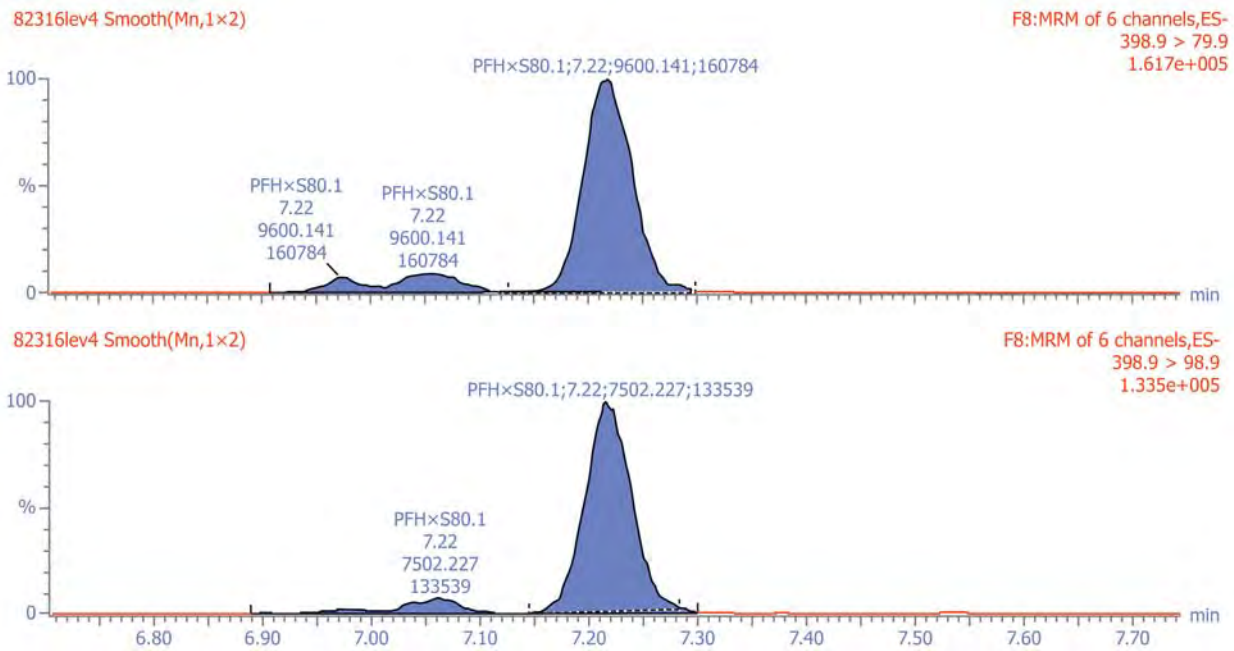


FIG. X1.3 PFHxS in Calibration Standard

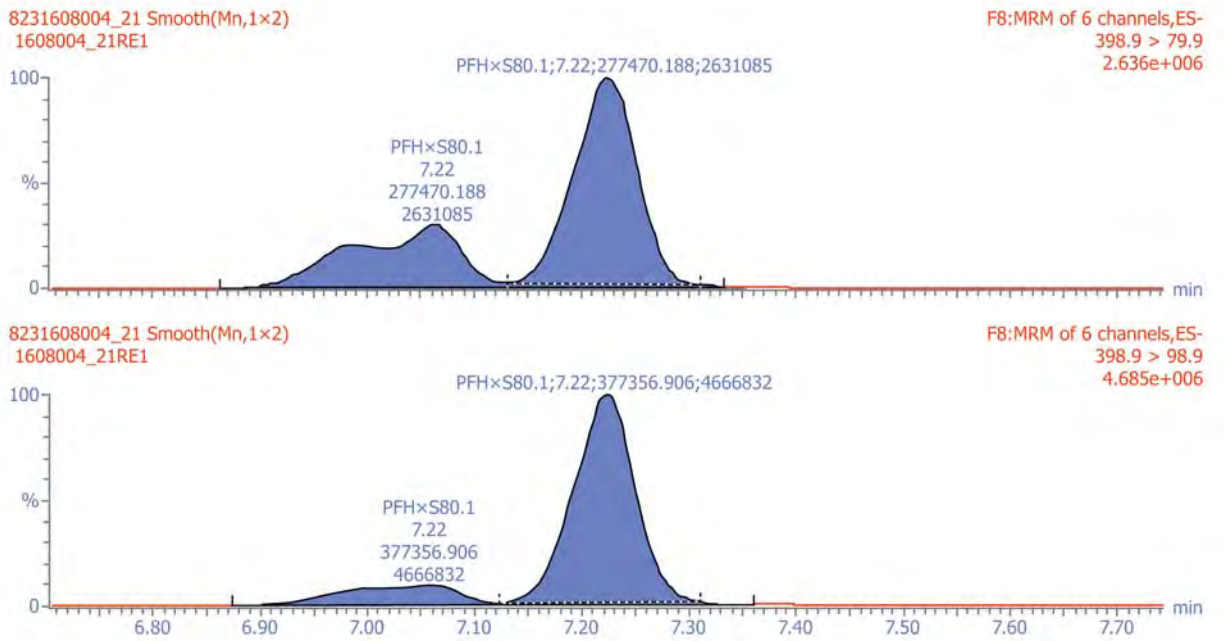


FIG. X1.4 PFHxS in Groundwater Sample

X2. HOLDING TIME STUDY

X2.1 This holding time study had two objectives related to the analysis of poly- and per-fluorinated alkyl substances (PFAS) in non-potable water matrices:

(1) To determine the sample stability or holding time, and

(2) To evaluate appropriate sample containers.

X2.1.1 The list of PFAS included in this study are shown in Table X2.1. These chemicals are of emerging concern and holding times for matrices listed in Test Method D7979 have not been established. Sample stability was evaluated by sampling spiked water samples. Three types of sample containers were compared: glass, high density polyethylene, and polypropylene. The purpose of this study was to determine noticeable loss of analytes with time, which is an important factor in the multi-laboratory validation of Test Method D7979.

TABLE X2.1 List of PFAS in Holding Time Study

NOTE 1—Concentration of each analyte in the individual samples.

Chemical/Analytes	Acronym	CAS	Initial Conc. in Each Container (ng/L)
Perfluorotetradecanoate	PFTreA	376-06-7	160
Perfluorotridecanoate	PFTriA	72629-94-8	160
Perfluorododecanoate	PFDoA	307-55-1	160
Perfluoroundecanoate	PFUnA	2058-94-8	160
Perfluorodecanoate	PFDA	335-76-2	160
Perfluorononanoate	PFNA	375-95-1	160
Perfluorooctanoate	PFOA	335-67-1	160
Perfluoroheptanoate	PFHpA	375-85-9	160
Perfluorohexanoate	PFHxA	307-24-4	160
Perfluoropentanoate	PFPeA	2706-90-3	800
Perfluorobutanoate	PFBA	375-22-4	800
Perfluorodecylsulfonate	PFDS	335-77-3	160
Perfluorononylsulfonate	PFNS	68259-12-1	160
Perfluorooctylsulfonate	PFOS	1763-23-1	160
Perfluoroheptylsulfonate	PFHpS	375-92-8	160
Perfluorohexylsulfonate	PFHxS	3871-99-6	160
Perfluoropentylsulfonate	PFPeS	2706-91-4	160
Perfluorobutylsulfonate	PFBS	29420-49-3	160
Perfluorooctanesulfonamide	PFOSA	754-91-6	160
Fluorotelomer sulfonate 8	FtS 8:2	39108-34-4	160
Fluorotelomer sulfonate 6:2	FtS 6:2	27619-97-2	160
Fluorotelomer sulfonate 4:2	FtS 4:2	757124-72-4	160
N-EtFOSAA	NEtFOSAA	2991-50-6	160
N-MeFOSAA	NMeFOSAA	2355-31-9	160

X2.1.2 The ASTM D19.06 Test Method D7979 task group performed analysis using an analyte list obtained from the Office of Superfund Remediation and Technology Innovation (OSRTI). The study was conducted using ASTM Type I reagent water and POTW influent. The study began December 6, 2016 and was completed February 6, 2017.

X2.2 Holding Time Study That Removed Aliquots From the Bottle (Aliquoting)

X2.2.1 This study evaluated holding times using three different types of containers at: 0, 3, 7, 14, 21, and 30 days for reagent water and 0, 4, 11, 18, and 27 days for POTW influent. The containers used were: 50-mL polypropylene Falcon[17] tubes, 120 mL amber glass (Amber Type III soda lime) bottles with hard plastic lids, and 250-mL high density polyethylene (HDPE) bottles. A 5-mL sample was removed from the specified container at each sampling day event, spiked with surrogates and analyzed in accordance with Test Method D7979. Three samples of reagent water from the three different sample containers were analyzed each day along with full quality control samples as specified in the test method. The average recoveries of three samples are shown in Figs. X2.1-X2.3. In addition, two samples for each POTW influent water from the three different sample containers were analyzed each day along with full quality control samples as specified in the test method. The average recoveries of the two influent water samples on the various days are shown in Figs. X2.1-X2.3.

[17] Falcon is a trademark of Corning Incorporate in Corning, NY.

X2.3 Holding Time Study Using the Entire Sample (No Aliquoting)

X2.3.1 This study evaluated PFC holding times using polypropylene and borosilicate glass tubes in POTW influent at 0, 3, 5, 11, 20, and 31 days for the polypropylene tubes and 0, 9, and 20 days for borosilicate glass tubes. The containers used were: 15-mL polypropylene Falcon tubes and 15 mL borosilicate centrifugal conical screw top glass tubes. Eighteen individual 5-mL samples were prepared in polypropylene tubes at the concentrations in Table X2.1 and twelve individual 5-mL samples were prepared in borosilicate glass tubes at the concentrations in Table X2.1. The entire 5 mL sample from each specified container at each sample event, was spiked with surrogates and analyzed in accordance with Test Method D7979. Three samples stored in polypropylene tubes were used each day along with full quality control samples as specified in the method. The average recoveries of three samples are shown in Fig. X2.4. Two samples stored in glass tubes were analyzed each day with full quality control samples as specified in the method. The average recoveries of two influent water samples stored in glass tubes on the various days are shown in Fig. X2.5 along with related polypropylene recovery data.

X2.4 Summary of Holding Time Study and Conclusions

X2.4.1 When taking aliquots (removing sample from the original sample container), the three different sample bottles all displayed unacceptable losses for many of the analytes. The POTW influent displayed more appreciable losses of than reagent water.

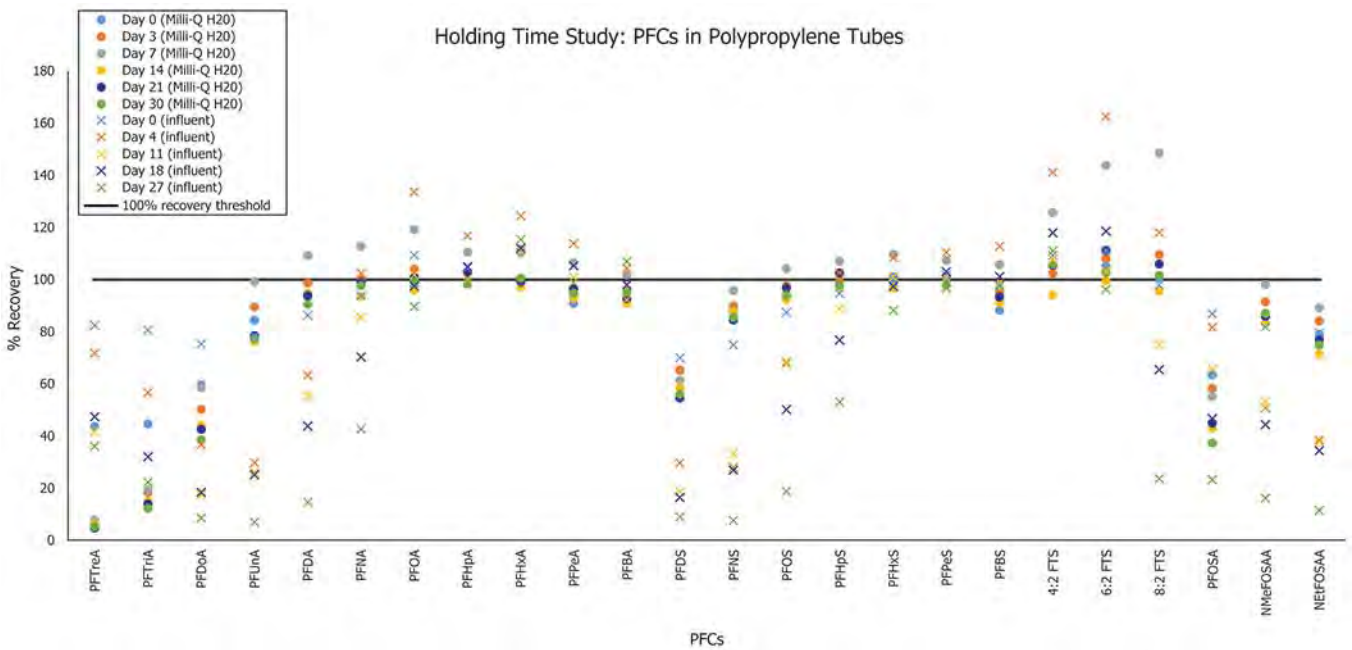


FIG. X2.1 Holding Time Study: PFCs in Polypropylene Tubes (Aliquot from Tubes)

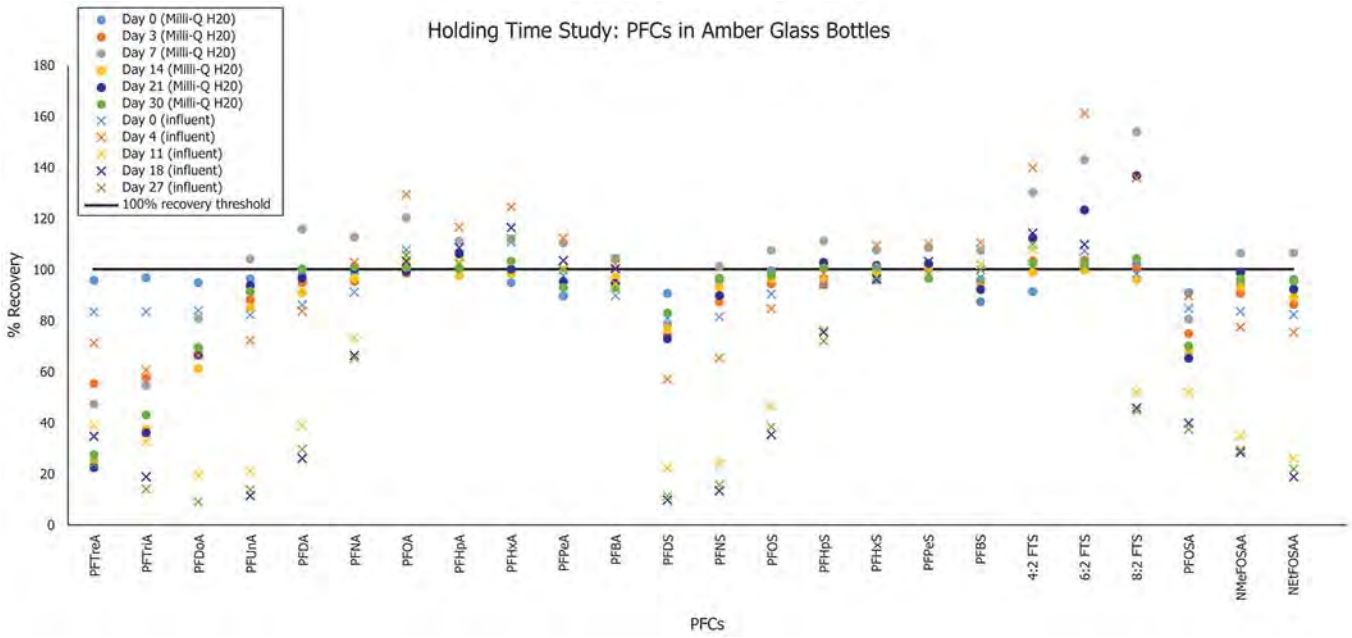


FIG. X2.2 Holding Time Study: PFCs in Amber Glass Bottles (Aliquot from Bottle)

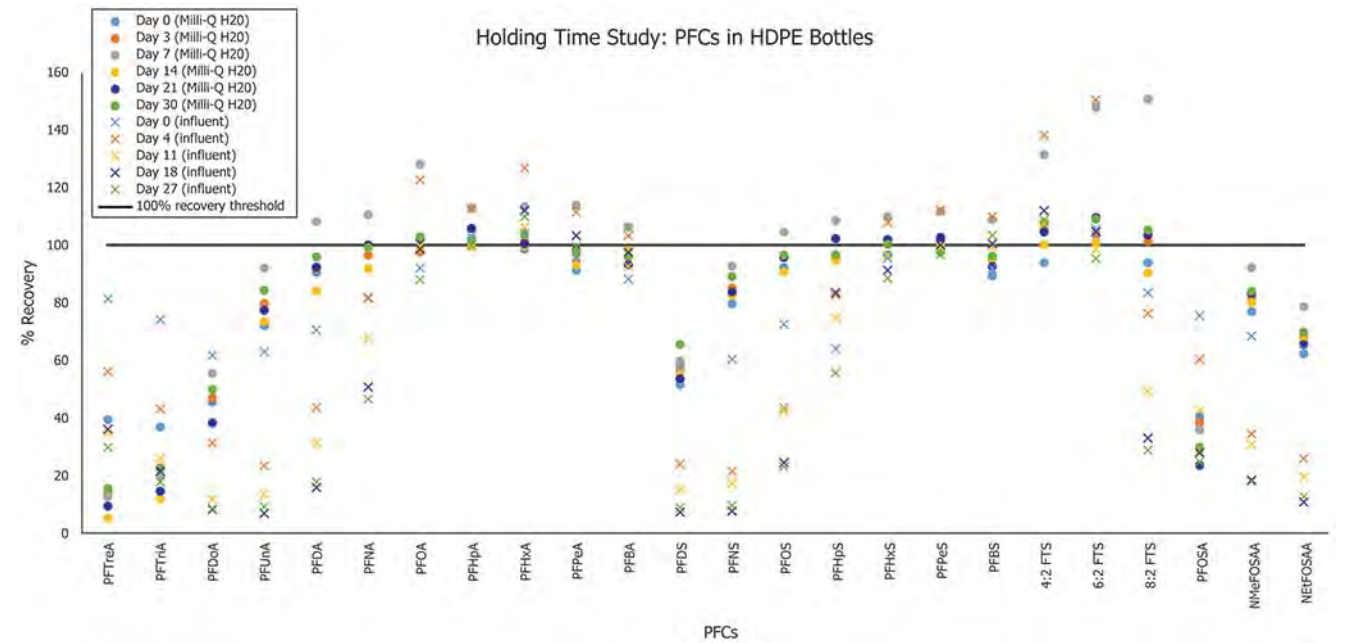


FIG. X2.3 Holding Time Study: PFCs in HDPE Bottles (Aliquot from Bottle)

X2.4.2 Acceptable recoveries were obtained for up to 31 days in samples collected and stored in polypropylene tubes with no aliquoting (processing the entire sample in the collection tube). Glass tubes compared well with spiked influent samples using the entire sample; however, for safety, Test Method D7979 recommends polypropylene. Sample contact with any surfaces outside of the sample container should be avoided. Aliquoting of samples and sample transfer before the addition of methanol must be minimized or absent from any sample collection or processing.

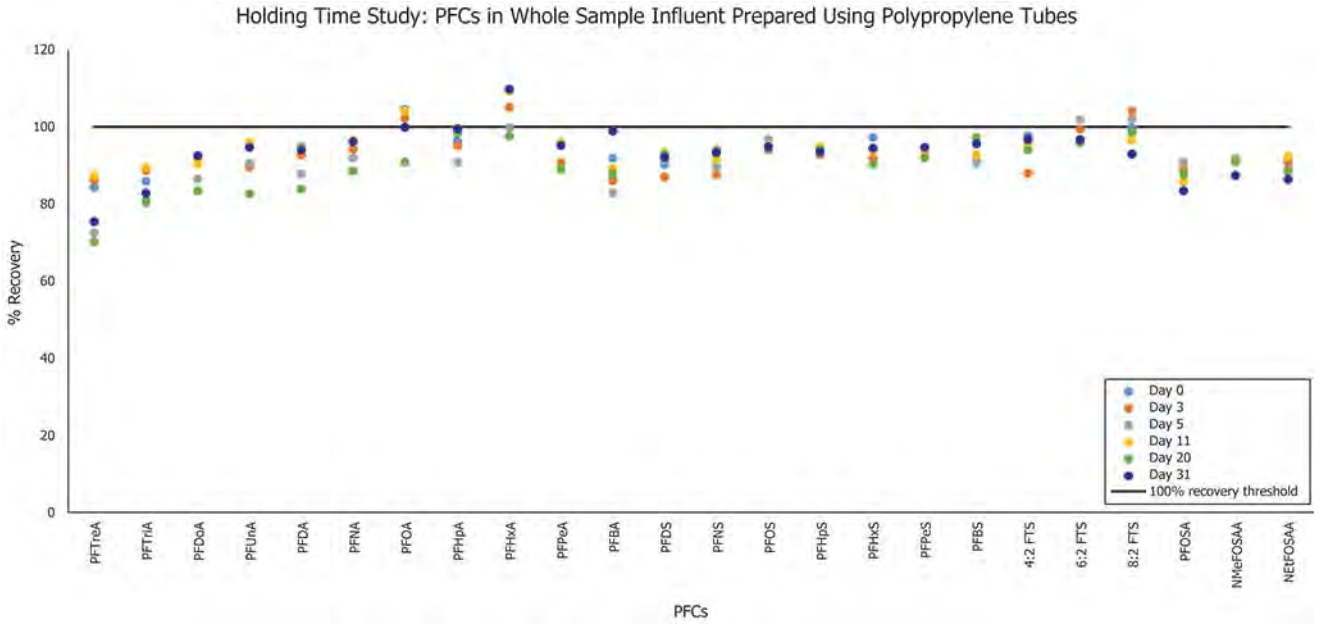


FIG. X2.4 Holding Time Study: PFCs in Whole Sample Influent Using Entire Sample (No Aliquot)

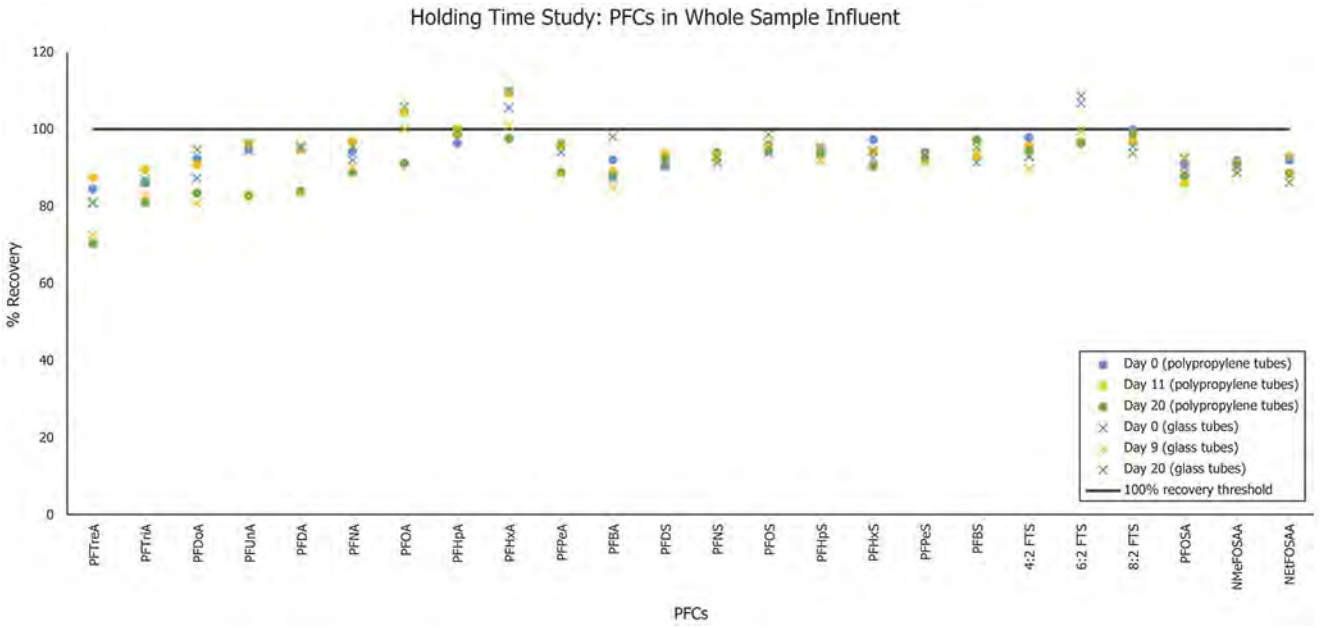


FIG. X2.5 Holding Time Study: Comparison of Polypropylene Tubes to Pyrex Glass Tubes for PFCs in Whole Sample Influent Using Entire Sample (No Aliquoting)

Attachment 12

METHOD 8327

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

TABLE OF CONTENTS

Disclaimer

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts formally trained in the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purposes of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

This method covers the analysis of selected per- and polyfluoroalkyl substances (PFAS) in prepared samples or sample extracts by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

The 24 PFAS that have been evaluated with this method are provided below. This method has been tested in surface water, groundwater, and wastewater matrices. Some precision and bias data are provided in Table 1 (Sec. 17.0). This determinative method may also be applicable to other PFAS target compounds and other matrices, provided that the laboratory can demonstrate adequate performance (refer to Sec. 9.0 or project-specific acceptance criteria) using representative sample matrices. Please refer to Method 8000 for additional information.

Analyte	Preparation Method 3512	CAS RN[†]
PFAS sulfonic acids		
Perfluoro-1-butanesulfonic acid (PFBS)	✓	375-73-5
Perfluoro-1-pentanesulfonic acid (PFPeS)	✓	2706-91-4
Perfluoro-1-hexanesulfonic acid (PFHxS)	✓	355-46-4
Perfluoro-1-heptanesulfonic acid (PFHpS)	✓	375-92-8
Perfluoro-1-octanesulfonic acid (PFOS)	✓	1763-23-1
Perfluoro-1-nonanesulfonic acid (PFNS)	✓	68259-12-1
Perfluoro-1-decanesulfonic acid (PFDS)	✓	335-77-3
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS)	✓	757124-72-4
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	*	27619-97-2

1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	✓	39108-34-4
PFAS carboxylic acids		
Perfluorobutanoic acid (PFBA)	✓*	375-22-4
Perfluoropentanoic acid (PFPeA)	✓*	2706-90-3
Perfluorohexanoic acid (PFHxA)	✓	307-24-4
Perfluoroheptanoic acid (PFHpA)	✓	375-85-9
Perfluorooctanoic acid (PFOA)	✓	335-67-1
Perfluorononanoic acid (PFNA)	✓	375-95-1
Perfluorodecanoic acid (PFDA)	✓	335-76-2
Perfluoroundecanoic acid (PFUnDA)	✓*	2058-94-8
Perfluorododecanoic acid (PFDoDA)	✓*	307-55-1
Perfluorotridecanoic acid (PFTrDA)	✓*	72629-94-8
Perfluorotetradecanoic acid (PFTeDA)	✓*	376-06-7
PFAS sulfonamides and sulfonamidoacetic acids		
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)	✓*	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)	✓*	2355-31-9
Perfluoro-1-octanesulfonamide (PFOSA)	✓	754-91-6

Performance data from a large multi-laboratory validation study were used to update this table (data can be found in Sec. 17.0, Table 1).

✓ Acceptable precision and bias can be obtained for this analyte with this preparation method.

✓* Acceptable precision and bias can be obtained for this analyte with this preparation method. However, this analyte may require special care to ensure analytical performance will meet the needs of the project. See Sec. 1.3 for specific information regarding this analyte.

* This analyte did not meet the criteria for acceptable performance using this preparation technique and determinative method and may require special care to ensure analytical performance will meet the needs of the project. See Sec. 1.3 for specific information regarding this analyte.

† Standards for some target analytes may consist of mixtures of structural isomers; however, the Chemical Abstracts Service (CAS) Registry Number (RN) listed in the table is for the linear isomer. All CAS RNs in the above table are for the acid form. Sulfonic acids in stock standard mixes are typically received as the sodium or potassium salt form. CAS RNs for the salt form are not included.

1.1 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600 and 8000) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and (ii) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this method is provided by the U.S. Environmental Protection Agency (EPA) as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.2 This method is restricted to use by, or under supervision of, appropriately experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.3 The following target compounds may require special treatment when being determined by this method:

1.3.1 During method development the following compounds showed a potential for loss either during standard preparation (resulting in low bias to calibration standards and high recoveries for samples) or during sample preparation (resulting in low recoveries). Extra care should be taken to ensure that the composition of the stock and intermediate standards (those above the high calibration standard) maintain a high enough proportion of organic cosolvent to limit loss from solution (See Sec. 7.4). Sub-sampling from aqueous sample containers prior to adding sufficient organic solvent will also result in a loss of these and potentially other compounds from solution, the extent of which will be container dependent (See Sec. 8.0).

Perfluoroundecanoic acid (PFUnDA)

Perfluorododecanoic acid (PFDoDA)

Perfluorotridecanoic acid (PFTrDA)

Perfluorotetradecanoic acid (PFTeDA)

N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)

N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)

1.3.2 The following compounds lack or have low abundance of secondary product ions, and interferences may make qualitative identification more difficult.

Perfluorobutanoic acid (PFBA).

Perfluoropentanoic acid (PFPeA)

Perfluorohexanoic acid (PFHxA)

Perfluoro-1-octanesulfonamide (PFOSA)

1.3.3 Background contamination must be carefully evaluated and managed to a level is acceptable for the project-specific data application. During validation of Method 3512, some laboratories had problems meeting quality control acceptance criteria for 1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS) due to high and/or sporadic background contamination. More information about identifying and minimizing sources of contamination is presented in Sec. 4.0.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared using an appropriate sample preparation method (e.g., solvent dilution or extraction). Prepared samples or extracts are then analyzed by LC/MS/MS using external standard calibration.

2.2 Target compounds are qualitatively identified in samples by comparing retention times (RTs) to RTs of isotopically labeled surrogates in the same samples or to RTs of target analytes in standards, as applicable, and by comparing product ion ratios to those in standards (Sec. 11.6, Sec. 17.0 Table 2). Qualitatively identified target compounds are then quantitated based on their primary product ion responses utilizing external standard calibration (Sec. 11.7).

3.0 DEFINITIONS

Refer to SW-846 Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure. See Glossary (Appendix A) for relevant terms and acronyms.

4.0 INTERFERENCES

4.1 In order to avoid compromising data quality, contamination of the analytical system by PFAS from the laboratory must be reduced to the lowest practical level. Method blanks (MBs) and reagent blanks (RBs) are prepared and analyzed with all samples and are used to demonstrate that laboratory supplies and preparation and analysis steps do not introduce interferences or PFAS artifacts at levels that would prevent the proper identification and integration of target analytes or bias quantitation, especially near the Lower Limit of Quantitation (LLOQ) or any project-specific concentration levels of interest. Careful selection of reagents and consumables is necessary because even low levels of PFAS contamination may alter the precision and bias of the method; background introduced by these materials (and variability thereof) is cumulative. See Sec. 9.5 for blank acceptance criteria. Refer to each method to be used for specific guidance on QC procedures and to SW-846 Chapter Four for general guidance on cleaning of reusable labware.

4.2 Refer to Methods 3500, 3600, and 8000 for discussions of interferences. Matrix interferences can be caused by contaminants from the sample, sampling devices, or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations of the sample matrix.

4.3 The following procedures are employed to minimize problems with measurement precision and bias.

4.3.1 All solvents should be LC/MS grade, or equivalent, to minimize interference problems. Solvents must be checked by lot prior to use.

4.3.2 PFAS contamination has been found in reagents, glassware, tubing, polytetrafluoroethylene (PTFE) LC vial caps, disposable pipets, aluminum foil, glass disposable pipettes, filters, degassers, and other apparatus that release fluorinated compounds. All supplies and reagents should be verified prior to use. If found, measures should be taken to remove the contamination, if possible, or find other suppliers or materials to use that meet method- or project-specified acceptance criteria.

4.3.3 The LC system used should have components replaced, where possible, with materials known to not contain PFAS target analytes of interest.

4.3.4 During method development, loss of some PFAS target analytes was observed during storage of standard solutions in 1:1 methanol-water containing 0.1% acetic acid in glass containers. Polypropylene containers should be used for preparation and storage of samples and standards. Other materials may be used, such as high density polyethylene (HDPE), if it can be shown the target analytes are not adversely affected (i.e., all quality control criteria in Sec. 9.0 can be met). Glass autosampler vials have been successfully used for solutions in 1:1 methanol-water containing 0.1% acetic acid during analysis.

4.3.5 An isolator column should be placed downstream of the solvent pumps and any mixer or degasser and before the sample injection valve to delay the elution of contaminants from the LC system to the analytical column.

4.3.6 If labware is re-used, the procedure described for labware cleaning (Sec. 6.2.4, or equivalent) should be followed to minimize risk of carryover contamination. The blank QC acceptance criteria in Sec. 9.5 can be used as a guideline for evaluating cleanliness.

4.4 Where measured analyte concentrations are suspected of being high-bias and/or false positive results due to contamination, the laboratory should inform the data user of any suspected data quality issues and qualify affected data appropriately.

4.5 High concentrations of the native 4:2 FTS, 6:2 FTS, and 8:2 FTS target analytes will interfere with the primary product ion signals listed in Sec. 17.0, Table 2 for the M2-4:2 FTS, M2-6:2 FTS and M2-8:2 FTS surrogates. This interference results from the natural abundance of the ^{34}S isotope (~4.2% abundance relative to ^{32}S) in the native FTS target analytes and can lead to high bias recovery of these surrogates. Using the secondary product ions identified in Table 2 for quantitation of these surrogates will minimize this interference because this

surrogate product ion loses the $^{13}C_2$-labeled fluorocarbon sidechain to produce $^{32}SO_3H^-$ (m/z 81), while the ^{34}S isotope of the target analyte produces $^{34}SO_3H^-$ (m/z 83) from the same nominal mass precursor ion.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of U.S. Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses.

5.2 Users of this method should operate a formal safety program.

5.3 The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. Exposure to these chemicals should be reduced to the lowest possible level, and the appropriate personal protective equipment (PPE) should be utilized. Review SDSs for specific physical and health hazards including appropriate PPE to be used. SDSs can be accessed at multiple locations (e.g., www.sigmaaldrich.com, www.well-labs.com, and www.isotope.com).

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those used during method development or subsequently evaluated by the Agency. Labware, reagents, supplies, equipment, and settings other than those listed in this method may be employed provided that method performance appropriate for the intended application has been demonstrated and documented, including meeting acceptance criteria for all categories of quality controls listed in Sec. 9.0. This section does not list all common labware (e.g., beakers and flasks) that might be used.

6.1 Equipment

6.1.1 Liquid chromatograph (LC) system: An ultra performance liquid chromatograph (UPLC®) with stainless steel flow through needle design was used to generate data during method development (PEEK needles may not puncture polyethylene caps; pre-slitting of caps is not allowed).

6.1.2 Analytical LC columns: The following columns were used to generate data during method development:

6.1.2.1 Acquity UPLC® CSHTM Phenyl-Hexyl, 2.1 x 100 mm and 1.7 µm particle size (Waters part no. 186005407)

6.1.2.2 ZORBAX RRHD Stable Bond C18, 2.1 x 100 mm and 1.8 µm particle size (Agilent part no. 858700-902)

6.1.2.3 Accucore RP 2.1 x 100 mm and 2.6 µm particle size (Thermo part no. 17626-102130)

6.1.2.4 Shim-pack SP-C18, 2.1 x 150 mm and 2.7 µm particle size (Shimadzu part no. 227-32003-04)

6.1.3 Isolator columns:

6.1.3.1 XBridge BEH C18, 2.1 x 50 mm and 3.5 µm particle size (Waters part no. 186003021)

6.1.3.2 ZORBAX RRHD Eclipse Plus C18, 50 × 3.0 mm, 1.8 µm (Agilent part no. 959757-302)

6.1.3.3 BDS HypersilC18, 2.1 x 50 mm and 5 µm particle size (Thermo part no. 28105-052130)

6.1.3.4 Shim-pack XR-ODS II, 2 x 75mm and 2.2 µm particle size (Shimadzu part no. 228-41605-93)

6.1.4 Tandem Mass Spectrometry (MS/MS) System: A mass spectrometer must be capable of MS/MS analysis with a cycle time sufficient to obtain at least ten mass spectra over each chromatographic peak. The system must be capable of documenting the performance of the MS/MS system against manufacturer specifications for mass resolution, mass assignment, and sensitivity using the internal calibrant (See Sec. 11). Sensitivity should be sufficient to meet project-specified needs in the matrices of interest, where practical (See Secs. 7.4.4.1 and 9.9). A triple quadrupole mass spectrometer with an electrospray ionization source was used to generate data during method development.

6.2 Support Equipment and Supplies

6.2.1 Adjustable volume pipettes, 10 µL to 10 mL with polypropylene tips.

6.2.2 Analytical balance, capable of weighing to 0.01 g for determining sample mass or to 0.0001 g for preparing standards from neat.

6.2.3 Sample containers and miscellaneous supplies; all supplies should meet blank criteria in Sec. 9.5 where practical.

6.2.3.1 Autosampler vials: HDPE, polypropylene or glass

6.2.3.2 Polyethylene autosampler vial caps (Waters Catalog # 186004169)

6.2.3.3 50-mL polypropylene tubes (BD Falcon, Catalog # 352098)

6.2.3.4 15-mL polypropylene tubes (BD Falcon, Catalog # 352097); pre-weighed tubes are recommended for collection of field samples and field QC

6.2.3.5 Polyethylene disposable pipettes (Samco Thermo Scientific, Catalog # 252)

6.2.3.6 Polypropylene pipette tips (Eppendorf, Catalog #s 022491997, 022492080, 022491954, 022491946, and 022491512)

6.2.4 Reusable labware cleaning instructions – If labware is re-used it should be washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. All labware is subsequently rinsed with organic solvent(s) such as acetone, methanol, and acetonitrile. Traces of target compounds should be reduced to a minimum.

7.0 REAGENTS AND STANDARDS

7.1 Chemicals used in all tests should be LC/MS grade if available, or reagent grade at a minimum. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be verified prior to use to ensure the blank acceptance criteria in Sec. 9.5 can be met.

7.2 Reagent water

All references to water in this method refer to reagent water as defined in SW-846 Chapter One, unless otherwise specified. Reagent water from in-house deionized water treatment systems may need additional treatment prior to use (e.g., with a point-of-use water purification system) to meet blank acceptance criteria (Sec. 9.5). Bottled reagent water should be evaluated in the same manner as reagent water from other sources.

7.3 Reagents and Gases

7.3.1 Acetonitrile, C_2H_3N (CAS RN 75-05-8)

7.3.2 Ultrapure argon and nitrogen

7.3.3 Methanol, CH_3OH (CAS RN 67-56-1)

7.3.4 Isopropyl alcohol, C_3H_8O (CAS RN 67-63-0)

7.3.5 Sodium hydroxide, NaOH (CAS RN 1310-73-2)

7.3.6 Ammonium acetate, $C_2H_7NO_2$ (CAS RN 631-61-8), neat

7.3.7 Glacial acetic acid, CH_3COOH (CAS RN 64-19-7)

7.4 PFAS standard solutions

The following sections describe preparation of stock solutions, spiking solutions, and calibration standards for the compounds of interest. This discussion is provided as an example. Other approaches and concentrations of the target compounds may be used if appropriate for

the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.4.1 Storage and Shelf-life

While not in use, store standard solutions at ≤6°C in the refrigerator or according to the manufacturer's recommended storage conditions. Bring standard solutions to room temperature and mix well prior to use. Keep standard solutions away from PFAS-containing packaging and materials.

Use the manufacturer's expiration date for purchased prepared standards and neat source materials, as applicable. The laboratory may develop QC practices for determining expiration dates for standards prepared from certified reference materials; otherwise, use one year from date of preparation for expiration of prepared standard solutions. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards or spiking solutions.

7.4.2 Stock standards

Solutions may be purchased as certified solutions or prepared from neat certified reference materials.

For standard solutions prepared from neat materials, the weight may be used without correction to calculate the concentration of the stock standard when standard compound purity is assayed to be 96% or greater. Commercially prepared stock standards may be used at any concentration if they are certified by an accredited supplier or third party.

NOTE: Esterification of fluorinated carboxylic acids in methanolic solutions is known to occur over time. If base is not already present, adding a small amount of strong base to stock standards will improve stability of these analytes in methanol. The Wellington stock standards listed below include 4 mole equivalents of sodium hydroxide (*i.e.*, 4 moles of OH^- per mole of PFAS target analytes). The equation below can be used to estimate an amount of sodium hydroxide to add, which uses an assumed molar mass of 250 g/mol and is reproduced from EPA Method 533 (Sec. 16.0 Reference 9).

$$\frac{Total\ PFAS\ mass\ (g) \times 160\ \left(\frac{g}{mol}\right)}{250\ \left(\frac{g}{mol}\right)} = Mass\ of\ NaOH\ Required\ (g)$$

7.4.2.1 Target compounds stock

A mixture of 24 target analytes from Wellington Laboratories was used for method validation (Catalog # PFAC-24PAR, 2000 ng/mL in methanol). See Table 1 for this list of target analytes. Sulfonic acids in this mixture were

prepared from salts, and some had certified concentrations of both linear and branched isomers.

NOTE: Correct concentrations of salt forms to acid (or base) concentrations for reporting purposes. For example, the certificate of analysis from a Wellington standard (PFAC-24PAR) included the concentration of PFBS as a potassium salt at 2000 ng/mL and as the acid at 1,770 ng/mL

7.4.2.2 Surrogates stocks

A mixture of 19 isotopically labeled surrogates from Wellington Laboratories was used for method validation (Catalog # MPFAC-24ES, 1000 ng/mL in methanol). See Sec. 17.0, Table 5 for this list of surrogates and suggested target analyte associations.

7.4.3 Spiking solutions

Spiking solutions should be prepared in 95:5 acetonitrile-water. Alternate solvents (e.g. 96:4 methanol-water) may be used provided that method performance is not adversely affected and the QC criteria in Sec. 9.0 can be met. The following sections have suggested spiking concentrations for 5 mL water samples that may be appropriate for use with Method 3512.

CAUTION: Loss of longer-chain PFAS from solution can occur at lower proportions of organic co-solvent.

7.4.3.1 PFAS target compounds spiking solution

PFAS target analytes (Sec. 7.4.2.1) are added to LLOQ verification, LCS and MS/MSD QC samples prior to preparation from the same source materials used to prepare ICAL standards. LCS and MS/MSD QC samples should be spiked at concentrations near the mid-point ICAL standard concentration after all sample preparation steps are complete, assuming 100% recovery. LLOQ verification QC samples should be spiked at concentrations near (0.5-2x) the established or anticipated LLOQ standard concentration after all sample preparation steps are complete, assuming 100% recovery.

Example preparation of a target compounds spiking solution for LCS and MS/MSD QC samples: A 100 µL aliquot of a stock PFAS target analytes mix at 2000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile-water produces a solution at 20 ng/mL concentration (nom.). Addition of 40 µL of this solution to 5 mL aqueous LCS and MS/MSD QC samples would result in target analyte concentrations of 160 ng/L.

Example preparation of target compounds spiking solution for LLOQ verification QC samples: A 10 µL aliquot of a stock PFAS target analytes mix at 2000 ng/mL concentration diluted to a final volume of 10 mL with 95:5 acetonitrile-water produces a solution at 2 ng/mL concentration (nom.). Addition

of 25 µL of this solution to a 5 mL aqueous LLOQ verification QC sample would result in target analyte concentrations of 10 ng/L.

7.4.3.2 PFAS surrogates spiking solution

Isotopically-labeled PFAS surrogates (Sec. 7.4.2.2) are added to field samples and associated QC samples prior to preparation using the same source materials as used to prepare ICAL standards. Surrogates should be spiked at near the mid-point ICAL standard concentration after all sample preparation steps are complete, assuming 100% recovery.

Example preparation of surrogates spiking solution: A 200 µL aliquot of a stock PFAS surrogates mix at 1000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile- water produces a solution at 20 ng/mL concentration (nom.). Addition of 40 µL of this solution to a 5 mL water sample would result in surrogate concentrations of 160 ng/L.

7.4.4 Calibration standards – Two types of calibration standards are used for this method: standards made from the primary source used for ICAL and continuing calibration verification (CCV), and standards made from a second source used for initial calibration verification (ICV). When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

NOTE: Both linear and branched isomers of some PFAS target analytes may be present in commercially available standards (e.g., PFHxS, PFOS, N-MeFOSAA, N-EtFOSAA), while others target analytes may be represented by only a linear isomer. Please see Sec. 11.3.3 for additional information.

7.4.4.1 ICAL

ICAL standards are recommended to be prepared using the same target analytes and surrogates spiking solutions used for sample preparation (See Sec. 7.4.3). These spiking solutions can be used to prepare a calibration standards stock that is further diluted to make individual calibration standards. A minimum of five different calibration standard concentrations is required for a linear (first-order) calibration model, and a minimum of six concentrations is required for a quadratic (second-order) model. Regardless of calibration model, the lowest ICAL standard concentration must be at or below the LLOQ (see Sec. 9.9 and Method 8000) and should be sufficient to meet any sensitivity DQOs identified for the project. The remaining standards should correspond to the range of concentrations found in actual samples but should not exceed the working range of the LC/MS/MS system. Each calibration standard should contain all the desired project-specific target analytes for which qualitative and quantitative results are to be reported by this method.

Example preparation of calibration standards stock solution: A 100 µL aliquot of a target compounds spiking solution at 20 ng/mL (nom., Sec. 7.4.3.1) and a 100 µL aliquot of a surrogates spiking solution at 20 ng/mL (nom., Sec.

7.4.3.2) brought to 10 mL with a 1:1 methanol-water solution containing 0.1% acetic acid would produce a solution of target analytes and surrogates at concentrations of 200 ng/L. This calibration standards stock can then be used to prepare lower concentration ICAL standards by diluting aliquots with appropriate volumes of 1:1 methanol-water containing 0.1% acetic acid.

NOTE: Acetic acid is added primarily because it improves chromatography for some target analytes.

NOTE: Calibration standards should not be reused once the caps are pierced unless the vial is immediately recapped. Volatile losses can occur rapidly because punctures of polyethylene caps leave large holes, and there are no septa to mitigate losses.

7.4.4.2 ICV: Second source standards for ICV are prepared using certified reference materials from a second manufacturer or from a manufacturer's batch prepared independently from the batch used for calibration. A second lot number from the same manufacturer may be adequate to meet this requirement. Target analytes in the ICV are recommended to be prepared at concentrations near the mid-point of the calibration range. The ICV standard must contain all calibrated target analytes that will be reported for the project, if readily available.

7.4.4.3 Continuing calibration verification (CCV): CCV standards should be prepared in the same manner as ICAL standards at concentrations near the middle of the calibration range.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation, and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in a regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation, and storage requirements.

8.1 Sample collection criteria – Grab samples are collected in polypropylene containers. Other types of container materials, such as high-density polyethylene (HDPE), may be used if performance is acceptable for the project. PTFE containers and contact surfaces with PTFE should be avoided. Depending on the needs of the project, field blanks may be required and should be collected according to recommended PFAS sampling practices, where available. The samplers should acquire pre-verified reagent water and containers from the analytical laboratory for preparing field blanks, where practical. Aqueous field samples and associated QC samples must be collected in separate containers, including field blanks, MS/MSDs, and duplicates. Volumes collected for water samples should match volumes consumed in the laboratory’s preparation procedure. Conventional laboratory practices

involving chain of custody, field sampling, laboratory custody beginning with receipt and transfer custody, and sampling protocols should be followed.

CAUTION: Surface binding of target compounds from aqueous solution to collection containers is known to occur. Subsampling or transfer of water from a container prior to addition of a sufficient proportion of organic solvent can result in significant loss of longer-chain PFAS target analytes (e.g., carboxylic acids ≥C_9, sulfonic acids ≥C_7). Aqueous samples and sample extracts containing significant amounts of water may only be subsampled or transferred to other containers if 50% organic co-solvent content is achieved beforehand. Quantitative transfer can be achieved by solvent-rinsing the empty container with methanol. If subsampling is performed prior to achieving 50% organic cosolvent content, i.e., when preparing the entire water sample is not possible or practical, the data must be qualified appropriately.

8.2 Sample preservation, storage and holding times

All samples are iced or refrigerated at ≤6 °C from the time of collection until sample analysis. In the laboratory, samples and sample extracts should be stored in the refrigerator at ≤6 °C while not being analyzed. Formal holding times have not yet been established for these analytes in various matrices. A 14-day limit from sample collection to preparation and a 30-day limit from preparation to analysis may be used as a guide until a more formal study is completed.

NOTE: There is some evidence that suggests freezing samples can prevent transformation of some PFAS analytes into others. Longer sample holding times may be appropriate for the PFAS target analytes in this method. See Sec. 16.0, References 1, 4, 8, and 9.

9.0 QUALITY CONTROL

9.1 General guidance – Refer to SW-846 Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving collection of analytical data should include development of a structured and systematic planning document, such as a quality assurance project plan (QAPP) or a sampling and analysis plan (SAP), which translates project objectives and specifications into directions for those implementing the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged. Procedures for handling QC failures and associated corrective actions should be defined in the laboratory's SOP or in project planning documents (e.g., QAPP, SAP). Refer to Method 8000 for more information and guidance on evaluation and reporting of sample data associated with non-compliant quality controls. All sample data files and QC data files should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 and 3600 for QC procedures to ensure the proper operation of sample preparation and cleanup techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3500, 3600, or 8000.

9.3 QC procedures necessary to evaluate the LC system operation are found in Sec.11.3 of this method and in Method 8000, including evaluation of RT windows, calibration verification, and chromatographic peak shapes in standards and samples.

9.4 Initial demonstration of proficiency (IDP) – An IDP must be performed by the laboratory prior to independently running an analytical method and should be repeated if other changes occur (e.g., significant change in procedure, change in personnel). Refer to Method 8000 Sec. 9.0 for additional information regarding instrument, procedure, and analyst IDPs. An IDP must consist of analysis of a minimum of four replicate reference samples for each sample preparation and determinative method combination utilized and by generating data of acceptable precision and bias for target analytes in a clean reference matrix taken through the entire preparation and analysis procedure.

9.5 Blanks

9.5.1 Before processing any samples, the analyst must demonstrate through the analysis of a MB or RB that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source of the contaminant peak and eliminate it, if possible. As a continuing check, each time a batch of samples is prepared and analyzed, and when there is a change in reagents, an additional MB must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. MBs and field blanks must be carried through all stages of sample preparation and analysis. At least one MB or RB must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples.

9.5.2 Blanks are generally considered to be acceptable if target analyte concentrations are less than one half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations/responses are ≥10X the blank). Other criteria may be used depending on the needs of the project.

9.5.3 If an analyte of interest is found in a sample in the batch near a concentration confirmed in the blank (refer to Sec. 9.5.2), the presence and/or concentration of that analyte should be considered suspect and may require qualification. Samples may require re-extraction and/or re-analysis if the blanks do not meet laboratory-established or project-specific criteria. Re-extraction and/or re-analysis is *not* necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.5.4 When new reagents or chemicals are received, the laboratory should monitor the blanks associated with samples for any signs of contamination. It may be necessary to test every new batch of reagents or chemicals prior to sample preparation as PFAS contamination is common. If reagents are changed during a preparation batch, separate blanks should be prepared for each set of reagents.

9.5.5 The laboratory should not subtract the results of the MB from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the MB results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the MB results, and the data qualified appropriately.

9.5.6 A minimum of one MB for every 20 field samples must be prepared in a blank matrix to investigate for PFAS contamination throughout sample preparation, extraction, and analysis.

9.5.7 A minimum of one RB should be analyzed daily and is prepared with a 1:1 methanol-water solution containing 0.1% acetic acid to investigate for system/reagent contamination. PFAS contamination at low levels is common in laboratory supplies and equipment. RBs are prepared and analyzed to help identify potential sources of contamination. Surrogates are not required to be added to RBs.

9.6 Sample QC for preparation and analysis

The laboratory must have procedures for documenting the effect of the matrix on method performance (precision, bias, sensitivity). At a minimum, this must include preparation and analysis of a MB and LCS, and where practical, an MS/MSD pair or MS and duplicate in each preparation batch, as well as monitoring the recovery of surrogates. An LLOQ verification QC sample is recommended to be included in each sample preparation batch, as needed for the project. All reported target analytes are recommended to be included in LLOQ verification, LCS and MS/MSD QC samples. Any MBs, LLOQ verifications, LCSs, MS/MSDs, and duplicate samples should be subjected to the same sample preparation and analysis procedures as those used on actual samples (See Sec. 11.0 and any relevant sample preparation and cleanup methods). Consult Method 8000 for more details on QC procedures for sample preparation and analysis, including information on developing statistically based acceptance criteria for sample preparation QC.

9.6.1 Matrix Spikes/Duplicates

Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one MS/MSD pair. The decision on whether to prepare and analyze an MS and duplicate or a MS/MSD pair should be based on knowledge of the samples and addressed in project planning documents. If samples are expected to contain target analytes, laboratories may use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, then laboratories should use a MS/MSD pair. Project defined acceptance limits are recommended for MS/MSD % recovery and MS/MSD or sample/duplicate relative % difference (RPD); statistically derived acceptance limits may be used in the

absence of project specifications.

9.6.1.1 When sufficient sample is available, MS/MSDs are prepared for each matrix at a minimum frequency of one MS/MSD pair for every 20 field samples to investigate for matrix interferences.

9.6.1.2 As part of a QC program, recovery of target analytes is monitored for each matrix type. Bias is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the MS, MSD (if performed), LCS, and any LLOQ verifications according to the following formula.

$$Recovery = \%R = \frac{(C_s - C_u)}{C_n} \quad x \quad 100$$

where:

C_s = Measured concentration of spiked sample aliquot
C_u = Measured concentration of unspiked sample aliquot (use 0 for LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS or LLOQ verifications).

NOTE: MS/MSD recoveries may not be meaningful if the amount of analyte in the sample is large relative to the amount spiked.

9.6.1.3 A duplicate sample or MSD is analyzed with every batch of 20 field samples, where available. The relative percent difference (RPD) between the sample and duplicate or MS and MSD should be ≤30% (or less than statistically derived acceptance limits or project defined acceptance limits). A laboratory control sample duplicate (LCSD) may be used to evaluate precision in the batch if extra field sample containers are not received for performing duplicates or MSD.

Calculate the RPD between the MS and MSD or sample and duplicate using the following equation:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \quad X \quad 100$$

where:

C_1 =Measured concentration of first sample aliquot
C_2 =Measured concentration of second sample aliquot.

NOTE: Using approximately the same sample size or scaling the spike amount to the

sample size for the MS/MSD will minimize bias in the RPD calculation. See Method 8000 for more information.

9.6.2 LCS

LCS QC samples are prepared at a minimum frequency of one per batch of 20 or fewer field samples. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume, like the MB. The LCS is spiked with the same analytes and at the same concentrations as the MS/MSD, near the midpoint of the initial calibration range, when appropriate, and is taken through all sample preparation steps. When the results of the MS/MSD analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. See Sec. 9.6.1.2 for recovery calculation and to Sec 9.6.1.3 for RPD calculation if LCSD data are acquired. Preliminary acceptance criteria for LCS recovery are 70-130% recovery and ≤30% RPD. Statistically derived acceptance limits or project defined acceptance limits are recommended.

9.6.3 Surrogates

Surrogates are added to all field samples and associated QC samples as described in Sec. 11. An isotopically labeled structural analog of each target analyte is recommended, if available. If an isotopically labeled surrogate of sufficient purity cannot be obtained, target analytes should be associated with surrogates that are as chemically similar as possible. See Sec. 17.0, Table 5 for examples of surrogates and suggested target analyte associations. Preliminary acceptance criteria are 70-130% recovery. Statistically derived acceptance limits or project defined acceptance limits are recommended.

9.6.4 LLOQ verification

LLOQ verification QC samples are recommended to be included at a frequency of one per batch of 20 or fewer field samples, as needed for the project. Refer to Sec. 9.9 for more information about LLOQ verifications. These QC samples are taken through all sample preparation steps like MB and LCS samples. Preliminary acceptance criteria are 50-150% recovery. Statistically derived acceptance limits or project defined acceptance limits are recommended. Refer to Sec. 9.6.1.2 for recovery calculation.

9.7 Initial Calibration Acceptance Criteria (ICAL) – The LC/MS/MS system must be calibrated as described in Sec. 11.3. Prior to analyzing samples, verify the ICAL standards using a second source ICV standard, if readily available (See Sec. 7.4.4.2).

9.8 CCV – ICAL of the LC/MS/MS system must be verified using the procedure and at the frequency described in Sec. 11.4.

9.9 Lower Limits of Quantitation (LLOQs)

General guidance for verifying LLOQs is provided in this section and in Method 8000. The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence. The LLOQ must be

≥ the lowest point in the calibration curve. The laboratory shall establish LLOQs at concentrations where both quantitative and qualitative requirements can consistently be met (see below and Sec. 11.6). The laboratory shall verify the LLOQ at least annually by matrix and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at or near established LLOQs. LLOQ verifications are also recommended on a project-specific basis (Sec. 9.6.4). Optimally, LLOQs should be less than the desired decision levels or regulatory action levels based on stated project-specific DQOs.

NOTE: If project-specific concentration levels of interest are sufficiently high, the LCS may be sufficient to evaluate bias.

9.9.1 Verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.9.2 LLOQ verifications are prepared by spiking a clean control material with the analyte(s) of interest at 0.5 - 2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5 - 2 times the established or anticipated LLOQ. LLOQ verifications are carried through the same preparation and analytical procedures as environmental samples and other QC samples.

9.9.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Preliminary acceptance criteria for the LLOQ verification are 50-150%. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Statistically based LLOQ verification acceptance criteria should be determined once sufficient data points have been acquired.

9.9.4 Reporting concentrations below LLOQ – Concentrations that are below the established LLOQ may still be reported; however, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analyte concentrations reported below the LLOQ should meet the qualitative identification criteria in Sec. 11.6.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. Specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample preparation methods that have been validated in conjunction with this determinative method are listed in Sec. 1.0.

11.2 Sample cleanup – Cleanup procedures should not be necessary for relatively clean sample matrices. Extracts from highly contaminated environmental, waste or biota samples may require additional cleanup steps prior to analysis to meet acceptance criteria for all QC categories. The specific cleanup procedure used will depend upon the analytes of interest, the nature of the interferences, and the DQOs for the project. At the time of publication, no cleanup methods have been validated in conjunction with this determinative method.

11.3 ICAL

11.3.1 Tune the mass spectrometer according to the manufacturer's specifications after major repair or maintenance to the system or when mass shifts > 0.2 Dalton. Acceptable system performance may be demonstrated by meeting manufacturer specifications for mass resolution, mass accuracy, and sensitivity using an internal calibrant. Other performance measures may be appropriate for some projects. Initial calibration must not begin until performance criteria are met.

Optimize other instrument settings as needed to obtain acceptable performance for all chromatographic peaks and monitored product ions. LC/MS/MS conditions used in method development are listed in Sec. 17.0, Tables 3 and 4. Chromatographic peaks should be inspected to ensure they are symmetrical, and significant peak tailing should be corrected.

11.3.2 Analyze initial calibration standards across a range of concentrations appropriate for the sensitivity and linear range of the instrument or as needed for the project. Quantitation is based on external standard calibration models, with a minimum of five standards at different concentrations for average calibration factor or linear (first-order) calibration models, and a minimum of six standards for a quadratic (second-order) model. The lowest calibration standard must be at or below the LLOQ (see Sec. 9.9). See Sec. 11.4 in Method 8000 for additional information and guidance for initial calibration. Standards and samples must be analyzed under the same LC/MS/MS conditions, including injection volume.

NOTE: Visually inspect target analyte peaks in the calibration standard at the LLOQ to ensure that peak signal is adequately distinguishable from background and meets the qualitative identification criteria outlined in Sec. 11.6. Product ions used for quantitation should have signal to noise (S/N) ≥10, and any product ions used to support qualitative identification should have S/N ≥3.

11.3.3 Identify target compounds using the optimized product ion responses from Sec. 11.3.1. A secondary product ion is identified for most of the target analytes (Sec. 17.0, Table 2).

NOTE: PFAS target analytes may be represented by both linear and branched isomers (e.g. PFHxS, PFOA, PFOS, N-MeFOSAA, and N-EtFOSAA) which can be calibrated using a summation of the responses for all of the isomer peaks if present in quantitative standards (for example, sum or integrate all of the C6 sulfonic acid linear and branched isomers as one calibration point) or by calibrating with only the linear isomer. If a quantitative standard containing both linear and branched isomers is not available, a separate qualitative standard may be used to identify retention times of isomer peaks. Quantitative results should only be reported for peaks that are also identified in quantitative or qualitative standards, and a quantitative standard must be used for calibration. The data must be reported such that the calibration and quantitation choices are clear to the data user. See Figure 1, Sec. 17.0, for examples of chromatograms with linear and branched isomer peaks. For a more detailed explanation of one approach see Method 533.

11.3.4 Initial calibration models and acceptance criteria

Average calibration factor, linear, or quadratic regression models may be used for initial calibration. Options for evaluation of initial calibration fit are presented in the following subsections and can be applied independently. Alternate acceptance criteria may be applied depending on the needs of the project; however, those criteria should be clearly defined in a laboratory SOP or a project planning document (e.g., QAPP, SAP, or equivalent).

11.3.4.1 Average Calibration Factor (CF) calibration model

Relative Standard Deviation (RSD) of calibration factors should be ≤20%. Refer to Sec. 11.5.1 of Method 8000 for calculations. The average CF should not be used for compounds that have an RSD >20%.

11.3.4.2 Linear or quadratic regression models

Correlation coefficient (r) should be ≥0.995 or Coefficient of Determination (COD; r^2) should be ≥0.99. For regression calculations see Sec. 11.5.2 in Method 8000. % Error (Sec. 11.3.4.3) should also be evaluated when r or r^2 is used as an ICAL acceptance criterion, especially near the LLOQ. Weighted regressions can improve calibration fit and reduce % error especially at lower ICAL standard concentrations.

11.3.4.3 % Error

Percent error between the calculated concentration of each calibration standard and its expected (prepared) concentration should be ≤50% at the LLOQ and ≤30% at higher concentrations. Refer to Sec. 11.5.4.1 of Method 8000 for calculations.

11.3.4.4 Relative Standard Error (RSE)

RSE should be ≤20%. Refer to Sec. 11.5.4.2 of Method 8000 for calculations.

11.3.5 When the calibration does not meet the acceptance criteria, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

NOTE: It is considered inappropriate once the calibration models have been finalized to select an alternate fit solely to meet QC acceptance criteria for samples on a case-by-case basis.

11.3.6 When the ICAL acceptance criteria are not met, qualify the affected data, or refer to Sec. 11.5.6 of Method 8000 for recommended corrective actions. If more than 10% of the target compounds included with the ICAL (or more than 10% of those that will be reported) do not meet the established ICAL acceptance criteria (Sec. 11.3.4), then the system is considered unacceptable for sample analysis to begin. Correct the source of the problem and repeat the calibration procedure beginning with Sec. 11.3. If the problem persists, more in-depth troubleshooting may be necessary.

11.3.7 ICV – Prior to analyzing samples, verify the ICAL using a standard obtained from a second source to the calibration standard (see Sec. 7.4.4.2). Suggested acceptance criteria for the analyte concentrations in this standard are 70 - 130% of the expected analyte concentration(s). Alternative acceptance criteria may be appropriate based on project-specific DQOs. Quantitative sample analyses should not proceed for those analytes that do not meet the ICV criteria. However, analyses may continue for those analytes that do not meet the criteria with an understanding that these results could be used for screening purposes and qualified appropriately.

11.4 Continuing Calibration Verification (CCV)

11.4.1 Verify the initial calibration by analyzing a mid-level CCV standard prior to any samples, after every 20 field samples as needed (or every 12 hours, whichever is shorter), and at the end of the analytical sequence. The CCV is prepared from the same stock solutions or source materials used for the ICAL standards. The results must be calculated with the most recent ICAL and should meet the acceptance criteria provided below.

NOTE: A CCV may be omitted from the beginning of the analysis sequence if samples are analyzed within 12 hours of ICAL, and the injection of the last ICAL standard may be used as the starting time reference for evaluation.

11.4.2 CCV Acceptance Criteria

The calculated concentration or amount of each analyte of interest in the CCV standard should fall within ±30% of the expected value, which is equivalent to percent

difference (%D) or percent drift ≤±30%. Refer to Sec. 11.7 of Method 8000 for %D and % drift calculations.

11.4.3 When the CCV acceptance criteria are not met, qualify the affected data, or refer to Sec. 11.7 of Method 8000 for guidance. Due to the number of compounds that may be analyzed by this method, some compounds may fail to meet the acceptance criteria. The analyst should strive to place more emphasis on meeting the acceptance criteria for those compounds that are critical to the project. If the criterion is not met (i.e., %D or % drift >±30%) for more than 10% of the compounds included in the ICAL (or more than 10% of those that will be reported), then corrective action must be taken prior to analysis of samples, e.g., by analyzing a separately prepared CCV, or reanalyzing a CCV or new initial calibration after performing instrument maintenance.

NOTE: The analyst must closely monitor responses and chromatography in the CCVs for signs that the system is unacceptable for analysis to continue (e.g., unusual tailing, loss of resolution). If significant losses of target analytes/surrogates occur (e.g., <50% recovery, other laboratory-defined criteria) or if significant degradation of chromatography occurs, corrective action must be taken prior to sample analysis, or the analyst must demonstrate there is adequate sensitivity to meet project objectives.

11.4.4 A MB or RB must be analyzed after the ICAL or CCV and prior to samples to ensure that the system (i.e., introduction device, transfer lines and LC/MS system) is free from levels of contaminants that would bias the results. If the blank indicates contamination, then it may be appropriate to analyze additional blanks to help determine the source of contamination. A MB or RB is not required after a CCV at the end of an analytical sequence. Refer to Sec. 9.5.2 regarding qualification of data and/or corrective actions related to MB or RB contamination.

NOTE: Background of PFAS target analytes may increase in some LC systems while they are held under initial conditions or while idle; re-started sequences should typically begin with at least one blank to bleed out any accumulated background and to provide information about the potential for any carryover in the system. Refer to Sec. 9.5 for associated acceptance criteria.

11.5 Sample analysis procedure

11.5.1 Analyze samples using the same LC/MS/MS conditions as used to generate the ICAL. Warm samples to room temperature and mix well prior to transferring to vials.

A suggested sequence order is:

RB
ICAL standards and ICV, or opening CCV
MB
LLOQ verification
LCS
Field samples (with a CCV followed by a MB or RB every 20 field samples or 12 hours,

whichever is shorter)
Duplicates
Matrix spike/matrix spike duplicate
Closing CCV

11.5.2 The laboratory must monitor recoveries of isotopically-labeled surrogates (Sec. 7.4.2.2). The percent recovery of each surrogate should fall within the acceptance criteria, especially for QC samples prepared in clean matrices like reagent water (e.g., MB, LCS, LLOQ verification). If multiple surrogates fail to meet the acceptance criteria and/or the target analytes associated with the failing surrogate(s) are important to the project, reanalysis and/or repreparation of samples may be warranted. Otherwise, the associated target analytes may be reported with appropriate data qualifiers. See additional guidance in Sec. 9.6 of Method 8000.

11.5.3 If the concentration of any target analyte exceeds the ICAL range of the system, the prepared sample or sample extract should be diluted with 1:1 methanol-water containing 0.1% acetic acid and reanalyzed. If dilutions cannot be performed, concentrations that exceed the calibration range must be qualified as estimated. When the response of a compound in the sample exceeds the calibration range, analysis of a RB can help determine the extent of any carryover that may occur under the conditions used at the laboratory. See the caution after Sec. 8.1 about ensuring organic solvent content is sufficient prior to subsampling.

11.6 Qualitative identification of target analytes – Target analytes are qualitatively identified by comparison of relative responses of primary and secondary product ions in a sample to standards and by comparison of RT in a sample to the isotopically labeled surrogate in the same sample and/or to the target analyte in standards.

11.6.1 Identify target analytes by comparing the relative responses of primary and secondary product ions in the sample to those in a standard. Secondary product ions are identified for most target analytes (Sec. 17.0, Table 2). The primary/secondary (or secondary/primary) product ion ratio should be within ±50% of the average of the ion ratios in the initial calibration standards or the ion ratio in in the mid-level ICAL standard or preceding CCV, as defined in the laboratory's SOP. The analyst should use professional judgment when interferences are observed or when ion ratios are not met to avoid misidentification.

NOTE: Depending on sensitivity and matrix interference issues, a secondary product ion response might be used for quantitation rather than a primary product ion. Clearly identify any changes to analyte quantitation to the end data user.

NOTE: The primary and secondary product ion ratios and RTs in samples may not match calibration standards as well if samples contain different proportions of branched and linear isomers. Figure 1 (Sec. 17.0) shows how relative abundances of branched and linear isomers can differ in samples and standards, which may lead to differences in product ion ratios. The complete isomer grouping present in standards must be integrated consistently in all samples. Refer to Sec. 11.3.3 for more information.

11.6.2 RTs of target analytes should fall within ±0.1 min of its isotopically labeled analog in the same sample, if present. Otherwise, RTs of target analytes in samples should fall within ±0.2 minutes of the RT of the same target analyte in the mid-level ICAL standard, average of the ICAL standards, or the preceding CCV, as defined in the laboratory's SOP. Alternatively, a relative RT deviation (in %) may be used for confirmation of target compounds. Qualitative identification of target analytes without isotopically labeled analogs may also be supported by standard additions (e.g., MS/MSD). RT shifts may result in peaks eluting outside the analytical time segment in which the characteristic product ions are monitored, which could produce false negative results. Differences in relative proportions of linear and branched isomers in samples can also complicate evaluation of RT shift, and the laboratory should take care to avoid misidentification of peaks. Time segments and RT windows must include branched isomers, where applicable.

11.7 Analyte quantitation – Once a target compound has been identified, the quantitation of that compound will be based on the integrated abundance of the primary product ion unless interference problems are observed. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually, particularly for integration of linear and branched isomers. Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g., RT updates, integration parameter files, maintaining chromatographic peak shapes, etc.). The analyst should seek to minimize manual integration where practical by properly maintaining the instrument, updating RTs, and configuring peak integration parameters.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations and documentation – Sample concentrations are quantitated using the following equations:

$$Concentration\ in\ \frac{ng}{L} = \frac{(X_s)(V_t)(D)}{(V_s)}$$

$$Concentration\ in\ \frac{ng}{g} = \frac{(X_s)(V_t)(D)}{(W_s)}$$

where:

V_t = Total volume of extract or diluted sample (in L).
V_s = Volume of aqueous sample prior to preparation (in L).
D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. This value is always dimensionless.

W_s = Weight of sample extracted (in grams). If kg units are used for this term, multiply results by 1000 g/kg. Unless otherwise requested, report results in solids on a dry-weight normalized basis.
X_s = Calculated concentration of analyte (ng/L) from the analysis. Type of calibration model used determines derivation of X_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3 of Method 8000.

12.2 See Secs. 11.5 and 11.10 of Method 8000 for additional information and formulas for quantitating results.

13.0 METHOD PERFORMANCE

Please refer to Sec. 17.0, Table 1 for a summary of method performance from a multi-laboratory validation study of aqueous samples prepared by Method 3512.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult Less is Better: Laboratory Chemical Management for Waste Reduction, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety at: https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* (Sec. 16.0).

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables, figures, and appendices referenced by this method.

TABLE 1. RECOVERY AND PRECISION OF TARGET ANALYTES IN MULTI-LABORATORY STUDY MATRICES PREPARED BY METHOD 3512[1]

200 ng/L (nominal) prepared concentration												
	Reagent Water			Groundwater			Surface Water			Wastewater		
Target Analyte	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	96.4	10.6	16.1	97.2	5.8	11.0	90.2	9.7	16.0	95.4	12.7	14.7
PFPeA	100	4.6	13.1	100	5.2	11.3	99.5	3.8	11.9	102	6.0	13.8
PFHxA	99.5	8.2	8.5	96.9	9.2	6.1	98.9	7.6	7.4	98.7	12.5	7.3
PFHpA	101	6.4	8.4	98.8	7.1	5.7	100	5.9	6.4	100	9.3	6.4
PFOA	104	7.9	9.3	102	9.8	7.8	103	6.7	8.3	104	8.0	8.8
PFNA	104	7.0	10.1	101	8.1	8.1	101	8.1	11.2	103	8.9	9.0
PFDA	106	14.3	9.3	102	9.4	7.2	104	9.8	9.3	106	11.6	8.9
PFUnDA	105	8.3	14.8	103	8.6	16.4	103	7.7	12.4	106	10.0	18.7
PFDoDA	103	7.7	13.9	103	5.6	20.4	101	9.3	18.3	101	10.7	17.8
PFTrDA	103	11.2	15.7	99.3	8.4	16.6	99.4	7.5	17.2	98.2	14.1	16.2
PFTeDA	101	12.6	15.7	92.9	12.0	12.6	94.1	7.9	14.9	91.8	13.1	13.9
PFBS	100	9.3	9.4	101	8.5	6.7	99.2	7.3	9.0	99.5	10.0	7.8
PFPeS	99.0	6.2	7.4	99.2	4.7	7.4	98.7	4.2	8.4	100	7.3	6.7
PFHxS	101	6.9	8.3	101	5.6	6.0	101	6.0	6.5	106	13.8	9.0
PFHpS	104	4.9	10.1	102	5.0	8.9	100	6.6	9.1	104	6.4	10.1
PFOS	104	5.8	11.2	105	6.6	11.1	103	6.4	9.4	120	51.9	31.8
PFNS	106	7.6	13.9	106	5.8	14.3	106	7.5	12.2	104	9.4	14.7
PFDS	104	7.2	15.2	102	6.4	16.1	100	6.1	15.9	95.5	15.1	17.5
PFOSA	92.1	4.2	10.0	97.3	4.0	10.0	94.2	4.0	6.9	98.6	4.9	10.9
FtS 4:2	104	6.5	8.3	97.6	6.3	11.4	101	10.4	11.5	106	10.0	18.6
FtS 6:2	92.3	17.0	23.5	97.1	77.4	44.8	86.2	11.9	28.6	93.9	11.0	31.5
FtS 8:2	109	11.1	11.1	105	10.3	10.5	107	12.8	12.1	118	13.4	19.7
NMeFOSAA	100	11.6	9.2	103	12.9	14.1	103	13.2	14.9	100	15.4	11.4
NEtFOSAA	104	15.7	18.3	108	11.8	21.4	105	8.7	19.1	109	9.3	20.2

	60 ng/L (nominal) prepared concentration											
	Reagent Water			Groundwater			Surface Water			Wastewater		
Target Analyte	$\bar{X}^2$ (%)	S_w [3] (%)	S_b [4] (%)	$\bar{X}^2$ (%)	S_w [3] (%)	S_b [4] (%)	$\bar{X}^2$ (%)	S_w [3] (%)	S_b [4] (%)	$\bar{X}^2$ (%)	S_w [3] (%)	S_b [4] (%)
PFBA	93.1	13.1	19.6	98.0	15.6	17.9	86.7	16.4	21.4	96.6	14.6	16.0
PFPeA	104	13.5	13.7	113	30.7	34.9	103	10.2	16.6	107	11.8	11.6
PFHxA	97.4	11.7	8.2	95.0	13.7	7.1	98.2	16.9	16.6	98.2	14.8	17.3
PFHpA	98.5	8.6	10.7	96.2	11.2	8.3	95.6	12.5	10.4	101	7.8	8.4
PFOA	100	11.4	9.7	98.8	11.4	9.6	101	14.2	9.8	100	11.3	7.8
PFNA	97.0	9.6	8.9	96.5	12.7	11.2	95.1	10.9	11.4	98.5	12.0	6.9
PFDA	102	16.6	14.4	98.2	16.4	14.0	97.2	14.0	11.3	100	17.5	8.8
PFUnDA	98.9	13.2	14.7	96.0	12.3	13.2	96.3	16.4	13.1	98.2	15.1	14.3
PFDoDA	95.8	17.5	17.9	98.0	15.4	22.7	93.9	12.3	17.0	95.6	14.9	25.6
PFTrDA	97.7	14.0	19.3	95.8	11.8	23.1	92.3	14.4	19.7	97.2	20.0	21.3
PFTeDA	95.5	15.1	22.6	88.2	13.9	18.6	83.8	13.5	16.1	90.7	17.4	25.7
PFBS	92.7	11.3	4.9	99.1	15.9	6.8	94.9	13.3	8.2	100	11.1	8.6
PFPeS	96.8	8.3	6.2	95.8	7.1	7.8	95.3	10.5	9.8	96.3	8.4	3.6
PFHxS	95.3	10.8	12.9	94.9	12.3	14.5	96.9	9.0	11.4	102	9.7	11.7
PFHpS	101	11.5	9.5	97.5	11.8	10.9	96.5	10.9	10.5	100	11.3	9.2
PFOS	100	15.8	14.0	103	13.6	14.3	104	13.8	10.8	108	14.3	15.3
PFNS	102	9.9	14.9	99.8	10.2	17.0	99.4	9.8	18.2	101	11.7	14.2
PFDS	97.7	12.2	14.6	95.6	9.2	18.3	94.4	13.8	17.6	95.1	14.5	16.5
PFOSA	87.7	8.2	8.4	89.6	7.7	10.9	85.2	10.2	13.2	92.4	6.2	8.2
FtS 4:2	98.5	15.9	6.5	91.0	16.6	13.2	92.2	12.4	13.7	98.7	16.9	15.7
FtS 6:2	85.5	32.9	32.0	75.6	17.8	21.2	130	363	153	88.4	23.2	27.7
FtS 8:2	105	12.6	9.6	101	19.4	12.1	93.9	14.9	13.8	111	17.4	19.1
NMeFOSAA	98.6	16.8	10.5	96.0	16.1	13.4	98.5	22.6	20.9	99.8	20.2	13.3
NEtFOSAA	96.5	18.3	17.2	97.6	20.9	22.4	99.0	18.8	20.7	109	13.8	25.4

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2]Pooled mean % recovery (n=40 samples, including 5 replicates of each matrix and spike level tested by each of 8 laboratories except for 60 ng/L wastewater where n=39 samples); For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf.

[3]Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4]Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

TABLE 2. RECOMMENDED PRECURSOR IONS AND PRODUCT IONS AND EXAMPLE CHROMATOGRAPHIC RETENTION TIMES

Target Analyte	Precursor Ion m/z	Product Ion m/z		Retention Time (min)[1]
		Primary	Secondary	
PFBA[2]	213	169	-	3.07
PFPeA[2]	263	219	-	4.16
4:2 FTS	327	307	81	5.13
PFHxA	313	269	119	5.46
PFBS	299	80	99	5.72
PFHpA	363	319	169	6.39
PFPeS	349	80	99	6.74
6:2 FTS	427	407	81	6.81
PFOA	413	369	169	7.08
PFHxS[3]	399	80	99	7.45
PFNA	463	419	219	7.68
8:2 FTS	527	507	81	7.92
PFHpS	449	80	99	8.08
PFDA	513	469	219	8.2
N-MeFOSAA	570	419	483	8.22
N-EtFOSAA	584	419	483	8.43
PFOS[3]	499	80	99	8.6
PFUnDA	563	519	269	8.7
PFNS	549	80	99	9.15
PFDoDA	613	569	169	9.17
PFTrDA	663	619	169	9.66
PFDS	599	80	99	9.67
PFOSA[2]	498	78	-	9.77
PFTeDA	713	669	169	10.12
Isotopically labeled surrogates[4]:				
M4PFBA	217	172		3.06
M5PFPeA	268	223		4.15
M2-4:2 FTS[5]	329	309	81	5.12
M5PFHxA	318	273		5.44
M3PFBS	302	80		5.72
M2PFHpA	367	322		6.38
M2-6:2 FTS[5]	429	409	81	6.8
M8PFOA	421	376		7.08
M3PFHxS	402	80		7.46
M9PFNA	472	427		7.66
M2-8:2 FTS[5]	529	509	81	7.92
M6PFDA	519	474		8.18
d3-NMeFOSAA	573	419		8.21
d5-NEtFOSAA	589	419		8.41
M8PFOS	507	80		8.63
M7PFUnDA	570	525		8.68
M2PFDoDA	615	570		9.17
M8PFOSA	506	78		9.77
M2PFTeDA	715	670		10.12

[1]RTs are based on conditions in Table 3 using the binary gradient.

[2]Secondary product ions with sufficient relative abundance were not identified for these chemicals using the conditions described in Tables 3 and 4.

[3]Branched isomers of PFHxS, PFOS, and potentially other perfluoroalkyl sulfonates may produce lower responses for product ions other than m/z 80, which may lead to low bias measurement of these isomers if used for quantitation.

[4]Secondary product ions are not identified for most isotopically labeled surrogates but may be useful.

[5]Using the m/z 81 product ion for quantitation of M2-4:2 FTS, M2-6:2 FTS, and M2-8:2 FTS will reduce interferences from high concentrations of the respective native target analytes compared to the primary product ions. Refer to Sec. 4.5 for more information.

TABLE 3. EXAMPLE LIQUID CHROMATOGRAPHY CONDITIONS

Analytical column: See Sec. 6.1.2.1

Isolator Column: See Sec. 6.1.3.1

Column temperature: 35-50ºC

Injection volume: 10-30µL

Needle wash: 60% acetonitrile / 40% 2-propanol

Binary Gradient

Time (min)	Flow rate (mL/min)	% Solvent Line A (20mM Ammonium Acetate in water)	% Solvent Line B (Acetonitrile)
0	0.3	100	0
1	0.3	80	20
6	0.3	50	50
13	0.3	15	85
14	0.4	0	100
17	0.4	0	100
18	0.4	100	0
21	0.4	100	0

Ternary Gradient:

Time (min)	Flow rate (mL/min)	% Solvent Line A (95:5 water-acetonitrile)	% Solvent Line B (Acetonitrile)	% Solvent Line C (400mM ammonium acetate in 95:5 water-acetonitrile)
0	0.3	95	0	5
1	0.3	75	20	5
6	0.3	50	45	5
13	0.3	15	80	5
14	0.4	0	95	5
17	0.4	0	95	5
18	0.4	95	0	5
21	0.4	95	0	5

TABLE 4. EXAMPLE MASS SPECTROMETER CONDITIONS

Instrument	Waters Xevo TQ-S
Ion Source	Electrospray Ionization (Negative mode)
Capillary voltage	0.75 kV
Source temp	150°C
Desolvation gas temp	450°C
Desolvation gas flow	800 L/hr
Cone gas flow	200 L/hr
Collision gas flow	0.15 mL/min
Collision energy	Optimized by analyte
Cone voltage	Optimized by analyte

TABLE 5. EXAMPLE SURROGATE AND TARGET ANALYTE ASSOCIATIONS

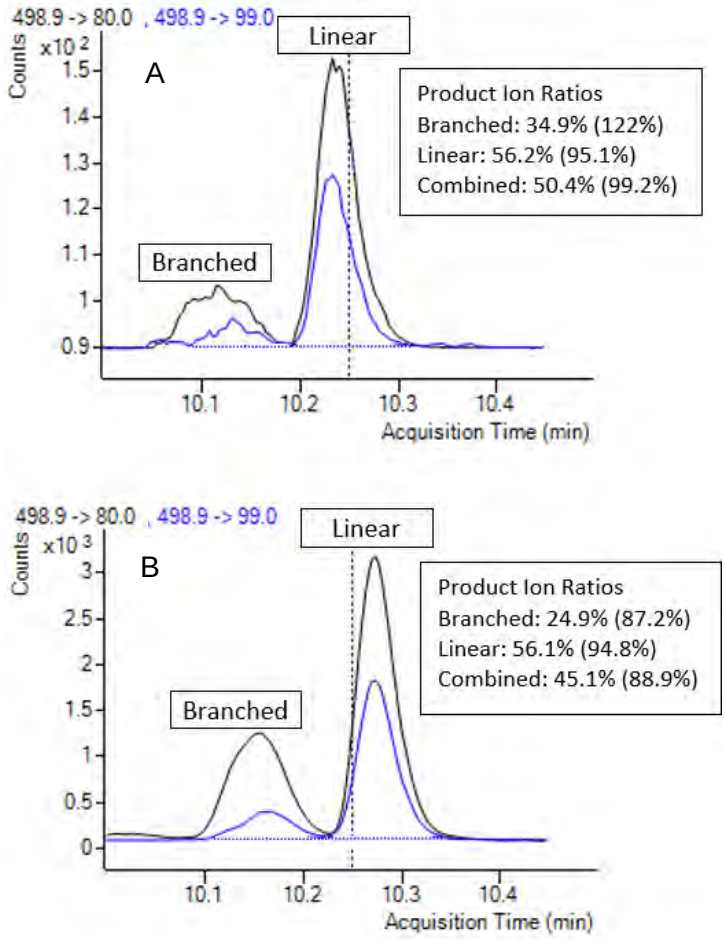
Examples of Isotopically Labeled PFAS Surrogates	Recommended target analyte association(s)
Sulfonic Acid Surrogates	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid (M3PFBS)	PFBS, PFPeS
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid (M3PFHxS)	PFHxS, PFHpS
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid (M8PFOS)	PFOS, PFNS, PFDS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] hexanesulfonic acid (M2-4:2 FTS)	4:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] octanesulfonic acid (M2-6:2 FTS)	6:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] decanesulfonic acid (M2-8:2 FTS)	8:2FTS
Carboxylic Acid Surrogates	
Perfluoro-n-[$^{13}C_4$]butanoic acid (M4PFBA)	PFBA
Perfluoro-n-[$^{13}C_5$]pentanoic acid (M5PFPeA)	PFPeA
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid (M5PFHxA)	PFHxA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid (M4PFHpA)	PFHpA
Perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA)	PFOA
Perfluoro-n-[$^{13}C_9$]nonanoic acid (M9PFNA)	PFNA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid (M6PFDA)	PFDA
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid (M7PFUnDA)	PFUnDA
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (M2PFDoDA)	PFDoDA, PFTrDA
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid (M2PFTeDA)	PFTeDA
Sulfonamide and Sulfonamidoacetic acid Surrogates:	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide (M8PFOSA)	PFOSA
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid (d3-N-MeFOSAA)	N-MeFOSAA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA)	N-EtFOSAA

TABLE 6. QC SUMMARY

Quality control category	Specification and minimum frequency	Acceptance criteria
Sample preservation, storage and holding time (Sec. 8.2)	Ice or refrigerate to ≤6ºC from sample collection to preparation; Recommended holding time from sample collection to preparation: 14 days Recommended holding time from preparation to analysis: 30 days	Use judgment to qualify data; formal holding time study pending
Initial Calibration (ICAL) (Sec. 9.7, 11.3)	Prior to analysis of samples	Mean CF: RSD ≤20% Linear or quadratic regression: r ≥0.995 or r^2 ≥0.99 %Error: ≤±50% at LLOQ and ≤±30% for higher concentrations RSE ≤20% ≥90% of target analytes and surrogates meet ICAL acceptance criteria
Initial calibration verification (ICV) (Sec. 9.7, 11.3.7)	After initial calibration and prior to analysis of samples	Target analytes are within ±30% of expected concentrations
Continuing calibration verification (CCV) (Sec. 9.8, 11.4)	Prior to analysis of field samples (unless ICAL analyzed in prior 12 hr), after every 20 samples and at end of sequence	≥90% of target analytes and surrogates within ±30% of expected concentrations
Reagent Blank (RB) (Sec. 9.5.7, 11.4.4)	One per day of analysis	Target analyte concentrations <1/2 LLOQ or <10% of sample concentrations
Method Blank (MB) (Sec. 9.5.6, 11.4.4)	One per preparation of 20 or fewer samples	Target analytes <1/2 LLOQ or <10% of sample concentration
Matrix spike/duplicate or matrix spike/matrix spike duplicate (MS/MSD) (Sec. 9.6.1)	One set per preparation of 20 or fewer field samples (if sufficient replicate samples are provided)	Meets laboratory derived or project specific recovery and RPD criteria
Laboratory Control Sample (LCS) (Sec. 9.6.2)	One per preparation batch of 20 or fewer samples	Within 70-130% recovery or within laboratory derived or project specific recovery criteria
Surrogates (Sec. 9.6.3)	Added to all field samples and prepared QC samples (MB, LLOQ Verification, LCS, MS/MSD)	Within 70-130% recovery or within laboratory derived or project specific recovery criteria

Quality control category	Specification and minimum frequency	Acceptance criteria
LLOQ verification (Sec. 9.6.4, 9.9.1)	Required annually. Recommended one per preparation batch of 20 or fewer samples	Within 50-150% recovery or within laboratory derived or project specific recovery criteria
Qualitative identification of target analytes (Sec. 11.6)	Evaluate each target analyte in field samples	RT in sample is within ±0.1 min of isotopically-labeled analog of target analyte, or otherwise within ±0.2 min of target analyte RT in midpoint ICAL standard, average of ICAL standards, or preceding CCV. For target analytes with secondary product ions, ratio of primary/secondary or secondary/primary product ion is within ±50% of expected ratio from midpoint ICAL standard, average of ICAL standards, or preceding CCV

FIGURE 1. PFOS PRIMARY AND SECONDARY PRODUCT IONS



NOTE: Product ion traces above show proportions of primary and secondary product ions for linear and branched PFOS isomers in (A) a calibration standard and (B) a field sample. Product ion ratios show the proportion of secondary product ion peak area to primary product ion peak area (in %), and the percentage in parentheses is the ratio in this standard or sample divided by the ratio in a reference standard. Refer to Sec. 11.3.3 for more information regarding integration of linear and branched isomer peaks in standards and samples and to Sec. 11.6.1 for more information regarding evaluation of product ion ratios to support qualitative identification.

APPENDIX A - GLOSSARY

ASTM	ASTM International, formerly American Society for Testing and Materials
CAS RN	Chemical Abstract Service Registry Number®
CCV	continuing calibration verification
DQOs	data quality objectives
EPA	U.S. Environmental Protection Agency
HDPE	high density polyethylene
ICAL	initial calibration
ICV	initial calibration verification
IDP	initial demonstration of proficiency
LC	liquid chromatography
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
LLOQ	lower limit of quantitation
MB	method blank
MS	matrix spike
MS/MS	tandem mass spectrometry – The process of separating precursor ions by m/z, followed by collisionally activated dissociation of a precursor ion at a given m/z into one or more product ions of smaller m/z.
MSD	matrix spike duplicate
m/z	mass-to-charge ratio
OSHA	U.S. Occupational Safety and Health Administration
PEEK	polyetheretherketone
PFAS	per- and polyfluoroalkyl substances
PPE	personal protective equipment
Precursor ion	Ion produced in the ion source that forms particular product ions or undergoes specified neutral losses during MS/MS analysis.
Product ion	Ion formed as the product of a reaction involving a particular precursor ion. See reference 9 in Section 16.0.
PTFE	polytetrafluoroethylene
QA	quality assurance
QAPP	quality assurance project plan

QC	quality control
RB	reagent blank
RSD	relative standard deviation
RT	retention time
SAP	sampling and analysis plan
SDS	safety data sheet
SOP	standard operating procedure
UPLC	Ultra Performance Liquid Chromatograph®

Attachment 13

METHOD 3512

SOLVENT DILUTION OF NON-POTABLE WATERS

TABLE OF CONTENTS

Disclaimer

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required methods used for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only and are not intended to be and must not be used as absolute QC acceptance criteria or for the purpose of laboratory accreditation.

1.0 SCOPE AND APPLICATION

This method is for preparation of non-potable water samples by dilution with an organic solvent prior to analysis by the appropriate determinative method.

The 24 PFAS that have been evaluated with this preparation method are provided below. This preparation method was validated in conjunction with determinative Method 8327. See Method 8327 and SW-846 website for performance data. This method has been tested in surface water, groundwater, and wastewater matrices. This preparation method may also be applicable to other target compounds and other aqueous matrices, provided that the laboratory can demonstrate adequate performance (refer to Sec. 9.0 of the applicable determinative method or to project-specific acceptance criteria) using representative sample matrices. Please refer to Method 8000 for additional information.

Analyte	CAS RN*
PFAS sulfonic acids	
Perfluoro-1-butanesulfonic acid (PFBS)	375-73-5
Perfluoro-1-pentanesulfonic acid (PFPeS)	2706-91-4
Perfluoro-1-hexanesulfonic acid (PFHxS)	355-46-4
Perfluoro-1-heptanesulfonic acid (PFHpS)	375-92-8
Perfluoro-1-octanesulfonic acid (PFOS)	1763-23-1
Perfluoro-1-nonanesulfonic acid (PFNS)	68259-12-1
Perfluoro-1-decanesulfonic acid (PFDS)	335-77-3
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS)	757124-72-4
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	27619-97-2
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	39108-34-4
PFAS carboxylic acids	
Perfluorobutanoic acid (PFBA)	375-22-4
Perfluoropentanoic acid (PFPeA)	2706-90-3
Perfluorohexanoic acid (PFHxA)	307-24-4
Perfluoroheptanoic acid (PFHpA)	375-85-9
Perfluorooctanoic acid (PFOA)	335-67-1
Perfluorononanoic acid (PFNA)	375-95-1
Perfluorodecanoic acid (PFDA)	335-76-2
Perfluoroundecanoic acid (PFUnDA)	2058-94-8
Perfluorododecanoic acid (PFDoDA)	307-55-1

Analyte	CAS RN*
Perfluorotridecanoic acid (PFTrDA)	72629-94-8
Perfluorotetradecanoic acid (PFTeDA)	376-06-7
PFAS sulfonamides and sulfonamidoacetic acids	
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)	2355-31-9
Perfluoro-1-octanesulfonamide (PFOSA)	754-91-6

*Standards for some target analytes may consist of mixtures of structural isomers; however, the Chemical Abstracts Service (CAS) Registry Number (RN) listed in the table is for the normal-chain isomer. All CAS RNs in the above table are for the acid form. Sulfonic acids in stock standard mixes are typically received as the sodium or potassium salt form. CAS RNs for the salt form are not included.

1.1 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.2 This method is restricted to use by, or under supervision of, appropriately experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.3 Refer to the appropriate determinative method for more information about method performance and related considerations. Note that this method may not be appropriate for aqueous samples with high levels of suspended solids. If significant particulate matter is present and the total sample is of concern, then the sample should be treated as a multi-phase sample per SW-846 Chapter Two. Larger sample collection volumes or centrifugation may aid phase separation.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared by adding isotopically labeled analogs of PFAS target analytes (as surrogates or as isotope dilution internal standards, depending on determinative method), diluting samples 1:1 with the appropriate organic solvent, filtering and pH adjustment, if necessary.

2.2 Determinative analysis is performed using the appropriate liquid chromatography/tandem mass spectrometry (LC/MS/MS) method.

3.0 DEFINITIONS

Refer to the SW-846 Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 In order to avoid compromising data quality, contamination from preparation procedure must be reduced to the lowest practical level. Method blanks (MBs) and reagent blanks (RBs) are prepared and analyzed with all samples and are used to demonstrate that laboratory supplies and preparation and analysis steps do not introduce interferences or PFAS artifacts at levels that would bias quantitation. Careful selection of reagents and consumables is necessary because even low levels of PFAS contamination may alter the precision and bias of the method, and background introduced by these materials (and variability thereof) is cumulative. Refer to each determinative method to be used for specific guidance on QC procedures and to SW-846 Chapter Four for general guidance on glassware cleaning.

4.2 Refer to determinative method for additional information on interferences.

4.3 Procedures employed to prevent or minimize problems (see determinative method for specific requirements and criteria).

4.3.1 All solvents should be of LC/MS grade, or equivalent, to minimize interference problems. Solvents must be checked by lot prior to use.

4.3.2 PFAS contamination has been found in reagents, glassware, tubing, polytetrafluoroethylene (PTFE) vial caps, aluminum foil, glass disposable pipettes, filters, and other apparatus that release fluorinated compounds. All supplies and reagents should be verified prior to use. If found, measures should be taken to remove the contamination, if possible, or find other suppliers or materials to use that meet method or project criteria.

4.3.3 Polyethylene disposable pipettes are recommended. Alternate materials may be used if the blank criteria in the determinative method are met. When a new batch of disposable pipettes is received, at least one should be checked for release of

target analytes or interferences.

4.3.4 During method development, loss of some PFAS target analytes was observed during storage of standard solutions in 1:1 methanol-water containing 0.1% acetic acid in glass containers. Polypropylene containers should be used for preparation and storage of samples and standards. Other materials may be used, such as high density polyethylene (HDPE), if it can be shown the target analytes are not adversely affected (i.e., all quality control criteria in Sec. 9.0 of the determinative method can be met). Glass autosampler vials have been successfully used for solutions in 1:1 methanol-water containing 0.1% acetic acid during analysis.

4.3.5 If labware is re-used, the procedure described for labware cleaning (Sec. 6.4) should be followed to minimize risk of contamination. The blank criteria in the appropriate determinative method can be used as a guideline for evaluating cleanliness.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of U.S. Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses.

5.2 Users of this method should operate a formal safety program.

5.3 The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. Exposure to these chemicals should be reduced to the lowest possible level and the appropriate personal protective equipment (PPE) should be utilized. Review SDSs for specific physical and health hazards including appropriate PPE to be used. SDSs may be accessed at multiple locations (e.g., www.sigmaaldrich.com, www.well-labs.com, and www.isotope.com).

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Labware, reagents, supplies, equipment, and settings other than those listed in this method may be employed provided that method performance appropriate for the intended application has been demonstrated and documented, including meeting acceptance criteria for all categories of quality controls listed in Sec. 9.0. This section does not list all common labware (e.g., beakers and flasks) that might be used.

Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented. This section does not list all common laboratory containers (e.g., beakers and flasks) that might be used.

6.1 Adjustable volume pipettes, 10-µL to 10-mL.

6.2 Analytical balance, capable of weighing to 0.01g

6.3 Miscellaneous Supplies

6.3.1 10- to 25-mL filter-adaptable syringe with luer lock: high density polyethylene (HDPE), polypropylene or glass (rubber tipped plungers are not to be used).

6.3.2 50-mL polypropylene tubes (BD Falcon, Catalog # 352098)

6.3.3 15-mL polypropylene tubes (BD Falcon, Catalog # 352097); use pre-weighed tubes for collection of field samples and field QC

6.3.4 Polyethylene disposable pipettes (Samco Thermo Scientific, Catalog # 252)

6.3.5 Pipette tips: polypropylene pipette tips of various sizes (Eppendorf, catalogue #s 022491997, 022492080, 022491954, 022491946, and 022491512)

6.3.6 Pall Acrodisc GxF/0.2-µm GHP or equivalent membrane syringe driven filter unit. Filters must be cleaned prior to use. A suggested protocol is to rinse each filter with 2 x 10 mL acetonitrile and then 2 x10 mL methanol prior to use.

6.3.7 Autosampler vials: HDPE, polypropylene or glass

6.3.8 Polyethylene autosampler vial caps (Waters Catalog # 186004169)

6.4 Labware cleaning instructions – If labware is reused it should be washed in hot water with detergent such as powdered Alconox, Detojet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. Rinse with organic solvents such as acetone, methanol, and/or acetonitrile. Traces of target compounds should be reduced to a minimum.

7.0 REAGENTS AND STANDARDS

7.1 Chemicals used in all tests should be LC/MS grade if available, or reagent grade at a minimum. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be verified prior to use to ensure the blank acceptance criteria in Sec. 9.5 can be met.

7.2 Reagent water – All references to water in this method refer to reagent water as defined in SW-846 Chapter One. Reagent water from in-house deionized water systems may need additional treatment prior to use (e.g., with a point-of-use water purification system) to meet blank acceptance criteria (Sec. 9.5). The laboratory should check for PFAS contamination coming from the point-of-use system (it should not contain fluoropolymers, where practical). Bottled reagent water should be evaluated in the same manner as reagent water from other sources.

7.3 Reagents – Items shown are for informational purpose only; equivalent reagents and standards may be used. All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems, preferably LC/MS grade or equivalent.

7.3.1 Methanol, CH_3OH (CAS RN 67-56-1)

7.3.2 Acetic acid, CH_3COOH (CAS RN 64-19-7)

7.3.3 Acetonitrile, C_2H_3N (CAS RN 75-05-8)

7.4 Standard Solutions

See the relevant determinative method for information about standards used for sample preparation (e.g., surrogates, internal standards, and/or target compounds spiking solutions).

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

See introductory material to SW-846 Chapter Four, “Organic Analytes”, Method 3500, and the specific determinative method to be used.

9.0 QUALITY CONTROL

9.1 General Guidance - Refer to SW-846 Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and the criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those who will implement the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Refer to Method 8000 and to the relevant determinative method for more information and guidance on evaluation and reporting of sample data associated with non-compliant quality controls.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 and 3600 for QC procedures to ensure the proper operation of sample preparation and cleanup techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3500, 3600 or 8000.

9.3 See Sec. 9.0 of the appropriate determinative method for QA/QC requirements specific to that analysis.

9.4 Initial demonstration of proficiency (IDP) -- Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000D, Sec. 9.3 for information on how to accomplish a demonstration of proficiency.

9.5 Blanks – Before processing any samples, the analyst must demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the preparation and analysis of method blanks (MBs). Each time samples are prepared, and when there is a change in reagents, a MB should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.5.1 At least one MB must be prepared with each batch of 20 or fewer field samples to investigate for PFAS contamination throughout sample preparation and analysis. MBs are reagent water samples that are subjected to all sample preparation steps in Sec. 11.0.

9.5.2 At least one RB should be prepared each day that samples are prepared to investigate for contamination in laboratory reagents and consumables. PFAS contamination at low levels is common in laboratory supplies and equipment. RBs contain the reagents (1:1 methanol-water containing 0.1% acetic acid) used in the preparation batch, but they are not subjected to sample preparation procedures like MBs (e.g., filtration). Surrogates are not required to be added to RBs.

9.6 Sample QC for preparation and analysis

The laboratory must have procedures for documenting the effect of the matrix on method performance (precision, bias, sensitivity). At a minimum, this must include preparation and analysis of a MB and LCS, and where practical, an MS/MSD pair or MS and duplicate in each preparation batch of 20 or fewer samples. An LLOQ verification QC sample is also recommended to be included in each sample preparation batch, as needed for the project. These QC samples are subjected to the same preparation procedures (Sec. 11.0) as those used on actual samples.

9.7 All field samples and QC samples should be spiked with an appropriate concentration of isotopically labeled analogs of PFAS target analytes used as surrogates or internal standards.

10.0 CALIBRATION AND STANDARDIZATION

There are no calibration or standardization steps directly associated with this preparation procedure.

11.0 PROCEDURE

The following sections are written assuming a container size of 15 mL and a 5 mL sample size. Alternate sample volumes and container sizes may be used provided the solvent proportions are maintained and the entire sample is prepared (e.g., 20 mL samples are collected in 50 mL polypropylene tubes and diluted with 20 mL of solvent). Refer to the appropriate determinative method for suggested concentrations for surrogates/internal standards and any target analytes by QC sample type. The analyst should limit standard additions to ≤1% of the final volume (e.g., ≤100 µL in 10 mL) to minimize errors in the dilution.

CAUTION: Surface binding of target compounds from aqueous solution to collection containers is known to occur. Subsampling or transfer of water from a container prior to addition of a sufficient proportion of organic solvent can result in significant loss of longer-chain PFAS target analytes (e.g., carboxylic acids ≥C_9, sulfonic acids ≥C_7). Aqueous samples may only be subsampled or transferred to other containers if 50% organic co-solvent content is achieved beforehand. Quantitative transfer can be achieved by solvent-rinsing the empty container with methanol. If subsampling is performed prior to achieving 50% organic cosolvent content, i.e., when preparing the entire water sample is not possible or practical, the data must be qualified appropriately.

11.1 Initial Sample Volume

11.1.1 Field Samples, MS/MSD, and duplicate QC samples – Use separately collected containers for each field sample and each MS, MSD and/or duplicate QC sample. Allow the samples to warm to room temperature and determine the sample volume using one of the options below. If the sample is transferred to another container prior to diluting 1:1 with methanol, the original container must be solvent rinsed and the rinsate included in the solvent dilution of the sample (Sec. 11.2). Hand-shake or vortex the rinse solvent in the original sample container for ~2 min to ensure quantitative transfer.

11.1.1.1 If sample containers were pre-weighed prior to collection, sample volume may be determined by weighing the container plus sample, calculating the sample mass by difference and converting to volume, assuming a density of 1.0 g/mL.

11.1.1.2 Sample volume may be determined either by mass difference of the original sample and the container after transfer (using assumed density as described above), by marking the original sample volume on the outside of the

container and determining after transfer, or by direct measurement (e.g., using certified graduation marks on sample containers).

11.1.2 MB, LCS, and LLOQ verification QC samples – Add 5.0 mL (or other appropriate volume) of reagent water to separate 15-mL polypropylene tubes.

NOTE: If field samples were collected at a different volume, measure a similar volume for MBs, LLOQ verifications, and LCSs into similarly sized containers.

11.2 Sample Preparation

11.2.1 Spike each field sample and associated QC sample with an appropriate volume of the surrogate/internal standard spiking solution; and spike each LLOQ verification, LCS, or MS/MSD sample with an appropriate volume of a target compounds spiking solution. For external standard calibration determinative methods, the spiking solution volumes should be scaled to the sample volumes if they differ significantly from the nominal expected volume (e.g., 5.0 mL).

11.2.2 Dilute each field sample and associated QC sample 1:1 with methanol by adding a volume equivalent to the initial sample volume (Sec. 11.1) to each tube (e.g., 5.0 mL).

11.2.3 Hand shake or vortex each sample for ~2 min.

11.2.4 Filter each diluted field sample and associated QC sample through separate rinsed Acrodisc GxF/0.2-µm GHP membrane syringe-driven filters (See Sec. 6.3.8 of Method 8327) to remove particulates in the samples. Centrifugation may aid in removal of particulates.

11.2.5 Add 0.1% (v/v) acetic acid to each field sample and associated QC sample after filtration (e.g., add 10 µL of glacial acetic acid to 10 mL). Transfer an aliquot of that solution to an LC vial and apply a polyethylene cap. The sample is now ready for analysis.

11.2.6 The final volume of each prepared field sample and associated QC sample may be calculated as the sum of the aqueous sample and methanol volumes or measured. The decrease in solution volume upon mixing of equal volumes of methanol and water is small (see Reference 4 in Sec. 16.0) and can be considered insignificant.

NOTE: To minimize PFAS contamination in subsequent samples, a suggested protocol is to soak reusable syringes in hot tap water and then rinse with 5 x 10 mL reagent water, 3 x 10 mL acetonitrile and 3 x 10 mL methanol.

12.0 DATA ANALYSIS AND CALCULATIONS

There are no data analysis and calculation steps directly associated with this procedure. Follow the directions given in the determinative method.

13.0 METHOD PERFORMANCE

Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the ACS, Committee on Chemical Safety at: https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available at: http://www.labsafetyinstitute.org/FreeDocs/WasteMgmt.pdf.

16.0 REFERENCES

1. ASTM Standard D7979-20, “Standard Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)”, ASTM International, West Conshohocken, PA, 2020. Available at www.astm.org.
2. U.S. Environmental Protection Agency, Region 5 Laboratory, “Standard Operating Procedure for the Analysis of Polyfluorinated Compounds of Interest to OSRTI in Water, Sludge, Influent, Effluent, and Wastewater by Multiple Reaction Monitoring Liquid Chromatography/Mass Spectrometry (LC/MS/MS),” 75 pp., 2016.
3. Standard Practices for Sampling Water, American Society for Testing and Materials, Philadelphia. ASTM Annual Book Standards, Part 31, D3370-76.
4. Mikhail, S.Z., & Kimel, W.R. (1961). Densities and Viscosities of Methanol-Water Mixtures. Journal of Chemical and Engineering Data. 6(4), 533-537. https://doi.org/10.1021/je60011a015

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION

Refer to the relevant determinative method for performance data.

Attachment 14

Additional Performance Data Associated with Multi-Laboratory Validation of SW-846 Methods 3512 and 8327

Date: 07-15-2021
Author: United States Environmental Protection Agency
Docket No. EPA-HQ-OLEM-2018-0846

Groundwater % Recovery statistics, 200 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	90.7	2.3	110	6.7	108	4.3	91.7	2.6	82.3	10.0	93.9	2.3	89.8	5.5	112	7.6	97.2	5.8	11.0
PFPeA	107	7.3	109	8.2	113	8.0	96.8	1.6	81.8	2.0	91.6	2.1	91.9	2.9	111	3.9	100	5.2	11.3
PFHxA	89.0	7.5	104	17.1	103	10.6	97.4	1.7	93.7	2.6	93.3	1.7	91.0	2.9	104	14.0	96.9	9.2	6.1
PFHpA	93.0	3.7	105	9.0	106	6.6	96.8	3.3	95.3	5.1	97.4	3.2	91.9	5.5	104	13.7	98.8	7.1	5.7
PFOA	98.8	12.3	107	5.5	111	9.0	97.3	2.4	93.4	3.9	107	2.7	91.2	5.9	111	21.0	102	9.8	7.8
PFNA	93.4	5.9	107	13.5	115	10.3	98.6	1.1	94.8	2.1	106	3.5	90.9	6.2	99.3	11.8	101	8.1	8.1
PFDA	95.7	4.4	104	17.2	109	5.7	95.4	2.7	95.6	5.5	111	7.1	95.3	8.1	110	14.4	102	9.4	7.2
PFUnA	87.0	12.4	101	6.9	115	6.2	100	2.4	89.7	4.0	107	6.0	89.7	7.3	136	15.4	103	8.6	16.4
PFDoA	83.0	3.9	101	3.6	106	5.3	97.4	2.1	91.4	4.0	99.9	5.4	93.5	5.9	150	10.7	103	5.6	20.4
PFTriA	73.2	6.7	103	5.5	124	8.3	95.7	3.0	91.7	4.4	99.6	4.1	87.4	14.7	120	12.4	99.3	8.4	16.6
PFTreA	76.1	6.7	90.3	9.9	99.5	11.0	89.3	4.0	87.8	4.5	100	3.0	83.0	15.2	117	24.7	92.9	12.0	12.6
PFBS	93.1	4.5	108	11.9	109	13.0	98.3	2.7	101	1.7	95.7	2.0	92.3	2.8	107	15.1	101	8.5	6.7
PFPeS	88.9	6.5	108	6.1	103	4.3	102	1.3	98.1	1.0	94.9	2.1	91.0	3.7	109	7.3	99.2	4.7	7.4
PFHxS	98.6	6.8	96.8	6.5	105	5.7	101	1.3	99.4	2.1	98.8	7.8	92.2	3.6	113	7.3	101	5.6	6.0
PFHpS	89.2	5.5	105	9.2	106	4.6	101	2.2	94.9	2.4	110	5.2	92.1	4.6	114	1.6	102	5.0	8.9
PFOS	95.9	8.5	108	11.7	116	7.1	99.8	2.4	99.5	2.0	107	3.8	90.7	5.5	125	5.6	105	6.6	11.1
PFNS	92.3	9.0	106	5.3	121	5.0	103	1.2	92.4	2.8	114	6.7	91.4	5.5	130	7.1	106	5.8	14.3
PFDS	88.0	3.6	100	9.4	121	9.1	97.8	2.0	85.8	2.1	107	4.3	88.2	5.6	130	9.3	102	6.4	16.1
PFOSA	90.8	3.0	99.5	5.4	111	7.1	96.8	1.3	90.0	3.4	93.4	2.7	84.5	2.2	112	3.8	97.3	4.0	10.0
FtS 4:2	91.3	7.7	106	9.0	111	6.6	93.3	2.0	79.4	4.0	92.8	3.4	93.9	7.3	113	6.9	97.6	6.3	11.4
FtS 6:2	94.4	20.4	103	9.1	112	15.7	102	2.8	188	217	38.1	2.8	84.6	7.5	54.4	3.5	97.1	77.4	44.8
FtS 8:2	88.5	13.5	109	16.7	119	10.0	107	3.2	101	8.6	115	4.6	93.0	8.9	109	10.0	105	10.3	10.5
NMeFOSAA	91.7	12.5	104	10.1	133	30.5	94.7	1.4	92.8	3.5	105	3.5	93.0	7.6	112	7.1	103	12.9	14.1
NEtFOSAA	91.3	10.2	111	16.8	155	24.7	96.2	1.7	93.9	4.4	102	3.5	92.7	6.6	120	5.7	108	11.8	21.4

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Groundwater % Recovery statistics, 60 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	88.0	13.7	124	6.7	95.9	11.6	74.7	14.9	87.2	9.3	102	7.7	87.6	7.1	124	34.0	98.0	15.6	17.9
PFPeA	97.6	13.1	124	5.5	109	9.5	89.5	4.5	83.5	5.5	98.5	9.5	106	6.9	194	84.1	113	30.7	34.9
PFHxA	85.9	13.4	106	11.3	99.0	13.2	86.5	7.8	99.5	4.1	99.6	7.9	91.0	8.4	92.4	28.3	95.0	13.7	7.1
PFHpA	89.3	15.5	108	8.7	100	7.9	85.7	3.3	94.1	7.2	105	15.4	87.8	6.8	99.7	16.7	96.2	11.2	8.3
PFOA	88.4	21.4	109	6.5	91.6	13.8	91.9	4.4	96.5	5.4	111	12.8	92.1	6.5	111	10.0	98.8	11.4	9.6
PFNA	87.8	12.8	105	12.5	100	10.1	84.9	4.7	90.1	5.6	114	16.8	84.2	8.0	105	21.7	96.5	12.7	11.2
PFDA	85.3	14.6	98.4	13.5	109	11.7	82.7	4.6	96.6	4.8	112	14.2	82.8	11.6	118	35.1	98.2	16.4	14.0
PFUnA	84.7	14.6	91.0	17.6	107	9.2	86.7	4.6	86.5	4.1	104	12.3	87.1	8.1	121	18.7	96.0	12.3	13.2
PFDoA	87.2	19.2	72.6	7.2	106	9.8	84.5	3.3	91.4	4.0	110	16.3	86.2	12.7	146	30.4	98.0	15.4	22.7
PFTriA	75.1	8.6	73.0	7.5	133	13.1	84.6	4.2	91.6	6.6	110	11.4	76.8	5.7	122	24.3	95.8	11.8	23.1
PFTreA	72.8	8.3	68.2	14.4	103	9.1	78.9	7.1	87.6	4.8	123	17.0	74.3	16.9	98.2	23.5	88.2	13.9	18.6
PFBS	92.8	15.4	108	22.2	99.4	13.2	87.9	2.1	104.8	10.2	96.0	8.7	104	21.9	99.0	21.0	99.1	15.9	6.8
PFPeS	81.4	12.3	105	7.6	101	4.8	90.8	2.8	95.5	4.7	101	4.6	90.7	2.5	102	10.9	95.8	7.1	7.8
PFHxS	88.5	15.1	66.8	10.5	103	9.7	93.2	3.4	99.3	7.6	102	23.2	89.7	2.0	117	12.9	94.9	12.3	14.5
PFHpS	82.1	21.9	98.7	15.4	98.7	9.3	90.9	5.3	94.6	5.7	110	12.8	89.8	3.7	116	8.6	97.5	11.8	10.9
PFOS	82.6	13.4	110	21.5	116	10.5	91.7	5.3	98.7	8.9	111	19.7	88.1	4.3	122	14.7	103	13.6	14.3
PFNS	81.4	19.5	87.0	8.3	118	5.2	92.1	5.0	92.8	7.6	126	9.5	86.3	5.9	115	12.2	99.8	10.2	17.0
PFDS	70.1	16.3	86.2	11.1	118	8.9	90.0	5.6	86.5	3.3	114	10.3	82.1	5.4	118	6.1	95.6	9.2	18.3
PFOSA	77.2	10.6	80.0	7.0	93.2	12.0	84.8	5.5	91.0	5.7	100	8.0	81.6	3.5	109	5.6	89.6	7.7	10.9
FtS 4:2	73.1	14.5	97.3	33.6	106	20.8	84.6	4.4	72.5	9.7	95.1	8.2	93.3	4.7	107	15.3	91.0	16.6	13.2
FtS 6:2	80.5	9.2	87.8	19.3	96.6	11.6	90.5	4.5	77.2	42.4	39.6	5.2	86.8	7.1	45.8	6.8	75.6	17.8	21.2
FtS 8:2	82.7	29.4	99.1	7.6	115	29.2	93.6	4.3	98.7	8.8	119	11.3	92.1	11.4	108	30.0	101	19.4	12.1
NMeFOSAA	79.6	17.0	116	29.9	91.7	16.2	83.8	4.3	94.8	5.1	107	19.0	85.2	8.8	110	11.7	96.0	16.1	13.4
NEtFOSAA	72.8	37.1	77.6	18.9	136	36.1	86.4	4.2	97.5	4.5	104	13.4	83.3	7.1	124	14.2	97.6	20.9	22.4

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Reagent water % Recovery statistics, 200 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	110	8.0	111	7.2	109	8.7	88.4	10.6	65.1	10.5	94.9	4.5	86.3	7.7	107	20.1	96.4	10.6	16.1
PFPeA	118	4.5	112	7.1	112	4.4	90.2	4.9	85.4	3.0	86.7	4.9	90.8	3.7	107	2.1	100	4.6	13.1
PFHxA	104	4.7	116	5.4	104	8.7	91.1	5.6	94.2	1.7	92.1	3.0	93.1	6.4	102	18.2	99.5	8.2	8.5
PFHpA	108	4.8	110	7.7	110	3.6	94.5	1.7	97.2	3.0	97.0	3.3	87.4	4.4	107	13.9	101	6.4	8.4
PFOA	111	9.3	110	7.8	115	7.9	93.6	1.7	94.5	1.5	102	5.3	94.4	6.1	114	14.6	104	7.9	9.3
PFNA	106	2.3	113	3.8	118	1.8	95.7	2.0	90.9	3.2	104	3.9	91.0	5.3	110	17.6	104	7.0	10.1
PFDA	106	10.8	116	35.8	119	10.6	98.0	1.6	94.9	3.0	101	2.5	97.1	7.3	111	7.5	106	14.3	9.3
PFUnA	96.9	15.6	107	8.8	119	4.1	99.1	1.9	93.8	3.0	97.5	4.9	89.5	9.9	134	8.9	105	8.3	14.8
PFDoA	90.5	10.0	101	7.3	110	7.5	99.3	3.3	95.6	1.8	100	3.8	93.4	7.0	134	13.6	103	7.7	13.9
PFTriA	85.5	5.6	97.5	21.2	130	12.0	104	3.5	97.8	2.8	101	9.2	84.7	11.3	121	12.2	103	11.2	15.7
PFTreA	84.5	9.7	83.8	21.1	115	12.9	104	3.9	96.5	3.0	111	11.3	88.1	8.6	127	18.5	101	12.6	15.7
PFBS	106	4.9	114	17.1	112	5.2	92.2	4.0	93.4	10.3	90.6	4.3	91.3	2.7	102	14.0	100	9.3	9.4
PFPeS	101	4.4	109	8.2	98.5	8.5	92.1	3.1	97.4	2.1	91.4	3.8	92.5	4.5	110	9.8	99.0	6.2	7.4
PFHxS	106	11.5	97.0	4.1	112	3.2	95.4	0.9	98.9	2.4	94.6	9.7	91.8	3.1	114	10.4	101	6.9	8.3
PFHpS	95.4	7.9	110	3.2	113	5.0	98.4	0.8	95.7	3.9	104	1.7	93.0	5.0	121	7.0	104	4.9	10.1
PFOS	96.8	4.8	113	11.5	118	3.4	98.2	2.3	99.0	2.9	102	4.1	87.3	5.6	119	6.3	104	5.8	11.2
PFNS	104	15.4	102	7.2	126	9.5	98.5	0.9	94.3	1.3	105	3.9	90.8	3.5	129	7.6	106	7.6	13.9
PFDS	96.8	11.8	98.9	5.3	127	13.0	100	2.2	87.6	4.6	102	4.1	89.4	3.9	127	4.4	104	7.2	15.2
PFOSA	88.8	5.0	96.2	7.3	106	1.8	94.3	1.5	87.0	3.0	90.8	4.2	72.9	2.4	101	5.1	92.1	4.2	10.0
FtS 4:2	109	9.0	115	6.1	108	8.1	92.9	4.3	105	4.0	94.0	3.0	96.9	7.9	111	7.3	104	6.5	8.3
FtS 6:2	105	7.3	105	9.4	112	9.6	104	5.2	113	27.7	53.3	34.3	85.3	8.6	60.6	4.7	92.3	17.0	23.5
FtS 8:2	99.7	13.1	109	8.4	126	12.4	105	2.2	111	9.7	111	4.1	90.0	10.1	120	19.2	109	11.1	11.1
NMeFOSAA	102	11.9	98.5	10.8	111	23.6	98.8	1.3	97.2	4.1	92.3	4.9	88.0	4.3	116	14.0	100	11.6	9.2
NEtFOSAA	89.5	11.4	103	30.7	141	23.7	98.9	1.9	99.5	2.4	89.6	3.2	89.6	7.3	121	16.2	104	15.7	18.3

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Reagent water % Recovery statistics, 60 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	103	13.6	119	10.7	112	16.7	85.4	3.3	58.5	17.0	96.3	5.8	75.5	4.7	96.4	20.8	93.1	13.1	19.6
PFPeA	104	6.0	120	18.1	111	12.2	87.5	3.5	88.4	9.3	115	16.7	89.3	2.1	117	23.7	104	13.5	13.7
PFHxA	99.8	10.2	111	15.9	105	14.2	90.2	5.2	97.8	12.7	99.2	6.2	87.2	6.9	89.2	16.1	97.4	11.7	8.2
PFHpA	101	8.6	111	8.0	113	4.5	87.8	5.5	94.7	9.1	104	6.0	83.5	5.6	92.6	15.9	98.5	8.6	10.7
PFOA	105	15.0	108	10.2	106	10.8	88.8	2.8	93.8	13.3	110	7.0	84.1	11.9	104	14.9	100	11.4	9.7
PFNA	97.0	9.9	103	5.6	111	9.8	90.0	4.9	93.7	12.9	105	9.2	83.5	7.1	92.6	13.8	97.0	9.6	8.9
PFDA	88.6	7.1	126	31.0	111	22.0	92.6	5.4	97.8	11.6	108	10.2	82.1	9.7	110	19.0	102	16.6	14.4
PFUnA	87.8	9.7	87.2	15.4	123	15.5	92.7	3.9	93.5	11.9	106	3.4	83.9	10.2	118	23.5	98.9	13.2	14.7
PFDoA	81.2	19.0	73.0	15.1	106	19.1	91.7	5.8	95.9	10.5	103	12.2	85.3	13.0	131	32.3	95.8	17.5	17.9
PFTriA	77.9	12.4	78.8	18.0	120	19.2	93.6	5.5	100	11.1	111	11.0	76.0	14.1	125	15.6	97.7	14.0	19.3
PFTreA	66.3	12.7	73.3	26.3	108	22.5	92.4	8.7	97.9	11.0	111	7.7	80.4	11.5	135	9.2	95.5	15.1	22.6
PFBS	94.6	11.6	102	16.4	96.5	9.7	87.0	5.4	91.7	16.1	92.4	8.9	89.1	3.0	88.6	11.7	92.7	11.3	4.9
PFPeS	98.4	11.5	107	8.9	99.4	6.0	88.3	3.4	96.6	11.0	95.6	4.9	88.6	4.2	100	11.3	96.8	8.3	6.2
PFHxS	99.3	11.2	68.2	13.6	111	14.5	92.9	5.8	100	12.4	99.3	9.1	87.9	4.4	104	10.8	95.3	10.8	12.9
PFHpS	95.7	16.5	104	10.4	107	13.0	92.8	5.8	95.4	9.1	112	8.9	88.4	4.9	115	16.8	101	11.5	9.5
PFOS	90.5	5.0	112	35.3	113	11.6	92.8	3.5	98.0	9.1	102	15.9	74.9	5.0	117	14.7	100	15.8	14.0
PFNS	80.4	10.2	103	4.5	121	13.4	92.9	4.9	97.2	4.8	113	8.1	87.4	3.0	119	18.9	102	9.9	14.9
PFDS	84.5	9.7	84.0	11.4	117	22.0	94.9	6.9	91.4	9.7	106	6.8	84.4	5.0	119	16.3	97.7	12.2	14.6
PFOSA	78.6	6.1	88.5	12.7	96.0	7.0	83.8	3.5	86.9	7.0	97.3	7.7	74.7	3.0	95.7	12.7	87.7	8.2	8.4
FtS 4:2	93.7	18.5	110	31.2	103	14.1	90.4	6.5	97.0	10.7	100	9.6	91.8	7.1	102	14.8	98.5	15.9	6.5
FtS 6:2	100	20.0	98.2	16.0	99.3	17.8	96.2	7.0	128	84.3	39.2	6.0	87.9	6.6	35.0	21.0	85.5	32.9	32.0
FtS 8:2	96.3	9.3	95.6	17.0	109	12.9	103	6.5	119	15.4	115	10.5	92.7	8.4	110	16.6	105	12.6	9.6
NMeFOSAA	100	15.3	92.3	29.3	120	25.6	93.0	3.1	101	11.6	95.4	10.0	84.6	10.1	103	12.6	98.6	16.8	10.5
NEtFOSAA	88.6	9.1	71.7	19.6	127	39.9	94.0	5.4	102	14.0	94.7	8.8	82.3	12.5	112	12.3	96.5	18.3	17.2

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Surface water % Recovery statistics, 200 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	85.0	6.8	109	7.3	113	5.7	76.4	14.0	64.2	5.8	93.6	2.8	87.4	6.4	93.3	18.3	90.2	9.7	16.0
PFPeA	98.6	3.7	115	3.6	113	4.9	109	1.6	83.7	4.1	88.3	4.5	88.7	3.1	100	4.0	99.5	3.8	11.9
PFHxA	91.9	3.4	109	8.0	111	10.7	102	2.5	92.4	2.7	95.4	3.3	94.4	8.4	95.6	13.2	98.9	7.6	7.4
PFHpA	98.8	4.0	108	7.1	110	4.0	102	3.2	96.3	4.6	99.0	5.1	90.7	1.9	95.1	11.6	100	5.9	6.4
PFOA	98.6	10.7	110	7.4	111	5.1	103	4.0	92.8	1.6	107	4.5	89.1	8.8	109	6.8	103	6.7	8.3
PFNA	95.0	9.7	110	9.0	117	5.9	108	3.4	92.3	2.9	107	7.1	83.1	8.4	95.4	12.9	101	8.1	11.2
PFDA	91.7	8.3	108	16.4	116	2.1	107	5.3	96.2	4.9	108	6.9	90.8	7.9	109	16.2	104	9.8	9.3
PFUnA	94.1	10.5	106	6.7	123	2.8	105	5.0	90.6	2.8	99.1	4.6	88.7	7.7	118	13.9	103	7.7	12.4
PFDoA	74.6	5.7	105	7.4	118	5.3	105	2.7	91.7	4.0	102	3.5	83.0	7.9	131	21.9	101	9.3	18.3
PFTriA	75.4	7.9	110	3.5	128	8.5	100	3.0	92.6	2.7	102	1.9	78.4	10.9	109	12.8	99.4	7.5	17.2
PFTreA	73.7	8.3	102	13.7	114	5.8	90.4	4.2	90.3	2.1	111	2.8	74.6	10.0	97.3	8.8	94.1	7.9	14.9
PFBS	93.8	3.7	115	13.7	112	7.6	96.6	2.7	98.2	1.3	94.6	5.7	89.8	2.0	94.1	11.2	99.2	7.3	9.0
PFPeS	86.4	6.4	111	4.8	106	3.5	99.1	1.9	95.1	1.9	96.7	3.5	89.8	1.0	105	6.5	98.7	4.2	8.4
PFHxS	99.7	7.2	104	9.6	110	7.1	99.1	1.0	96.3	2.3	101	5.5	90.8	2.9	109	7.0	101	6.0	6.5
PFHpS	86.1	7.7	103	5.3	112	2.8	101	1.4	93.3	2.4	106	3.0	91.2	2.6	109	15.3	100	6.6	9.1
PFOS	94.2	4.0	103	12.7	119	5.6	101	2.0	98.3	1.5	108	4.1	90.7	3.7	112	9.2	103	6.4	9.4
PFNS	99.1	15.6	109	9.9	125	5.2	101	0.9	93.2	2.7	112	3.1	88.6	1.6	117	8.0	106	7.5	12.2
PFDS	80.0	11.0	103	3.3	126	9.0	99.2	1.8	85.7	1.8	107	2.7	85.3	3.0	115	8.0	100	6.1	15.9
PFOSA	90.4	3.4	95.1	5.6	104	5.1	93.9	1.1	88.9	3.1	93.6	2.9	84.0	2.3	103	5.9	94.2	4.0	6.9
FtS 4:2	83.3	10.7	107	10.6	113	3.2	118	22.2	93.6	6.8	95.1	3.4	94.7	6.4	104	6.1	101	10.4	11.5
FtS 6:2	106	12.6	102	5.6	103	4.7	125	26.6	74.8	10.1	39.8	2.3	84.8	10.0	54.4	3.2	86.2	11.9	28.6
FtS 8:2	95.7	13.6	103	20.9	130	14.0	118	13.6	105	9.8	107	4.4	93.1	9.3	103	10.4	107	12.8	12.1
NMeFOSAA	89.7	11.6	106	21.3	135	23.2	101	2.4	95.4	3.3	101	4.2	87.3	8.0	109	13.3	103	13.2	14.9
NEtFOSAA	92.5	11.8	108	9.6	149	12.2	102	3.3	97.2	4.4	97.2	3.1	88.2	7.1	110	11.9	105	8.7	19.1

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Surface water % Recovery statistics, 60 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	78.9	12.9	124	9.2	111	10.7	76.5	24.5	54.2	9.0	85.0	4.1	84.0	12.6	80.8	30.3	86.7	16.4	21.4
PFPeA	84.8	13.2	130	7.7	104	9.2	114	8.3	76.4	6.7	101	16.8	101	8.4	109	6.6	103	10.2	16.6
PFHxA	84.2	11.9	131	24.3	114	28.3	97.4	9.3	89.5	6.1	93.8	6.8	93.7	10.1	82.0	21.6	98.2	16.9	16.6
PFHpA	86.1	13.4	114	15.7	107	12.9	96.8	2.3	84.5	3.6	96.2	6.4	91.4	9.9	88.5	22.3	95.6	12.5	10.4
PFOA	95.7	27.7	111	10.4	113	18.6	104	4.8	84.7	5.3	103	10.9	90.0	9.2	103	11.3	101	14.2	9.8
PFNA	80.7	12.9	105	17.7	109	12.5	104	3.7	82.9	6.5	104	8.0	86.8	6.6	88.5	12.7	95.1	10.9	11.4
PFDA	82.5	8.9	101	31.0	113	10.2	102	5.5	88.1	5.9	104	2.9	82.5	12.9	104	13.8	97.2	14.0	11.3
PFUnA	80.6	15.4	95.3	13.0	104	24.7	104	4.7	80.2	4.8	102	4.1	86.7	13.2	117	29.9	96.3	16.4	13.1
PFDoA	76.7	18.4	76.5	8.0	108	12.9	103	5.6	83.0	2.1	96.7	5.9	83.6	21.8	125	9.5	93.9	12.3	17.0
PFTriA	62.7	16.0	77.7	21.7	124	24.9	98.6	4.7	89.6	6.9	101	4.2	76.9	11.8	108	9.9	92.3	14.4	19.7
PFTreA	52.4	16.5	85.5	13.7	98.5	13.1	84.5	10.3	90.6	5.8	105	6.2	74.1	18.3	79.6	17.9	83.8	13.5	16.1
PFBS	90.5	14.8	110	13.5	99.4	16.6	97.5	4.8	87.8	3.3	90.8	4.8	99.3	12.8	84.3	22.8	94.9	13.3	8.2
PFPeS	77.3	14.8	109	11.2	102	14.0	97.9	3.7	87.2	5.1	94.5	2.9	93.4	8.0	101	15.2	95.3	10.5	9.8
PFHxS	84.6	15.6	114	11.1	108	9.0	95.5	4.0	88.1	4.1	84.3	6.5	94.3	9.0	107	7.1	96.9	9.0	11.4
PFHpS	82.3	20.5	96.4	12.5	102	14.5	99.9	4.9	82.7	3.6	107	4.6	91.2	7.6	111	6.4	96.5	10.9	10.5
PFOS	95.5	18.6	107	26.4	119	14.5	101	3.5	89.2	2.3	101	6.4	97.5	11.1	119	10.1	104	13.8	10.8
PFNS	67.4	5.9	98.4	15.2	119	15.7	99.6	2.4	87.0	4.2	114	8.2	89.2	8.9	120	9.2	99.4	9.8	18.2
PFDS	70.2	11.8	77.6	24.6	116	17.7	101	4.4	82.3	3.1	104	10.1	87.1	10.2	117	15.5	94.4	13.8	17.6
PFOSA	67.0	9.0	68.7	6.3	94.7	9.1	88.6	3.4	76.5	6.1	98.6	22.8	85.6	6.8	101	3.7	85.2	10.2	13.2
FtS 4:2	72.0	17.9	89.2	17.0	113	18.7	106	6.5	79.0	6.4	86.1	6.2	91.9	8.6	101	9.0	92.2	12.4	13.7
FtS 6:2	93.1	15.8	102	28.0	119	12.4	113	10.0	-4.3	18.5	494	1030	85.5	6.9	41.2	6.5	130	363	153
FtS 8:2	75.6	10.3	104	22.3	92.9	26.5	118	12.1	88.4	6.6	104	9.0	83.5	12.0	84.8	7.3	93.9	14.9	13.8
NMeFOSAA	74.0	21.2	94.0	14.3	143	53.7	97.8	1.8	85.5	3.9	92.4	3.6	89.8	8.3	111	21.0	98.5	22.6	20.9
NEtFOSAA	75.3	25.7	88.8	37.5	137	20.4	105	2.9	87.1	6.0	91.9	7.4	86.0	9.1	121	12.8	99.0	18.8	20.7

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Wastewater % Recovery statistics, 200 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=5) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	82.0	6.6	110	8.2	109	9.0	77.8	18.3	80.7	9.9	96.4	3.5	92.0	5.2	115	25.0	95.4	12.7	14.7
PFPeA	100	6.5	118	4.9	115	6.3	103	6.1	81.3	3.8	86.2	2.0	93.6	3.6	115	10.5	102	6.0	13.8
PFHxA	95.4	3.8	110	9.1	109	9.6	100	6.6	91.1	6.0	95.6	3.7	98.8	5.9	90.0	30.7	98.7	12.5	7.3
PFHpA	95.7	3.4	108	10.3	110	6.0	103	5.0	92.1	4.0	98.4	2.8	94.6	3.7	98.7	21.9	100	9.3	6.4
PFOA	99.6	10.9	107	6.6	114	10.2	107	4.5	89.1	5.2	109	4.6	93.4	6.4	111	11.8	104	8.0	8.8
PFNA	97.3	5.0	107	7.1	115	8.8	106	3.7	90.8	3.9	110	4.7	91.4	10.2	109	18.1	103	8.9	9.0
PFDA	99.3	8.7	113	21.7	119	1.3	112	4.4	93.4	4.0	111	9.7	97.7	15.0	105	13.5	106	11.6	8.9
PFUnA	87.7	8.9	103	11.8	125	3.8	109	6.5	88.2	4.6	103	3.8	91.5	10.8	141	19.4	106	10.0	18.7
PFDoA	81.3	5.3	103	7.3	101	17.5	108	5.8	88.8	4.8	98.5	3.6	88.9	15.0	140	15.0	101	10.7	17.8
PFTriA	69.1	11.3	109	11.4	109	26.4	106	5.4	92.6	5.3	100	4.1	81.8	10.0	119	21.5	98.2	14.1	16.2
PFTreA	69.7	11.0	99.6	15.3	83.6	18.3	89.8	10.1	88.2	4.5	107	6.5	84.5	13.7	113	18.2	91.8	13.1	13.9
PFBS	95.4	7.4	109	7.4	113	7.7	103	5.9	96.8	3.1	92.2	6.7	96.2	2.2	91.2	23.2	99.5	10.0	7.8
PFPeS	93.0	4.4	110	4.7	101	3.3	102	4.7	95.2	3.3	97.6	2.6	93.4	2.8	109	18.0	100	7.3	6.7
PFHxS	101	4.1	115	36.6	115	2.7	103	4.2	97.2	2.4	97.6	4.1	97.3	3.4	119	10.1	106	13.8	9.0
PFHpS	91.9	9.0	107	6.3	115	6.2	102	5.2	95.3	5.9	109	4.0	93.1	5.4	118	7.9	104	6.4	10.1
PFOS	102	10.6	113	6.7	120	4.3	101	15.1	194	145	111	11.8	92.3	7.3	124	7.0	120	51.9	31.8
PFNS	85.5	9.0	106	10.5	112	17.3	103	5.3	92.4	3.0	116	3.2	90.9	5.5	130	11.7	104	9.4	14.7
PFDS	66.7	10.9	101	6.5	92.4	35.6	95.8	3.7	85.8	3.0	107	5.5	89.0	8.2	128	16.2	95.5	15.1	17.5
PFOSA	91.5	2.1	100	3.8	115	1.7	99.2	4.8	88.7	4.0	94.4	1.5	85.8	4.4	114	10.6	98.6	4.9	10.9
FtS 4:2	88.2	5.1	118	10.0	118	8.6	134	16.1	78.7	6.6	99.2	3.2	93.9	5.5	115	16.0	106	10.0	18.6
FtS 6:2	104	8.2	111	12.7	118	13.3	141	18.6	89.5	7.4	41.8	2.5	83.0	9.8	63.4	7.4	93.9	11.0	31.5
FtS 8:2	149	23.5	116	9.7	112	11.0	144	12.3	95.3	11.7	117	1.5	94.0	11.5	120	15.8	118	13.4	19.7
NMeFOSAA	85.1	11.8	110	15.4	110	35.6	101	5.0	90.8	6.2	99.2	3.3	88.0	9.5	116	9.9	100	15.4	11.4
NEtFOSAA	101	6.2	103	10.1	152	16.0	117	5.4	92.0	3.9	96.8	5.7	92.0	7.3	122	13.0	109	9.3	20.2

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Wastewater % Recovery statistics, 60 ng/L (nom.) concentration[1]

Target Analyte	Lab 2 (n=5) % Recovery		Lab 4 (n=5) % Recovery		Lab 5 (n=5) % Recovery		Lab 6 (n=5) % Recovery		Lab 10 (n=4) % Recovery		Lab 11 (n=5) % Recovery		Lab 12 (n=5) % Recovery		Lab 16 (n=5) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	82.8	7.7	123	11.0	115	17.0	84.5	9.5	76.0	19.8	92.9	11.0	98.4	8.9	96.8	23.3	96.1	14.6	15.9
PFPeA	99.6	6.6	122	7.5	124	11.6	99.9	10.9	90.8	15.7	112	15.8	103	13.8	101	9.0	107	11.8	11.6
PFHxA	101	10.0	131	21.9	116	15.2	91.8	9.5	85.8	9.6	90.0	12.0	90.3	12.9	78.4	21.2	97.9	14.8	17.3
PFHpA	98.4	7.3	114	7.5	112	13.1	101	6.9	91.8	2.0	91.6	10.6	97.9	4.7	102	4.0	101	7.8	8.4
PFOA	110	21.4	104	7.9	107	5.8	99.8	6.3	84.5	4.7	95.9	15.5	97.1	8.0	101	10.0	99.8	11.3	7.7
PFNA	91.3	10.3	103	10.1	110	10.1	96.4	8.1	90.2	3.8	104	18.0	92.7	9.6	98.9	18.6	98.3	12.0	6.9
PFDA	97.5	10.2	92.4	24.9	117	17.7	103	9.6	89.5	8.6	96.2	15.1	96.7	21.2	106	24.1	99.8	17.5	8.8
PFUnA	86.3	11.4	83.8	12.3	123	11.3	101	9.2	86.3	7.3	98.6	17.1	89.8	11.2	115	29.2	97.9	15.1	14.3
PFDoA	83.2	9.1	57.4	9.3	97.7	20.0	99.3	7.5	87.8	8.8	98.1	19.7	90.5	10.1	149	24.0	95.4	14.9	25.6
PFTriA	75.9	11.3	75.9	10.3	98.7	29.5	99.8	8.7	93.7	7.0	100	13.1	88.8	14.9	144	39.6	97.1	20.0	21.3
PFTreA	66.0	18.3	58.2	20.7	90.7	26.0	91.0	8.1	90.2	4.4	104	14.3	82.7	15.1	143	21.4	90.7	17.4	25.7
PFBS	105	8.7	110	24.4	111	9.4	97.3	8.5	93.5	1.7	87.4	6.6	102	3.0	92.4	9.5	99.8	11.1	8.6
PFPeS	93.6	11.6	96.6	12.1	100	7.6	100	8.7	90.7	6.8	95.4	2.7	93.0	4.7	99.3	8.4	96.1	8.4	3.6
PFHxS	96.5	10.9	102	5.5	115	12.3	101	10.7	95.8	4.1	81.8	15.5	102	3.3	120	8.4	102	9.7	11.7
PFHpS	95.4	18.0	94.8	15.6	107	11.8	100	8.3	88.9	3.2	101	9.5	94.1	4.8	118	11.0	100	11.3	9.2
PFOS	116	16.4	130	17.2	108	6.0	92.9	10.4	93.9	4.1	95.9	28.5	96.9	6.2	128	7.2	108	14.3	15.2
PFNS	80.8	14.0	90.1	12.0	110	13.3	100	8.0	90.0	2.0	117	2.1	93.5	7.2	121	21.2	100	11.7	14.2
PFDS	80.9	11.5	76.5	10.3	98.9	32.8	93.4	8.9	83.7	5.8	106	7.6	91.0	5.3	128	13.1	94.8	14.5	16.5
PFOSA	88.2	5.2	85.1	7.4	98.5	7.8	91.9	5.4	85.0	5.9	90.5	1.7	88.7	3.1	109	9.2	92.2	6.2	8.2
FtS 4:2	84.9	10.4	98.7	32.3	132	24.8	102	14.7	81.2	3.7	90.4	10.3	93.8	10.0	104	9.0	98.3	16.9	15.7
FtS 6:2	109	6.1	110	18.2	115	24.1	94.3	10.9	107	54.9	39.2	6.5	76.7	11.7	59.6	7.1	88.8	23.2	27.7
FtS 8:2	124	22.5	102	24.5	151	26.6	112	10.5	95.0	14.7	106	4.6	94.0	8.7	98.8	13.8	110	17.4	19.1
NMeFOSAA	93.8	29.1	115	14.0	124	42.6	89.9	6.7	89.3	5.9	87.2	10.8	94.4	7.2	103	12.6	99.5	20.2	13.3
NEtFOSAA	93.9	22.2	74.2	17.1	159	17.6	107	8.2	93.1	7.3	93.6	10.2	95.5	9.4	117	10.4	104	13.8	25.4

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Lowest Acceptable LLOQ Verification QC Sample Concentrations by Laboratory and Sample Preparation Batch, in ng/L[1]

Lab	Lab 2			Lab 4			Lab 5			Lab 6			Lab 10			Lab 11			Lab 12			Lab 16			Median LLOQ (ng/L); n=24	Median 95% low CI	Median 95% high CI
Batch #	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3			
PFBA	10	10	10	80	80	40	10	20	20	10	10	10	10	20	10	20	20	20	10	10	10	10	10	10	10	10	20
PFPeA	10	10	10	80	80	40	10	20	10	10	10	10	10	20	20	20	20	20	10	10	10	10	10	10	10	10	20
PFHxA	10	10	20	80	80	40	20	10	20	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFHpA	10	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	10
PFOA	20	10	20	80	80	40	10	10	20	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFNA	10	10	10	80	80	40	10	10	10	10	10	10	20	20	10	20	20	20	10	10	10	10	10	10	10	10	20
PFDA	20	10	***160***	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	20	10	10	10	10	10	10	10	20
PFUnA	10	20	20	80	80	40	10	10	20	10	10	10	10	20	10	20	20	20	10	10	10	10	10	10	10	10	20
PFDoDA	20	20	***160***	80	80	40	10	20	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFTrDA	10	20	20	80	80	40	***160***	40	40	10	10	10	10	20	10	20	20	20	10	10	10	10	10	10	15	10	20
PFTeDA	20	20	20	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	20	10	10	10	20
PFBS	20	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFPeS	10	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	10
PFHxS	10	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	10
PFHpS	10	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	20	20	10	10	20
PFOS	20	10	20	80	80	40	10	10	20	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFNS	10	20	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
PFDS	10	20	20	80	80	40	20	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	20	10	10	10	20
PFOSA	10	10	10	80	80	40	10	10	10	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	10
4:2 FTS	10	10	20	80	80	40	10	10	20	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	10	10	20
6:2 FTS	20	10	10	80	80	40	20	10	10	10	10	***160***	***160***	***160***	***160***	none	none	none	20	20	20	***160***	***160***	***160***	60	20	160
8:2 FTS	10	10	20	80	80	40	40	40	40	10	10	10	10	***160***	10	20	20	20	10	10	10	***160***	10	10	15	10	40
NMeFOSAA	20	***160***	***160***	80	80	40	40	20	20	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	15	10	20
NEtFOSAA	***160***	20	***160***	80	80	40	20	20	40	10	10	10	10	10	10	20	20	20	10	10	10	10	10	10	15	10	20

[1]NOTES:

Lower Limits of Quantitation (LLOQs) in the table above are nominal; Preliminary acceptance criterion for LLOQ Verification was 50-150% recovery

95% Confidence Interval (CI) of median is calculated as described in Section 1.3.1 of the Method 8327 Statistical Report; the 95% low and high CI are equivalent to the 7th and 18th ranked values sorted from low to high, respectively.

Values in bold did not meet preliminary LLOQ verification acceptance criteria (50-150% recovery) except at the LCS level (160 ng/L, nominal)

Values in red did not meet preliminary LLOQ verification acceptance criteria (50-150% recovery) at any concentration; These values were considered to be >160 ng/L for determination of median LLOQ and 95% CI

Labs 2, 10, 12, and 16 included LLOQ verifications at 10 and 20 ng/L in each preparation batch

Lab 4 included LLOQ verifications at 80 ng/L in preparation batches 1 and 2 and at 40 ng/L in preparation batch 3

Lab 5 included LLOQ verifications at 10, 20 and 40 ng/L in each preparation batch

Lab 6 included two replicate LLOQ verifications at 10 ng/L in each preparation batch

Lab 11 included LLOQ verifications at 20 ng/L in each preparation batch

LCS % Recovery statistics by Laboratory, 160 ng/L (nom.) concentration

Target Analyte	Lab 2 (n=6) % Recovery		Lab 4 (n=6) % Recovery		Lab 5 (n=6) % Recovery		Lab 6 (n=6) % Recovery		Lab 10 (n=6) % Recovery		Lab 11 (n=6) % Recovery		Lab 12 (n=6) % Recovery		Lab 16 (n=6) % Recovery		Summary Statistics All Labs		
	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	Mean (%)	Std dev (%)	$\bar{X}$ (%)[2]	S_w (%)[3]	S_b (%)[4]
PFBA	101	5.6	98.1	6.5	100	5.3	86.3	4.5	59.3	2.5	94.3	1.6	85.5	5.1	107	10.9	91.5	2.8	14.9
PFPeA	102	7.8	99.3	5.0	106	4.7	88.5	6.8	76.8	2.0	91.3	2.8	90.7	3.6	98.5	8.6	94.1	2.4	9.2
PFHxA	100	9.4	98.8	4.5	104	11.5	86.4	4.8	98.9	3.1	97.1	2.7	91.5	6.8	89.2	19.6	95.8	5.7	6.2
PFHpA	94.8	8.6	99.0	4.0	105	6.6	90.7	4.8	98.9	2.2	98.7	2.8	91.3	5.2	93.2	18.3	96.4	5.2	4.7
PFOA	94.9	12.8	94.1	7.5	116	9.6	92.1	7.1	95.0	2.7	110	4.5	92.7	7.1	109	12.2	101	3.5	9.6
PFNA	101	15.7	101	4.6	125	6.0	92.2	6.6	96.9	5.1	111	4.7	96.5	5.1	98.8	9.6	103	3.8	10.4
PFDA	95.7	13.8	111	16.4	113	7.4	91.8	6.3	102	4.0	107	7.6	95.0	9.2	100	7.6	102	4.1	7.5
PFUnA	90.8	11.1	98.3	7.9	115	9.1	95.5	7.3	95.6	2.7	104	4.8	93.2	10.7	113	15.2	101	3.9	9.1
PFDoA	86.5	12.9	95.9	6.2	121	9.2	94.7	5.7	100	3.1	104	7.3	100.9	10.0	129	21.7	104	5.7	14.1
PFTriA	82.9	9.8	101	8.5	153	21.2	96.7	5.3	103	3.6	111	3.9	98.1	16.2	113	17.8	107	6.8	20.7
PFTreA	73.6	8.3	110	9.9	121	15.3	99.1	7.0	103	4.9	120	7.6	102.0	15.3	99.0	27.2	103	7.2	14.8
PFBS	91.1	7.5	105	10.1	95.5	6.0	90.2	5.9	90.3	4.1	92.6	1.6	91.4	2.6	89.8	20.0	93.2	5.8	5.1
PFPeS	93.9	7.9	99.9	5.2	105	1.7	90.8	4.6	102	2.5	98.5	3.4	93.1	4.7	101	11.6	98.0	3.2	4.9
PFHxS	90.6	4.4	91.9	4.4	110	5.2	92.4	4.7	103	4.2	102	3.8	92.7	3.9	101	8.7	97.9	1.6	6.9
PFHpS	92.7	8.5	98.9	9.3	109	7.4	93.4	5.8	98.6	3.0	112	3.3	93.6	2.2	106	6.3	101	2.6	7.5
PFOS	89.9	5.1	103	8.7	104	7.7	91.8	3.4	105	2.6	107	3.9	91.3	5.7	108	6.8	99.9	2.1	7.5
PFNS	99.8	16.6	103	8.8	126	1.1	95.3	5.3	101	3.1	110	3.2	92.9	5.2	112	11.1	105	5.1	10.6
PFDS	92.0	10.5	97.1	7.1	115	2.8	94.9	3.8	94.8	1.3	104	2.6	92.6	4.5	111	10.5	100	3.6	8.8
PFOSA	92.4	6.0	101	4.9	114	6.3	93.5	4.1	97.1	3.3	98.5	1.9	90.4	3.3	103	4.7	98.7	1.5	7.4
FtS 4:2	100	14.6	88.7	12.1	98.6	9.4	97.7	20.2	103	10.7	95.6	2.2	94.3	6.0	105	11.2	98.0	5.4	5.2
FtS 6:2	99.9	3.8	90.2	11.8	105	6.0	118	26.1	135	31.1	40.0	1.5	85.0	8.1	55.1	8.2	91.1	10.7	31.3
FtS 8:2	90.1	8.6	103	5.2	105	22.5	110	19.3	117	11.9	111	4.8	93.3	9.7	105	14.6	104	6.4	8.9
NMeFOSAA	87.2	7.4	104	12.1	132	11.8	95.6	6.2	107	3.7	98.4	5.1	91.5	9.1	101	10.8	102	3.2	13.6
NEtFOSAA	93.5	7.4	92.2	17.5	129	22.1	98.9	8.0	109	3.8	94.8	2.3	92.7	4.1	106	4.9	102	7.2	12.5

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Surrogate % Recovery statistics across study sample matrices by Laboratory, 160 ng/L (nom.) concentration

	Lab 2 (n=60) % Recovery		Lab 4 (n=59)[1] % Recovery		Lab 5 (n=60) % Recovery		Lab 6 (n=59) [1] % Recovery		Lab 10 (n=59)[1] % Recovery		Lab 11 (n=60) % Recovery		Lab 12 (n=60) % Recovery		Lab 16 (n=60) % Recovery		Summary Statistics All Labs		
Target Analyte	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
MPFBA	89.3	6.4	105	6.7	106	5.5	91.1	6.2	87.3	11.9	99.1	5.2	91.2	6.9	96.4	15.7	95.6	3.7	7.1
M5PFPeA	94.7	8.1	108	4.4	105	5.8	95.3	5.0	97.7	7.1	92.8	3.3	95.7	3.6	100	5.6	98.6	1.7	5.3
M5PFHxA	87.8	10.8	110	9.6	103	11.8	95.4	4.4	102	4.4	100	3.2	96.6	4.2	84.6	15.1	97.3	4.5	8.2
M4PFHpA	92.4	9.0	110	11.7	104	5.4	96.3	4.5	104	5.8	104	3.4	93.6	4.9	86.1	13.2	98.7	3.6	7.9
M8PFOA	94.5	10.5	107	8.3	106	7.1	97.6	4.5	102	4.6	110	4.4	94.9	7.4	95.1	10.6	101	2.6	6.2
M9PFNA	87.4	10.8	109	9.1	107	4.9	97.8	5.0	102	5.3	118	4.5	94.2	5.7	102	12.0	102	3.0	9.4
M6PFDA	89.2	9.5	109	8.0	110	7.3	99.8	5.7	104	5.9	120	6.8	99.3	10.3	104	12.9	104	2.5	9.0
M7PFUnDA	92.4	13.1	106	7.2	113	9.0	101	5.4	102	5.6	111	6.8	94.1	8.9	108	14.6	103	3.4	7.5
MPFDoDA	83.6	10.3	98.7	6.9	110	6.3	99.5	5.4	99.7	5.6	109	5.8	89.2	11.9	116	21.7	101	5.6	10.8
M2PFTeDA	75.9	10.3	84.2	14.7	105	16	92.2	9.4	101	5.8	120	7.0	89.2	11.8	107	23.7	96.8	5.8	14.2
M3PFBS	84.6	8.6	108	10.3	105	6.8	96.8	4.4	98.2	15.5	98.0	4.7	97.4	2.7	86.2	13.9	96.8	4.6	8.1
M3PFHxS	92.4	8.7	103	9.0	105	4.9	98.6	3.6	105	4.0	110	6.9	96.8	3.1	103	6.2	102	2.3	5.5
M8PFOS	92.3	11.5	103	9.7	113	7.0	99.1	3.6	103	3.6	119	6.9	95.3	4.6	108	8.0	104	2.9	9.0
M8PFOSA	90.6	5.9	98.3	5.4	113	12.2	99.2	3.7	102	4.6	101	3.6	95.2	3.0	105	7.4	101	3.0	6.7
M2-4:2FTS	89.4	11.4	102	46.2	103	8.4	102	18.9	96.9	11.7	94.7	5.9	97.3	4.7	96.9	7.9	97.8	13.6	4.5
M2-6:2FTS	93.2	11.3	105	10.9	105	11.7	108	19	108	12.0	93.1	25.1	95.1	5.7	96.6	8.0	100	6.2	6.5
M2-8:2FTS	95.3	18.0	105	13.2	111	12.4	111	14.8	108	13.0	112	9.9	98.0	7.7	106	9.3	106	3.3	6.2
d3-N-MeFOSAA	83.1	11.0	106	17.2	125	20.6	96.3	5.0	102	5.4	109	11.2	97.4	4.8	102	7.4	103	5.9	12.0
d5-N-EtFOSAA	91.3	16.2	98.6	16.1	130	16.3	102	7.7	103	6.1	104	9.7	95.3	5.2	108	8.6	104	4.7	11.7

[1] A study sample from labs 4 and 6 with recovery near 200% for all surrogates and a study sample from lab 10 with no recovery of target analytes or surrogates were excluded from this summary; a preparation error was presumed for these samples

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Surrogate % Recovery statistics across method blank, LCS, and LLOQ verification quality control samples by Laboratory, 160 ng/L (nom.) concentration

Target Analyte	Lab 2 (n=18) % Recovery		Lab 4 (n=15) % Recovery		Lab 5 (n=21) % Recovery		Lab 6 (n=18) % Recovery		Lab 10 (n=18) % Recovery		Lab 11 (n=15) % Recovery		Lab 12 (n=17) % Recovery		Lab 16 (n=18) % Recovery		Summary Statistics All Labs		
	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	Avg (%)	Std dev (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
MPFBA	97.9	3.4	98.6	4.5	101	5.7	92.4	5.1	71.9	4.7	95.4	3.3	86.4	5.7	102	12.7	93.3	3.0	10.0
M5PFPeA	103	6.8	100	4.2	102	6.5	91.2	5.3	87.8	5.7	91.9	3.9	92.3	4.5	94.8	9.4	95.5	1.8	5.6
M5PFHxA	102	10.7	102	3.8	92.6	12.0	91.2	3.9	101	3.4	97.5	2.1	94.8	5.6	82.1	15.4	95.2	4.9	6.8
M4PFHpA	97.6	8.8	102	7.5	103	4.9	93.6	5.1	103	4.9	102	2.2	91.1	7.0	84.3	15.6	97.0	4.0	6.9
M8PFOA	103	10.8	99.2	4.6	105	7.8	94.2	4.5	103	4.4	106	3.4	94.9	7.4	92.1	13.1	99.7	3.5	5.4
M9PFNA	93.8	10.7	100	5.8	104	5.7	94.5	5.1	101	4.4	113	4.7	93.6	4.8	97.3	13.4	99.5	3.3	6.6
M6PFDA	95.5	6.5	99.2	7.4	114	9.2	95.7	5.6	104	4.4	111	4.0	97.9	11.9	97.7	11.5	102	3.0	7.1
M7PFUnDA	97.0	11.2	101	6.9	112	10.7	98.0	6.4	103	3.3	104	4.6	93.9	7.7	98.1	14.1	101	3.6	5.6
MPFDoDA	88.2	10.0	101	7.1	113	13.7	97.6	6.0	102	4.6	106	3.4	94.4	9.7	109	20.2	102	5.5	8.0
M2PFTeDA	73.5	10.8	105	7.6	110	23.9	98.6	6.8	104	5.8	124	3.9	101	13.2	91.8	19.3	101	7.0	14.5
M3PFBS	93.5	6.4	103	6.1	99.7	4.7	92.4	4.7	92.1	7.6	96.4	4.7	95.0	8.4	84.5	15.7	94.5	3.7	5.5
M3PFHxS	97.6	6.2	96.1	8.5	100	4.0	94.6	3.6	104	2.8	110	6.9	93.7	4.5	97.5	9.0	99.1	2.3	5.4
M8PFOS	98.4	10.4	98.7	7.6	106	6.4	96.0	3.7	103	3.9	117	5.5	93.5	3.0	103	10.0	102	2.8	7.3
M8PFOSA	97.0	4.8	101	6.9	111	13.7	97.0	4.1	102	4.5	101	3.5	92.1	4.5	97.2	6.7	100	3.3	5.5
M2-4:2FTS	101	10.2	105	46.6	95.4	7.5	103	18.0	105	8.2	92.0	4.9	92.4	4.2	93.3	12.9	98.4	13.9	5.7
M2-6:2FTS	99.8	11.6	99.4	11.6	105	10.8	114	22.2	121	18.2	85.6	5.9	92.9	5.8	94.5	14.0	102	5.6	11.5
M2-8:2FTS	94.3	12.3	96	13.7	105	14.1	109	13.9	116	13.9	104	6.8	95.5	7.4	98.5	13.7	103	3.1	7.6
d3-N-MeFOSAA	95.6	9.3	100	16.7	115	22.6	98.1	5.8	105	5.0	99.5	6.3	94.8	4.5	93.8	11.4	101	6.5	7.0
d5-N-EtFOSAA	95.0	13.7	108	14.6	119	18.1	99.9	6.0	110	4.5	96.6	8.2	94.0	4.6	104	9.6	104	5.1	8.7

[1] One QC sample with recovery near 200% for all surrogates was excluded from this summary; a preparation error was presumed for these samples

[2] Pooled mean % recovery across 8 laboratories; For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf. Individual % recoveries were calculated after subtracting the average concentration across unspiked replicates by matrix and laboratory.

[3] Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4] Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

Attachment 15

United States Environmental Protection Agency

Office of Water

www.epa.gov December 2022

3rd Draft Method 1633

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

U.S. Environmental Protection Agency
Office of Water (4303T)
Office of Science and Technology
Engineering and Analysis Division
1200 Pennsylvania Avenue, NW
Washington, DC 20460

EPA 821-D-22-003

Method 1633
Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

December 2022

Notice

This document represents the third draft of Method 1633 for PFAS currently under development by the EPA Office of Water, Engineering and Analysis Division (EAD), in collaboration with the Department of Defense (DoD), and includes the wastewater results of the multi-laboratory validation study. Overall, the method demonstrated good recovery for all the spiked wastewaters. The multi-laboratory validation study of the method is still underway, and the Office of Water will use the final results of the multi-laboratory validation study to finalize the method and add formal performance criteria for all of the matrices.

Issuing this third draft version of the method does not require its use for Clean Water Act compliance monitoring at the Federal level; that will only occur after it has been proposed and promulgated through rulemaking (e.g., added to 40 CFR Part 136). However, EPA recommends the use of this method, and it is currently the only PFAS method that has been validated for wastewater by 8 laboratories in 6 diverse and challenging wastewater matrices.

EPA anticipates issuing two more versions of the method in the next year:

1. 4th Draft: Once all the groundwater and surface water data are reviewed and analyzed, the QC acceptance criteria in the method generated from the wastewater data will be reexamined and the method may be revised to apply those criteria to all aqueous matrices. Alternatively, EPA may develop separate QC acceptance criteria for groundwater and surface water samples. (Wastewater is generally a more difficult matrix to analyze than groundwater or surface water, and the wastewater data often drives the statistical determinations of the upper and lower limits of QC criteria. Preliminary review of the surface water and groundwater data indicates this may be the case for Method 1633 as well.)

2. Final: When the data for all the solid matrices and landfill leachate are reviewed and analyzed, final QC criteria for the solid matrices (soil, sediment, biosolids, and tissue) and landfill leachate will be added to the method to produce the version of the method that EPA expects to propose through rulemaking.

Those future versions are unlikely to involve substantive changes to the procedure. They will update the tables that dictate the required performance criteria for the relevant matrices. EPA decided to release multiple draft of the method in response to stakeholder requests to update the method with the best data as soon as practical.

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This method was prepared under the direction of Adrian Hanley of the Engineering and Analysis Division, Office of Science and Technology, within EPA's Office of Water, in collaboration with the Department of Defense.

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Adrian Hanley	EPA Office of Water, Office of Science and Technology, Engineering and Analysis Division
S. Bekah Burket	EPA Office of Water, Office of Science and Technology, Engineering and Analysis Division
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Ivona Zysk	SGS-AXYS Analytical
Ting Chen	SGS-AXYS Analytical
Henry Huang	SGS-AXYS Analytical
Mirna Alpizar	General Dynamics Information Technology
Harry McCarty	General Dynamics Information Technology

Disclaimer

See the notice on the title page regarding the status of this method.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Contact

Please address questions, comments, or suggestions to:

CWA Methods Team, Engineering and Analysis Division (4303T)
Office of Science and Technology
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, DC 20460

https://www.epa.gov/cwa-methods
https://www.epa.gov/cwa-methods/forms/contact-us-about-cwa-analytical-methods

Table of Contents

Method 1633 - Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

1.0 Scope and Application

1.1 Method 1633 is for use in the Clean Water Act (CWA) for the determination of the per- and polyfluoroalkyl substances (PFAS) in Table 1 in aqueous, solid (soil, biosolids, sediment) and tissue samples by liquid chromatography/mass spectrometry (LC-MS/MS).

1.2 The method calibrates and quantifies PFAS analytes using isotopically labeled standards. Where linear and branched isomers are present in the sample and either qualitative or quantitative standards containing branched and linear isomers are commercially available, the PFAS analyte is reported as a single result calculated from the combined responses of the linear and branched isomers.

1.3 The instrumental portion of this method is for use only by analysts experienced with LC-MS/MS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.4 By their very nature, many components of PFAS present analytical challenges unique to this class of analytes. For example, PFAS analytes readily adhere to the walls of the sample containers and may also stratify in the container. EPA has included procedures in the method that must be employed to address such challenges (see Section 11.0 and Appendices A and B).

1.5 This method is "performance-based," which means that modifications may be made without additional EPA review to improve performance (e.g., overcome interferences, or improve the sensitivity, accuracy, or precision of the results) *provided that* all performance criteria in this method are met. Requirements for establishing equivalency are in Section 9.1.2 and include 9.1.2.2c. For CWA uses, additional flexibility is described at 40 CFR 136.6. Changes in performance, sensitivity, selectivity, precision, recovery, etc., that result from modifications within the scope of 40 CFR Part 136.6, and Section 9.0 of this method must be documented, as well as how these modifications compare to the specifications in this method. After promulgation, changes outside the scope of 40 CFR Part 136.6 and Section 9.0 of this method may require prior review or approval by EPA under the Clean Water Act Alternate Test Procedure program described at 40 CFR 136.4 and 136.5.

1.6 The target analytes in Table 1 were included in this method based in part on the availability of standards for both unlabeled and isotopically labeled PFAS compounds at the time that the method was first developed. Data from the single-laboratory and multi-laboratory validation studies suggest that the method does not perform as well for some of the PFAS listed in Table 1 as for others, which is not surprising given the wide range of structures across the nine classes of compounds in that table. EPA has identified the analyte classes that are poor performers in Table 1 and data users and laboratories should take that information into account during project planning.

2.0 Summary of Method

Environmental samples are prepared and extracted using method-specific procedures. Sample extracts are subjected to cleanup procedures designed to remove interferences. Analyses of the sample extracts are conducted by LC-MS/MS in the multiple reaction monitoring (MRM) mode. Sample concentrations are determined by isotope dilution or extracted internal standard quantification (see Section 10.3) using isotopically labeled compounds added to the samples before extraction.

2.1 Extraction

2.1.1 Aqueous samples are spiked with isotopically labeled standards, extracted using solid-phase extraction (SPE) cartridges and undergo cleanup using carbon before analysis.

2.1.2 Solid samples are spiked with isotopically labeled standards, extracted into basic methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.1.3 Tissue samples are spiked with isotopically labeled standards, extracted in potassium hydroxide and acetonitrile followed by basic methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.2 This method measures the analytes as either their anions or neutral forms. The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms, using the equations in Section 15.2, although the differences between the anion and acid form concentrations are minimal (See Table 2). Other project-specific reporting schemes may be used where required.

2.3 Individual PFAS analytes are identified through peak analysis of the quantification and confirmation ions, where applicable.

2.4 Quantitative determination of target analyte concentrations is made with respect to an isotopically labeled PFAS standard; the concentrations are then used to convert raw peak areas in sample chromatograms to final concentrations.

2.5 By virtue of the use of isotope dilution and extracted internal standard quantification (see Section 10.3), the results for the target analytes are corrected for any losses that may occur during sample extraction, extract cleanup, and concentration. Isotope dilution calibration also may address matrix effects that lead to signal suppression or enhancement in the LC-MS/MS system and would otherwise lead to measurement bias. Isotopically labeled compound recoveries are determined by comparison to the responses of one of seven non-extracted internal standards (a.k.a., the "recovery" standards) and are used as general indicators of overall analytical quality.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and LC-MS/MS systems.

3.0 Definitions

Definitions are provided in the glossary at the end of this method.

4.0 Contamination and Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and solvents may be required.

4.2 Clean all equipment prior to, and after each use to avoid PFAS cross-contamination. Typical cleaning solvents used include water, methanol, and methanolic ammonium hydroxide. The residual PFAS content of disposable plasticware and filters must be verified by batch/lot number

and may be used without cleaning if the mass of any PFAS analyte found in a nominal 500-mL aqueous sample is less than half the Minimum Level (ML, see Table 6).

4.2.1 All glass equipment that is used in the preparation or storage of reagents is cleaned by washing with detergent and baking in a kiln or furnace (Section 6.2.2). After detergent washing, glassware should be rinsed immediately with reagent water. Prior to use, baked glassware must be solvent rinsed and then air dried. A solvent rinse procedure using methanolic ammonium hydroxide (1%), toluene, and methanol is recommended.

4.2.2 All parts of the SPE manifold must be cleaned between samples with methanolic ammonium hydroxide (1%) and air dried prior to use. Sonication with methanolic ammonium hydroxide (1%) may be used for components that will fit in an ultrasonic bath. Smaller parts, like the needles, adapters, reservoirs, and stopcocks associated with the manifold, require rinsing with tap water prior to manual cleaning or sonicating with methanolic ammonium hydroxide (1%) and air drying. When in use, after loading the samples but prior to elution procedures, the chamber must be rinsed with methanolic ammonium hydroxide (1%).

4.2.3 All equipment used in the filleting, dissecting, shucking, compositing, and homogenization of tissue must be cleaned with detergent and hot water, then rinsed with ultra-pure water followed by a series of solvent rinses. A typical solvent rinse procedure would be acetone, followed by toluene, and then methanol.

4.3 All materials used in the analysis must be demonstrated to be free from interferences by running method blanks (Section 9.5) at the beginning and with each sample batch (samples started through the extraction process in a given batch during the same work shift, to a maximum of 20 field samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix being tested. Ideally, the reference matrix should not contain PFAS in detectable amounts (i.e., above the laboratory's method detection limits (MDLs).

4.3.2 For tissue, chicken breast or other similar animal tissue (see Section 7.2.3) may be used as the reference matrix. The laboratory must verify that the source product used does not contain PFAS in detectable amounts.

4.3.3 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.2.1) can be used to simulate water samples and Ottawa sand and/or reagent-grade sand (Section 7.2.2) can be used to simulate soils.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the native PFAS. Because low levels of PFAS are measured by this method, elimination of interferences is essential. The cleanup steps given in Section 12.0 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PFAS at the levels shown in Table 6. The most frequently encountered interferences are fluoropolymers; however, bile salts (e.g., Taurodeoxycholic Acid [TDCA]) may be present in various matrices, including fish and wastewaters, and can interfere in the chromatography. For this reason, analysis of a standard containing TDCA is required as part of establishing the initial chromatographic conditions (see Sections 10.2.2.5 and 10.3.5).

4.5 Each piece of reusable glassware may be numbered to associate that glassware with the processing of a particular sample. This may assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded. If that approach is used, the numbered glassware should be assigned to field samples, QC samples, and method blanks in a random manner (e.g., do not use the same glassware for method blanks in every batch).

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Several PFAS, including PFOA, have been described as likely to be carcinogenic to humans. Pure standards and materials known or suspected to contain PFAS should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

5.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they must be prepared in a hood, following universal safety measures.

5.2 The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDS) should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1-4. The references and bibliography at the end of Reference 3 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling these compounds.

5.3.1 Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate face velocity. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment – Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood with adequate face velocity should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full-face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands.

5.3.3 Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene – Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift) using soaps or detergents that are free of PFAS. Before starting work, staff should avoid the use of personal-care products on exposed skin, because such products may be a source of some PFAS.

5.3.5 Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Waste Handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste.

5.3.7 Laundry – Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.4 Biosolids samples may contain high concentrations of biohazards and must be handled with gloves and opened in a fume hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this method is the responsibility of the laboratory. All equipment described below must be constructed of materials that will not react with or sorb PFAS constituents and before use must be demonstrated to be free of PFAS at levels that would be detectable in blanks or samples. Where available, certification of the PFAS levels of the materials provided by the supplier will suffice. However, in the absence of such certification from the supplier, and in the event of persistent problems with method blanks and other QC samples, the laboratory is responsible for independent testing of all equipment and supplies.*

6.1 Sampling equipment for discrete or composite sampling.

6.1.1 Sample bottles and caps

Note: *Do not use PTFE-lined caps on sample containers. All containers must be demonstrated to be PFAS-free at the laboratory's MDLs for the target analytes by testing one or more representative containers from each lot.*

6.1.1.1 Liquid samples (waters, sludges, and similar materials containing < 50 mg solids per sample) – Sample bottle, HDPE, 500-mL, 250-mL, and 125-mL, with linerless HDPE or polypropylene caps.

6.1.1.2 Solid samples (soils, sediments, and biosolids that contain more than 50 mg solids) – Sample bottle or jar, wide-mouth, HDPE, 500-mL, with linerless HDPE or polypropylene caps.

6.1.1.3 Tissue samples – Sample jar, wide-mouth HDPE, 100-mL, with linerless HDPE or polypropylene caps.

6.1.2 Grab sampling equipment – Sample containers may be attached to a metal or wooden pole with stainless steel hose clamps or cable ties in order to reach into flowing waters. Stainless steel scoops or spoons may be used to collect samples of soils, sediments, and biosolids.

6.1.3 Compositing equipment –Because some PFAS are known surfactants, EPA does not recommend composite sampling for compliance monitoring (see Section 8.2), but if composite sampling is approved for given project, the equipment described below may be used.

Automatic or manual compositing system incorporating properly cleaned containers . An integrating flow meter is used to collect proportional composite samples. Only HDPE tubing must be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, each lot of tubing must be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. The final reagent water rinse should be collected and analyzed for PFAS to confirm that the tubing is suitable for use.

6.2 Equipment for glassware cleaning

Note: *If blanks from other glassware show no detectable PFAS contamination when using fewer cleaning steps than required above, unnecessary cleaning steps and equipment may be eliminated.*

6.2.1 Laboratory sink with overhead fume hood

6.2.2 Kiln – Capable of reaching 450 ºC within 2 hours and maintaining 450 - 500 ºC ± 10 ºC, with temperature controller and safety switch (Cress Manufacturing Co., Santa Fe Springs, CA, B31H, X31TS, or equivalent). For safety, the kiln or furnace should be vented outside the laboratory, or to a trapping system.

6.3 Equipment for sample preparation

6.3.1 Polyethylene gloves

6.3.2 Laboratory fume hood (of sufficient size to contain the sample preparation equipment listed below)

6.3.3 Glove box (optional)

6.3.4 Meat grinder – Hobart, or equivalent, with 3- to 5-mm holes in inner plate

6.3.5 Equipment for determining percent moisture

6.3.5.1 Oven – Capable of maintaining a temperature of 105 ± 5 ºC

6.3.5.2 Desiccator

6.3.6 Balances

6.3.6.1 Analytical – Capable of weighing 0.1 mg

6.3.6.2 Top loading – Capable of weighing 10 mg

6.3.7 Aluminum foil

6.3.8 Disposable spoons, 10 mg, polypropylene or stainless steel

6.3.9 Ultrasonic mixer (sonicator) – Immersion style, for use with tissue samples

6.3.10 HDPE bottles, with linerless HDPE or polypropylene caps – 60 mL

6.3.11 pH Paper, range 0-14 - (Whatman® Panpeha™ or equivalent), 0.5-unit readability (papers with other pH ranges may be suitable as well)

6.3.12 Analog or digital vortex mixer, single or multi-tube (Fisher Scientific 02-215-452, or equivalent)

6.3.13 Volumetric flasks, Class A

6.3.14 Disposable polypropylene collection tubes (13 x 100 mm, 8 mL)

6.3.15 Variable speed mixing table (Fisherbrand™ Nutating mixer or equivalent)

6.4 Filtration

6.4.1 Silanized glass wool (Sigma-Aldrich, Cat # 20411 or equivalent) – store in a clean glass jar and rinsed with methanol (2 times) prior to use.

6.4.2 Disposable syringe filter, 25-mm, 0.2-µm Nylon membrane, PALL/Acrodisc or equivalent

6.4.3 Glass fiber filter, 47-mm, PALL A/E or equivalent, for use in determining total suspended solids

6.5 Centrifuge apparatus

6.5.1 Centrifuge (Thermo Scientific Legend RT+, 16-cm rotor, or equivalent), capable of reaching at least 3000 rpm

6.5.2 Centrifuge tubes – Disposable polypropylene centrifuge tubes (50 mL)

6.6 Pipettes

6.6.1 Norm-Ject® syringe (or equivalent), polypropylene/HDPE, 5 mL

6.6.2 Variable volume pipettes with disposable HDPE or polypropylene tips (10 µL to 5 mL) – used for preparation of calibration standards and spiked samples.

6.6.3 Disposable glass pipets

6.6.4 Calibrated mechanical pipettes or Hamilton graduated syringes

6.7 Solid-phase extraction

6.7.1 Solid-phase extraction (SPE) cartridges (Waters Oasis WAX 150 mg, Cat # 186002493 or equivalent). The SPE sorbent must have a pKa above 8 so that it remains positively charged during the extraction.

Note: *SPE cartridges with a different bed volume (e.g., 500 mg) may be used; however, the laboratory must demonstrate that the bed volume does not negatively affect analyte absorption and elution, by performing the initial demonstration of capability analyses described in Section 9.2.*

6.7.2 Vacuum manifold for SPE Cartridges (Waters™ extraction manifold #WAT200607 or equivalent)

6.8 Evaporation

6.8.1 Automatic or manual solvent evaporation system (TurboVap® LV or equivalent)

6.8.2 Evaporation/concentrator tubes: 60 mL clear glass vial, 30 x 125 mm, without caps (Wheaton Cat # W226060 or equivalent). Cover with foil if required.

6.9 Vials

6.9.1 Snap cap/crimp top vials, 300 µL, polypropylene (12 x 32 mm) – used in sample pre-screening (DWK Life Sciences Cat # 225180 or equivalent)

6.9.2 Polypropylene crimp/snap vials, 1 mL (Agilent Cat # 5182-0567 or equivalent)

6.9.3 Clear snap cap, polyethylene, 11 mm (Fisher Scientific # 03-375-24E, or equivalent)

6.9.4 Single step filter vials (Restek Thomson SINGLE StEP® Standard Filter Vials, 0.2-µm Nylon membrane, with Black Preslit caps Cat # 25891 or equivalent) – used in sample pre-screening.

6.10 Instrument

6.10.1 Ultra high-performance liquid chromatograph (UPLC, also called UHPLC) or high-performance liquid chromatograph (HPLC) equipped with tandem quadrupole mass spectrometer (Waters Xevo TQ-S Micro or equivalent) capable of collecting at least 10 scans across a chromatographic peak

6.10.2 C18 column, 1.7 µm, 50 x 2.1 mm (Waters Acquity UPLC® BEH or equivalent)

6.10.3 Guard column (Phenomenex Kinetex® Evo C18 or equivalent)

6.10.4 Trap/delay column (Purospher Star RP-18 endcapped [3 µm] Hibar® RT 50-4 or equivalent)

6.11 Bottles, HDPE or glass, with linerless HDPE or polypropylene caps. Various sizes. To store prepared reagents.

7.0 Reagents and Standards

7.1 Reagents

Reagents prepared by the laboratory may be stored in either glass or HDPE containers. Proper cleaning procedures (Section 4.2) must be followed prior to using the containers. Before use, all reagents described below must be demonstrated to be free of PFAS at levels that would be detectable in blanks or samples Where available, certification of the PFAS levels of the reagents provided by the supplier will suffice. However, in the absence of such certification from the supplier, and in the event of persistent problems with method blanks and other QC samples, the laboratory is responsible for independent testing of each lot.

7.1.1 Acetic acid (concentrated) – ACS grade or equivalent, store at room temperature

7.1.2 Acetic acid (0.1%) – dissolve acetic acid (1 mL) in reagent water (1 L), store at room temperature, replace after 3 months.

7.1.3 Acetonitrile – UPLC grade or equivalent, verified before use, store at room temperature

7.1.4 Ammonium acetate – (Caledon Ultra LC/MS grade, or equivalent), store at 2-8 °C, replace 2 years after opening date

7.1.5 Ammonium hydroxide – certified ACS+ grade or equivalent, 30% in water, store at room temperature, and replace 2 years after opening date

7.1.6 Aqueous ammonium hydroxide (3%) – add ammonium hydroxide (10 mL, 30%) to reagent water (90 mL), store at room temperature, replace after 3 months

7.1.7 Methanolic ammonium hydroxide

7.1.7.1 Methanolic ammonium hydroxide (0.3% v/v) – add ammonium hydroxide (1 mL, 30%) to methanol (99 mL), store at room temperature, replace after 1 month

7.1.7.2 Methanolic ammonium hydroxide (1% v/v) – add ammonium hydroxide (3.3 mL, 30%) to methanol (97 mL), store at room temperature, replace after 1 month

7.1.7.3 Methanolic ammonium hydroxide (2% v/v) – add ammonium hydroxide (6.6 mL, 30%) to methanol (93.4 mL), store at room temperature, replace after 1 month

7.1.8 Methanolic potassium hydroxide (0.05 M) – add 3.3 g of potassium hydroxide to 1 L of methanol, store at room temperature, replace after 3 months

7.1.9 Methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid (v/v) – add ammonium hydroxide (3.3 mL, 30%), reagent water (1.7 mL) and acetic acid (0.625 mL) to methanol (92 mL), store at room temperature, replace after 1 month. This solution is used

to prepare the instrument blank (Section 7.3.6) and is used to dilute the extracts of samples that exceed the calibration range (see Section 15.3).

7.1.10 Eluent A – Acetonitrile, Caledon Ultra LCMS grade or equivalent

7.1.11 Eluent B – 2 mM ammonium acetate in 95:5 water/acetonitrile. Dissolve 0.154 g of ammonium acetate (Section 7.1.4) in 950 mL of water and 50 mL of acetonitrile (Caledon Ultra LCMS grade, or equivalent). Store at room temperature, shelf life 2 months.

7.1.12 Formic acid – (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature

7.1.13 Formic acid

7.1.13.1 Formic acid (aqueous, 0.1 M) – dissolve formic acid (4.6 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.2 Formic acid (aqueous, 0.3 M) – dissolve formic acid (13.8 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.3 Formic acid (aqueous, 5% v/v) – mix 5 mL formic acid with 95 mL reagent water, store at room temperature, replace after 2 years

7.1.13.4 Formic acid (aqueous, 50% v/v) – mix 50 mL formic acid with 50 mL reagent water, store at room temperature, replace after 2 years

7.1.13.5 Formic acid (methanolic 1:1, 0.1 M formic acid/methanol) – mix equal volumes of methanol and 0.1 M formic acid, store at room temperature, replace after 2 years

7.1.14 Methanol – (HPLC grade or better, 99.9% purity), verified by lot number before use, store at room temperature

7.1.15 Potassium hydroxide – certified ACS or equivalent, store at room temperature, replace after 2 years

7.1.16 Reagent water – Laboratory reagent water, test by lot/batch number for residual PFAS content

7.1.17 Carbon – EnviCarb® 1-M-USP or equivalent, verified by lot number before use, store at room temperature. Loose carbon allows for better adsorption of interferent organics.

Note: *The single-laboratory validation laboratory achieved better performance with loose carbon than carbon cartridges. Loose carbon was used for the multi-laboratory validation to establish statistically based method performance criteria. Now that the method has been validated for wastewater matrices, laboratories have the flexibility to implement the use carbon cartridges for wastewater samples, as long as all method QC criteria applicable to wastewater analyses are met (see 40 CFR 136.6). (This flexibility may be extended to other matrices in subsequent revisions of this method.)*

7.1.18 Toluene – HPLC grade, verified by lot number before use. Store at room temperature.

7.1.19 Acetone – Pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.1.20 Dichloromethane (methylene chloride) – Pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.2 Reference matrices – Matrices in which PFAS and interfering compounds are not detected by this method. These matrices are to be used to prepare the batch QC samples (e.g., method blank, and ongoing precision and recovery sample).

7.2.1 Reagent water – purified water, Type I

7.2.2 Solids reference matrix – Ottawa or reagent-grade sand

7.2.3 Tissue reference matrix – chicken breast or similar animal tissue

7.3 Standard solutions – Prepare from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. Observe the safety precautions in Section 5.

Purchase of commercial standard solutions or mixtures is highly recommended for this method; however, when these are not available, preparation of stock solutions from neat materials may be necessary. Some PFAS, notably the fluorinated carboxylic acids, will esterify in anhydrous acidic methanol. To such prevent esterification, standards must be stored under basic conditions. If base is not already present, this may be accomplished by the addition of sodium hydroxide (approximately 4 mole equivalents) when standards are diluted in methanol. If the chemical purity is 98% or greater, the weight may be used without correction to calculate the concentration of the standard. Dissolve an appropriate amount of assayed reference material in the required solvent. For example, weigh 10 to 20 mg of an individual compound to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with the required solvent. Once the compound is completely dissolved, transfer the solution to a clean vial and cap.

When not being used, store standard solutions in the dark at less than 6 °C, but not frozen, unless the vendor recommends otherwise, in screw-capped vials with foiled-lined caps. Place a mark on the vial at the level of the solution so that solvent loss by evaporation can be detected. Discard the solution if solvent loss has occurred.

Note: *Native PFAS standards are available from several suppliers. Isotopically labeled compounds are available from Cambridge Isotope Laboratories and Wellington Laboratories, but may also be available from other suppliers. Listing of these suppliers does not constitute a recommendation or endorsement for use. All diluted solutions must be stored in glass or HDPE containers that have been thoroughly rinsed with methanol.*

^{18}O-mass labeled perfluoroalkyl sulfonates may undergo isotopic exchange with water under certain conditions, which lowers the isotopic purity of the standards over time. Similarly, some of the deuterated standards may undergo isotopic exchange in protic solvents such as methanol.

The laboratory must maintain records of the certificates for all standards, as well as records for the preparation of intermediate and working standards, for traceability purposes. Copies of the certificates must be provided as part of the data packages in order to check that proper calculations were performed.

7.3.1 Extracted Internal Standard (EIS) – (a.k.a. isotopically labeled compounds) Prepare the EIS solution containing the isotopically labeled compounds listed in Table 4 as extracted internal standards in methanol from prime stocks. An aliquot of EIS solution, typically 50 µL, is added to each sample prior to extraction. Table 3 presents the nominal amounts of the EIS compounds added to each sample.

Note: *Larger EIS amounts may be added to samples for which pre-screening results (see Section 11.0) indicate that the sample extract will require dilution, provided that the extract dilution will result in approximately the same masses of the EISs as are found in the calibration standards (assuming 100% recovery).*

The list of EIS compounds in Table 3 represents the compounds that were available at the time this method was validated. Additional isotopically labeled PFAS compounds may be included as EISs as soon as practical, once they become commercially available.

7.3.2 Non-Extracted Internal Standard (NIS) – The NIS solution containing the isotopically labeled compounds listed in Table 3 as non-extracted internal standards is prepared in methanol from prime stock. An aliquot of NIS solution, typically 50 µL, is added to each sample prior to instrumental analysis. Table 3 presents the nominal amounts of NIS compounds added to each sample. As with the EIS solution above, larger amounts of the NIS compounds may be used for samples known to require extract dilution.

7.3.3 Native Standards Solution – Prepare a spiking solution, containing the method analytes listed in Table 4, in methanol from prime stocks. The solution is used to prepare the calibration standards and to spike the known reference QC samples that are analyzed with every batch. Quantitative standards containing a mixture of branched and linear isomers must be used for method analytes if they are commercially available. Currently, these include PFOS, PFHxS, PFOSA, NMeFOSAA, NEtFOSAA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE. Additional mixtures of branched and linear isomers must be included as soon as practical, once they become commercially available

7.3.4 Calibration standard solutions – A series of calibration solutions containing the target analytes and the ^{13}C-, ^{18}O-, and deuterium-labeled extracted internal standards (EIS) and non-extracted internal standards (NIS) is used to establish the initial calibration of the analytical instrument. The concentration of the method analytes in the solutions varies to encompass the working range of the instrument, while the concentrations of the EIS and NIS remain constant. The calibration solutions are prepared using methanol, 2% methanolic ammonium hydroxide, reagent water, acetic acid, and the target analyte and isotopically labeled compound standard solutions. After dilution, the solvent composition of the final calibration solutions will approximate the solvent composition of the sample extracts, which contain methanol with roughly 4% water (due to the solubility of water from the sample in the methanolic extraction fluid), 1% ammonium hydroxide and about 0.6% acetic acid (also see Section 7.1.9). Calibration standard solutions do not undergo solid-phase extraction/cleanup.

Concentrations for seven calibration solutions are presented in Table 4. A minimum of six contiguous calibrations standards are required for a valid analysis when using a linear calibration model, with at least five of the six calibration standards being within the quantitation range (e.g., from the Limit of Quantitation [LOQ] to the highest calibration standard). If a second-order calibration model is used, then a minimum of seven calibration standards are required, with at least six of the seven calibration standards within the

quantitation range. The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 for the quantitation ions and confirmation ions, and 10:1 for quantitation ions that have no confirmation ion and be at a concentration less than or equal to the LOQ. The calibration is verified with a standard in the middle of the laboratory's calibration range, i.e., the CS4 standard in Table 4 if using the default calibration range in that table.

Note: *Additional calibration standards, at levels lower than the lowest calibration standard listed in the method, may be added to accommodate a lower limit of quantitation if the instrument sensitivity allows. Calibration standards at the high end of the calibration may be eliminated if the linearity of the instrument is exceeded or at the low end if those calibration standards do not meet the S/N ratio criterion of 3:1, or 10:1 for analytes without a confirmation ion, as long as the required number of calibration points is met. All analytes with commercially available stable isotope analogues must be quantified using isotope dilution.*

7.3.5 Qualitative Standards – Standards that contain mixtures of the branched and linear isomers of the method analytes and that are used for comparison against suspected branched isomer peaks in field samples. These qualitative standards are **not** required for those analytes where the quantitative standards in Section 7.3.3 already contain the branched and linear isomers. Qualitative standards that are currently commercially available include PFOA and PFNA. Additional qualitative standards must be included as soon as practical, once they become commercially available.

7.3.6 Instrument Blank – During the analysis of a batch of samples, a solvent blank is analyzed after standards (e.g., calibration, CV) and based on screening results or prior knowledge of the source, after samples containing high levels of target compounds to monitor carryover from the previous injection. The instrument blank consists of the solution in Section 7.1.9 fortified with the EIS and NIS for quantitation purposes.

7.3.7 Stability of solutions – Standard solutions used for quantitative purposes (Sections 7.3.1 through 7.3.5) should be assayed periodically (e.g., every 6 months) against certified standard reference materials (SRMs) from the National Institute of Science and Technology (NIST), if available, or certified reference materials from a source accredited under ISO Guide 17034 that attests to the concentration, to assure that the composition and concentrations have not changed.

7.4 Mass calibration solution – Use the mass calibration solution specified by the instrument manufacturer.

7.5 Bile salt interference check standard containing Taurodeoxycholic Acid (TDCA) or Sodium taurodeoxychloate hydrate – (Sigma Aldrich 580221-5GM, or equivalent). This standard is used to evaluate the chromatographic program relative to the risk of an interference from bile salts in samples when using acetonitrile as the mobile phase in the instrument. Prepare solution at a concentration of 1 µg/mL in the same solvent as the calibration standards. If using other mobile phases, it will be necessary to evaluate taurochenodeoxycholic acid (TCDCA) (Sigma Aldrich T6260-1G, or equivalent) and tauroursodeoxycholic acid (TUDCA) (Sigma Aldrich 580549-1GM, or equivalent) as well.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Collect samples in HDPE containers following conventional sampling practices (Reference 5). All sample containers must have linerless HDPE or polypropylene caps. Other sample collection techniques, or sample volumes may be used, if documented.

8.2 Aqueous samples

8.2.1 Because some PFAS are known surfactants, EPA does not recommend composite sampling for compliance monitoring. Therefore, samples that flow freely are collected as grab samples. Collect multiple sample aliquots in HDPE bottles that have been lot-certified to be PFAS-free. Do not fill the bottle past the shoulder, to allow room for expansion during frozen storage.

For aqueous sources other than leachates that have not been analyzed previously, the nominal sample size is 500-mL. For sources that are known or expected to contain levels of any target analytes above the calibration range, samples may be collected in smaller size containers, provided that the volume analyzed is sufficient to meet any regulatory limits. Because the target analytes are known to bind to the interior surface of the sample container, the entire aqueous sample that is collected must be prepared and analyzed and subsampling avoided whenever possible. Therefore, if a sample volume smaller than 500 mL is to be used for analysis, collect the sample in an appropriately sized HDPE container.

Note: *In the absence of source-specific information (e.g., historical data) on the levels of PFAS or project-specific requirements, collect at least three aliquots of all aqueous samples to allow sufficient volume for an original whole-volume analysis, a re-extraction and second analysis, and for the determination of percent solids and for pre-screening analysis. That third aliquot may be collected in a smaller sample container (e.g., 250-mL or 125-mL).*

If composite sampling is approved for given project, the equipment described in Section 6.1.2 may be used to collect samples in refrigerated bottles using automated sampling equipment.

8.2.2 Leachate samples from landfills can present significant challenges and therefore only 100 mL of sample is collected for the analysis. Collect three 100-mL leachate sample aliquots in a similar manner as described in Section 8.2.1, using appropriately sized containers that have been lot-certified to be PFAS-free.

8.2.3 Maintain all aqueous samples protected from light and at 0 - 6 ºC from the time of collection until shipped to the laboratory. Samples must be shipped with sufficient ice to maintain the sample temperature below 6 ºC during transport for a period of at least 48 hours to allow for shipping delays. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples may be stored at 0 - 6 ºC or at ≤ -20 ºC, until sample preparation. However, the allowable holding time for samples depends on the storage temperature, as described in Section 8.5, so samples should be shipped to the laboratory as soon as practical.

8.3 Solid (soil, sediment, biosolids), excluding tissue

8.3.1 Collect samples using wide-mouth HDPE jars that have been lot-certified to be PFAS-free, and fill no more than ¾ full (see Section 6.1.1.2 for container size and type).

8.3.2 Maintain solid samples protected from light (in HDPE containers) from the time of collection until receipt at the laboratory. Samples must be shipped with sufficient ice to maintain the sample temperature below 6 ºC for a period of at least 48 hours to allow for shipping delays. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples may be stored at 0 - 6 ºC or at ≤ -20 ºC, until sample preparation. However, the allowable holding times for samples depend on the storage temperature, as described in Section 8.5, so samples should be shipped to the laboratory as soon as practical.

8.4 Fish and other tissue samples

The nature of the tissues of interest may vary by project. Field sampling plans and protocols should explicitly state the samples to be collected and if any processing will be conducted in the field (e.g., filleting of whole fish or removal of organs). All field procedures must involve materials and equipment that have been shown to be free of PFAS.

8.4.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.4.2 If whole fish are collected, wrap the fish in aluminum foil or food-grade polyethylene tubing, and maintain at 0 - 6 ºC from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport time is necessary, freeze the sample before shipping. Ideally, fish should be frozen upon collection and shipped to the laboratory on dry ice.

8.4.3 Once received by the laboratory, the samples must be maintained protected from light at ≤ -20 ºC until prepared. Store unused samples in HDPE containers or wrapped in aluminum foil at ≤ -20 ºC.

8.5 Holding times

8.5.1 Aqueous samples (including leachates) should be analyzed as soon as possible; however, samples may be held in the laboratory for up to 28 days from collection, when stored at 0 - 6 ºC and protected from the light, with the caveat that issues have been observed with certain perfluorooctane sulfonamide ethanols and perfluorooctane sulfonamidoacetic acids after 7 days. These issues are more likely to elevate the observed concentrations of other PFAS compounds via the transformation of these precursors if they are present in the sample (see Reference 10).

When stored at ≤ -20 ºC and protected from the light, aqueous samples may be held for up to 90 days.

8.5.2 Soil and sediment samples may be held for up to 90 days, if stored by the laboratory in the dark at either 0 - 6 ºC or ≤ -20 ºC, with the caveat that samples may need to be extracted as soon as possible if NFDHA is an important analyte for a given project (see Reference 10). However, some soils and sediments may exhibit microbial growth when stored at 0 - 6 ºC.

8.5.3 Tissue samples may be held for up to 90 days, if stored by the laboratory in the dark at ≤ -20 ºC, with the same caveat regarding NFDHA.

8.5.4 Biosolids samples may be held for up to 90 days, if stored by the laboratory in the dark at 0 - 6 ºC, but preferably at ≤ -20 ºC (see Reference 10). Because microbiological activity in

biosolids samples at 0 - 6 ºC may lead to production of gases which may cause the sample to be expelled from the container when it is opened, as well as producing noxious odors, EPA recommends that samples be frozen if they need to be stored for more than a few days before extraction.

8.5.5 Store sample extracts in the dark at less than 0 - 6 ºC until analyzed. If stored in the dark at ≤ 0 ºC, sample extracts may be stored for up to 90 days, with the caveat that issues were observed for some ether sulfonates after 28 days (see Reference 10). These issues may elevate the observed concentrations of the ether sulfonates in the extract over time. Samples may need to be extracted as soon as possible if NFDHA is an important analyte.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 6). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with isotopically labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

If the method is to be applied to a sample matrix other than water (e.g., solids and tissues), the appropriate alternative reference matrix (Sections 7.2.2 - 7.2.3) is substituted for the reagent water matrix (Section 7.2.1) in all performance tests.

9.1.1 The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this method. This demonstration is given in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, and cleanup procedures, and changes in sample volumes, columns, and detectors. Alternative determinative techniques and changes that degrade method performance, are *not* allowed without prior review and approval (see 40 CFR 136.4 and 136.5).

Note: *For additional flexibility to make modifications without prior EPA review, see 40 CFR Part 136.6.*

9.1.2.1 Each time a modification is made to this method, the laboratory is required to repeat the procedure in Section 9.2. If calibration will be affected by the change, the instrument must be recalibrated per Section 10. Once the modification is demonstrated to produce results in a relevant reference matrix and are equivalent or superior to results produced by this method as written, that modification may be used routinely thereafter, so long as the other requirements in this method (e.g., isotopically labeled compound recovery) are met in both the initial demonstration in Section 9.2 and in field samples and other QC samples.

9.1.2.2 The laboratory is required to maintain records of any modifications made to this method. These records include the following, at a minimum:

a) The names, titles, business addresses, and telephone numbers of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

b) A listing of pollutant(s) measured, by name and CAS Registry number.

c) A narrative stating reason(s) for the modifications (see Section 1.5).

d) Results from all quality control (QC) tests comparing the modified method to this method, including:

i. Calibration (Section 10)
ii. Calibration verification (Section 14.3)
iii. Initial precision and recovery (Section 9.2.1)
iv. Isotopically labeled compound recovery (Section 9.3)
v. Analysis of blanks (Section 9.5)
vi. Accuracy assessment (Section 9.4)

e) Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:
i. Sample numbers and other identifiers
ii. Extraction dates
iii. Analysis dates and times
iv. Analysis sequence/run chronology
v. Sample weight or volume (Section 11)
vi. Extract volume prior to each cleanup step (Section 12)
vii. Extract volume after each cleanup step (Section 12)
viii. Injection volume (Section 13.3)
ix. Dilution data, differentiating between dilution of a sample or an extract (Section 15.3)
x. Instrument
xi. Column (dimensions, liquid phase, solid support, film thickness, etc.)
xii. Operating conditions (temperatures, temperature program, flow rates)
xiii. Detector (type, operating conditions, etc.)
xiv. Chromatograms, printer tapes, and other recordings of raw data
xv. Quantitation reports, data system outputs, and other data to link the raw data to the results reported

9.1.2.3 Alternative columns and column systems – If a column or column system other than those specified in this method is used, that column or column system must meet all the requirements of this method.

Note: *The use of alternative columns or programs will likely result in a different elution order.*

9.1.3 Analyses of method blanks are required on an on-going basis to demonstrate the extent of background contamination in any reagents or equipment used to prepare and analyze field samples (Section 4.3). The procedures and criteria for analysis of a method blank are described in Section 9.5.

9.1.4 The laboratory must spike all samples with isotopically labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes

indicate atypical method performance for samples, the samples are diluted to evaluate whether the performance issue is caused by the sample matrix. Procedures for dilution are given in Section 15.3.

9.1.5 The laboratory must, on an ongoing basis, demonstrate that the analytical system is in control through calibration verification and the analysis of ongoing precision and recovery standards (OPR), spiked at low (LLOPR) and mid-level, and blanks. These procedures are given in Sections 14.3 through 14.7.

9.1.6 The laboratory must maintain records to define the quality of data generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial Demonstration of Capability

9.2.1 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery, the laboratory must perform the following operations for each sample matrix type to which the method will be applied by that laboratory.

9.2.1.1 Extract, concentrate, and analyze four aliquots of the matrix type to be tested (Section 7.2.1 through 7.2.3), spiked with 200 µL of the native standard solution (Section 7.3.3), 50 µL of the EIS solution (Section 7.3.1), and 50 µL of NIS solution (Section 7.3.2). At least one method blank, matching the matrix being analyzed, must be prepared with the IPR batch. In the event that more than one MB was prepared and analyzed with the IPR batch, all blank results must be reported. All sample processing steps that are to be used for processing samples, including preparation and extraction (Sections 11.2 – 11.4), cleanup (Section 12.0) and concentration (Section 12.0), must be included in this test.

9.2.1.2 Using results of the set of four analyses, compute the average percent recovery (R) of the extracts and the relative standard deviation (RSD) of the concentration for each target and EIS compound.

9.2.1.3 For each native and isotopically labeled compound, compare RSD and % recovery with the corresponding limits for initial precision and recovery in Table 5 and 5A. Table 5 includes the required QC acceptance limits for wastewater samples that were derived from the multi-laboratory validation study. Table 5A includes example performance data for solids and tissues from the single-laboratory validation study and are provided for illustrative purposes (e.g., those figures are not required acceptance criteria). For wastewater matrices, if RSD and R for all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and wastewater samples may begin. If, however, any individual RSD exceeds the precision limit or any individual R falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

9.2.2 Method detection limit (MDL) - Each laboratory must also establish MDLs for all the target analytes using the MDL procedure at 40 CFR Part 136, Appendix B. The minimum level of quantification (ML) can be calculated by multiplying the MDL by 3.18 and rounding the result to the nearest 1, 2 or 5 x 10^n, where n is zero or an integer (see the Glossary for alternative derivations). Example matrix-specific method detection limits are listed in Table 6.

9.3 To assess method performance on the sample matrix, the laboratory must spike all samples with the EIS standard solution (Section 7.3.1) and all sample extracts with the NIS spiking solution (Section 7.3.2).

9.3.1 Analyze each sample according to the procedures in Sections 11.0 through 16.0.

9.3.2 Compute the percent recovery of the EIS using the non-extracted internal standard method (Section 15.2) and the equation in Section 14.5.2.

9.3.3 The recovery of each EIS in a wastewater sample must be within the limits in Table 8, which are the required QC acceptance limits for wastewater samples that were derived from the multi-laboratory validation study. If the recovery of any EIS falls outside of these limits, method performance is unacceptable for that EIS in that wastewater sample. Additional cleanup procedures or limited dilution of the sample extract may be employed to attempt to bring the EIS recovery within the acceptance normal range. If the recovery cannot be brought within the acceptance limits after extract dilution or additional cleanup procedures have been employed, wastewater samples are diluted and prepared and analyzed, per Section 15.3. Table 8A includes example performance data for solids and tissues from the single-laboratory validation study and are provided for illustrative purposes (e.g., those figures are not required acceptance criteria). EIS recoveries in solids and tissues that fall well outside of the ranges in Table 8A are a potential cause for concern and laboratories should take similar steps to those described for wastewater samples to improve EIS recoveries.

9.4 Records of the recovery of EISs from samples must be maintained, and should be assessed periodically.

9.4.1 After the analysis of 30 samples of a given matrix type (water, solids, tissues, etc.), compute the recovery (R) and the standard deviation of the percent recovery (S_R) for the isotopically labeled compounds only. Express the assessment as a percent recovery interval from R - $2S_R$ to R + $2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for 30 analyses of soil, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each isotopically labeled compound in each matrix on a regular basis (e.g., after each five to ten new preparation batches).

9.5 Method blanks – A method blank is analyzed with each sample batch (Section 4.3) to demonstrate freedom from contamination. The matrix for the method blank must be similar to the sample matrix for the batch (e.g., reagent water blank [Section 7.2.1], solids blank [Section 7.2.2], or tissue blank [Section 7.2.3]).

9.5.1 Analyze the cleaned extract (Section 12.0) of the method blank aliquot before the analysis of the OPRs (Section 14.5).

9.5.2 If any PFAS is found in the blank at 1) at a concentration greater than the ML for the analyte, 2) at a concentration greater than one-third the regulatory compliance limit, or 3) at a concentration greater than one-tenth the concentration in a sample in the extraction batch, whichever is greatest, analysis of samples must be halted, and the problem corrected. Other project-specific requirements may apply; therefore, the laboratory may adopt more stringent acceptance limits for the method blank at their discretion. If the contamination is traceable to the extraction batch, samples affected by the blank must be re-extracted and

analyzed, provided enough sample volume is available and the sample are still within holding time.

If continued re-testing results in repeated blank contamination, the laboratory must document and report the failures (e.g., as qualifiers on results), unless the failures are not required to be reported as determined by the regulatory/control authority. Results associated with blank contamination for an analyte regulated in a discharge cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results.

9.6 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for initial calibration (Section 10.3), calibration verification (Section 14.3), and for initial (Section 9.2.1) and ongoing (Section 14.5) precision and recovery may be prepared from the same source; however, the use of a secondary source for calibration verification is highly recommended whenever available. If standards from a different vendor are not available, a different lot number from the same vendor can be considered a secondary source. A LC-MS/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for determination of PFAS by this method.

9.7 Laboratory duplicates – A second aliquot of one sample is prepared and analyzed with each sample batch to demonstrate within-laboratory precision for the analytes present in the sample. Use one of the additional containers for a field sample. Do **not** divide the contents of a single bottle of an aqueous sample into two smaller portions.

9.8 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique.

9.9 Matrix spikes generally are not required for methods that employ isotope dilution quantification because any deleterious effects of the matrix should be evident in the recoveries of the EIS compounds spiked into every sample. However, because some of the compounds are quantified by a non-analogous EIS (e.g., PFPeS is quantified by $^{13}C_3$-PFHxS), the analysis of matrix spike samples can help determine the accuracy of the analysis for such compounds, and may help diagnose matrix interferences for specific compounds.

10.0 Calibration and Standardization

10.1 Mass Calibration

The mass spectrometer must undergo mass calibration to ensure accurate assignments of m/z's by the instrument. This mass calibration must be performed at least annually or as recommended by the instrument manufacturer, whichever is more frequent, to maintain instrument sensitivity and stability. Mass calibration must be repeated on an as-needed basis (e.g., QC failures, ion masses fall outside of the required mass window, major instrument maintenance, or if the instrument is moved). Mass calibration must be performed using the calibration compounds and procedures prescribed by the manufacturer.

Multiple Reaction Monitoring (MRM) analysis is required to achieve better sensitivity than full-scan analysis. The default parent ions, quantitation ions (Q1), and confirmation (Q2) ions that were monitored during the validation of this method are listed in Table 7 for each native analyte, EIS, and NIS.

10.1.1 During the development of this method, instrumental parameters were optimized for the precursor and product ions of the linear isomers of the target analytes listed on Table 7. If a qualitative or quantitative standard containing an isomeric mixture (branched and linear isomers) of an analyte is commercially available for an analyte, the quantification ion used must be the quantification ion identified in Table 7, unless interferences render the product ion unusable as the quantification ion. In cases where interferences render the product ion unusable, consult the client before using the alternative product ion and document the reason for the change when reporting results. However, the use of ions with lower masses or common ions that may not provide sufficient discrimination between analytes of interest and co-eluting interferences must be avoided.

10.1.2 Optimize the response of the precursor ion $[M-H]^-$ or $[M-CO_2]^-$ for each method analyte following the manufacturer's guidance. MS parameters (e.g., source voltages, source and desolvation temperatures, gas flow, etc.) must be methodically changed until optimal analyte responses are determined. Typically, carboxylic acids have similar MS/MS conditions and sulfonic acids have similar MS/MS conditions. However, since analytes may have different optimal parameters, some compromise on the final operating conditions may be required.

10.1.3 Establish suitable operating conditions using the manufacturer's instructions and use the table below of MS conditions used during the development of this method as guidance.

Operating Conditions for Waters Acquity UPLC, TQ-S Xevo MS/MS

Injection volume	2.0 µL	(This is the default volume, and may be changed to improve performance)
MS/MS Conditions	Source Temp (ºC)	140
	Desolvation Temp (°C)	500
	Capillary Voltage (kV)	0.70
	Cone Gas (L/h)	~70
	Desolvation gas (L/h)	~800

10.1.4 As noted above, perform the mass calibration following the instrument manufacturer's instructions, using the calibrant prescribed by the manufacturer.

10.1.5 Regardless of the calibrant used, mass calibration is judged on the basis of the presence or absence of the exact calibration masses (e.g., a limit on the number of masses that are "missed"). If peaks are missing or not correctly identified, adjust the MS/MS, and repeat the test. Only after the MS/MS is properly calibrated may standards, blanks, and samples be analyzed.

10.1.6 Mass spectrometer optimization – Prior to measurements of a given analyte the mass spectrometer must be separately optimized for that analyte.

10.1.6.1 Using the post-column pump, separately infuse a solution containing each compound in methanol into the MS.

10.1.6.2 Optimize sensitivity for the product ion m/z for each compound. Precursor-product ion m/z's other than those listed may be used provided requirements in this method are met.

10.1.6.3 After MS calibration and optimization and LC-MS/MS calibration, the same LC-MS/MS conditions must be used for analysis of all standards, blanks, IPR and OPR standards, and samples.

10.1.7 Mass Calibration Verification

The mass calibration must be verified prior to the analysis of any standards and samples and after each subsequent mass calibration. Each laboratory must follow the instructions for their instrument software to confirm the mass calibration, mass resolution, and peak relative response.

10.1.7.1 Check the instrument mass resolution to ensure that it is at least unit resolution. Inject a mid-level calibration standard under LC-MS/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into segments or retention time ranges, each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$) for the analytes in each window and choose the most abundant product ion. The product ions (also the quantitation ions) chosen during method development are listed in Table 7, although these will be instrument dependent. Unit resolution must meet the manufacturer's criteria.

10.1.7.2 Check the mass calibration by measuring the amount of peak drift from the expected masses. If the peak apex has shifted more than approximately 0.2 Da, recalibrate the mass axis following the manufacturer's instructions.

10.2 Chromatographic conditions

10.2.1 The chromatographic conditions should be optimized for compound separation (including analytes with both linear and branched isomers) and for sensitivity. The same optimized operating conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples. The following table gives the suggested chromatographic conditions for this method using the specified instrument and column. Different instruments may require slightly different operating conditions. **Modification of the solvent composition of the standard or extract by increasing the aqueous content to prevent poor peak shape is not permitted.** The peak shape of early eluting compounds may be improved by increasing the volume of the injection loop or increasing the aqueous content of the initial mobile phase composition.

General LC Conditions

Column Temp (°C) 40

Max Pressure (bar) 1100.0

LC Gradient Program

Time (min)	Flow mixture [1,2]	Flow Rate Program	Gradient Curve
0.0	2% eluent A, 98% eluent B	0.35 mL/min	Initial
0.2	2% eluent A, 98% eluent B	0.35 mL/min	2
4.0	30% eluent A, 70% eluent B	0.40 mL/min	7
7.0	55% eluent A, 45% eluent B	0.40 mL/min	8
9.0	75% eluent A, 25% eluent B	0.40 mL/min	8
10.0	95% eluent A, 5% eluent B	0.40 mL/min	6
10.4	2% eluent A, 98% eluent B	0.40 mL/min	10
11.8	2% eluent A, 98% eluent B	0.40 mL/min	7

General LC Conditions

12.0	2% eluent A, 98% eluent B	0.35 mL/min	1

[1] Eluent A = Acetonitrile
[2] Eluent B = 2 mM ammonium acetate in 95:5 water/acetonitrile

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Note: *LC system components, as well as the mobile phase constituents, may contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep baseline levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, priming the mobile phase and flushing the column with at least 90% methanol before initiating a sequence may reduce background contamination.*

10.2.2 Retention time calibration

10.2.2.1 Inject compound solution(s) to determine its retention time. The laboratory may want to inject compounds separately the first time they perform the calibration. All native compounds for which there is an isotopically labeled analog will elute slightly before or with the labeled analog. Store the retention time (RT) for each compound in the data system.

10.2.2.2 Once RT windows have been confirmed for each analyte, once per ICAL and at the beginning of the analytical sequence, the position of all target analyte, EIS, and NIS peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. When ICAL is not performed, the initial CV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.

10.2.2.3 The RTs for the target analytes, EISs, and NISs must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. All branched isomer peaks identified in either the calibration standard or the qualitative (technical grade) standard also must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV.

10.2.2.4 For all method analytes with exact corresponding isotopically labeled analogs, target analytes must elute within 0.1 minutes of the associated EIS. (The laboratory may use relative retention times (RRTs) of the target analytes and their labeled analogs as an alternative, provided that they also develop corresponding RRT acceptance criteria that are at least as stringent as those described here.)

10.2.2.5 When establishing the chromatographic conditions, it is important to consider the potential interference of bile salts during analyses of samples. Inject the bile salt interference check standard containing TDCA (see Section 7.5 if the mobile phase is not acetonitrile) during the retention time calibration process and adjust the conditions to ensure that TDCA (or TDCA, TCDCA and TUDCA) does not coelute with any of the target analytes, EIS, or NIS standards. Analytical conditions must be set to allow a separation of at least 1 minute between the bile salts and the retention time window of PFOS as described in Section 7.3.3. In

order to ensure adequate chromatographic separation of the target analytes, the method requires this evaluation when establishing the chromatographic conditions, **regardless of the sample matrices to be analyzed**.

10.3 Initial calibration

Initial calibration is performed using a series of at least six solutions, with at least five of the six calibration standards being within the quantification range, and with the lowest standard at or below the LOQ. (If a second-order calibration model is used, then one additional concentration is required, with at least six of the seven calibration standards within the quantitation range.) The initial calibration solutions contain the entire suite of EISs, NISs, and target compounds. Calibration is verified with a calibration verification (CV) standard at least once every ten injections of a field sample extract, by analysis of a mid-level calibration solution. Calibration verification uses the mean RRs or RFs determined from the initial calibration to calculate the analyte concentrations in the verification standard.

Note: *Six calibration standards is the minimum number that must be used in the initial calibration; however, the laboratory may use more standards, as long as the criteria in Section 10.3.3.3 can be met.*

Prior to the analysis of samples, and after the mass calibration check has met all criteria in Section 10.1.4, each LC-MS/MS system must be calibrated at a minimum of 6 standard concentrations (Section 7.3.4 and Table 4). This method procedure calibrates and quantifies 40 target analytes, using the isotopically labeled compounds added to the sample prior to extraction, by one of two approaches:

- True isotope dilution quantification (ID), whereby the response of the target compound is compared to the response of its isotopically labeled analog. Twenty-four target compounds are quantified in this way.
- Extracted internal standard quantification (EIS), whereby the response of the target compound is compared to the response of the isotopically labeled analog of another compound with chemical and retention time similarities. Sixteen target compounds are quantified in this way.

10.3.1 Initial calibration frequency

Each LC-MS/MS system must be calibrated whenever the laboratory takes an action that changes the chromatographic conditions or might change or affect the initial calibration criteria, or if either the CV or Instrument Sensitivity Check (ISC) acceptance criteria have not been met.

10.3.2 Initial calibration procedure

Prepare calibration standards containing the native compounds, EISs, and NISs, at the concentrations described in Table 4. Analyze each calibration standard by injecting 2.0 µL (this volume may be changed to improve performance).

Note: *The same injection volume must be used for all standards, samples, blanks, and QC samples.*

10.3.3 Initial calibration calculations

10.3.3.1 Instrument sensitivity

Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 for the quantitation ions and the confirmation ions, or ≥ 10:1 if the analyte only has a quantitation ion, can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity (Table 4).

10.3.3.2 Response Ratios (RR) and Response Factors (RF)

The response ratio (RR) for each native compound calibrated by isotope dilution is calculated according to the equation below, separately for each of the calibration standards, using the areas of the quantitation ions (Q1) with the m/z shown in Table 7. RR is used for the 24 compounds measured by true isotope dilution quantification.

$$RR = \frac{Area_n\, M_{EIS}}{Area_{EIS}\, M_n}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS
$Area_{EIS}$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS used as the EIS in the calibration standard
M_{EIS} = The mass of the isotopically labeled PFAS used as the EIS in the calibration standard
M_n = The mass of the native compound in the calibration standard

Similarly, the response factor (RF) for each native compound calibrated by extracted internal standard is calculated according to the equation below. RF is used for the 16 compounds measured by extracted internal standard quantification.

$$RF = \frac{Area_s\, M_{EIS}}{Area_{EIS}\, M_s}$$

where:

$Area_s$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS
$Area_{EIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the EIS in the calibration standard
M_{EIS} = The mass of the isotopically labeled PFAS used as the EIS in the calibration standard
M_s = The mass of the native (unlabeled) PFAS in the calibration standard

A response factor (RF_s) is calculated for each isotopically labeled EIS compounds in the calibration standard using the equation below. RF_s is used for the 24 isotopically labeled compounds measured by non-extracted internal standard quantification.

$$RF_s = \frac{Area_{EIS}\, M_{NIS}}{Area_{NIS}\, M_{EIS}}$$

where:

$Area_{EIS}$ = The measured area of the Q1 m/z for the isotopically labeled EIS added to the sample before extraction
$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the NIS in the calibration standard
M_{NIS} = The mass of the isotopically labeled compound used as the NIS in the calibration standard

M_{EIS} = The mass of the isotopically labeled EIS added to the sample before extraction

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Note: *Other calculation approaches may be used, such as a weighted linear regression or non-linear regression, based on the capability of the data system used by the laboratory. If used, the regression must be weighted inversely proportional to concentration and must not be forced through zero. Analysts should consult their instrument vendor for details on regression calibration models. When using a weighted regression calibration, linearity must be assessed using Option 2 below.*

10.3.3.3 Instrument Linearity

One of the following two approaches must be used to evaluate the linearity of the instrument calibration:

Option 1: Calculate the relative standard deviation (RSD) of the RR or RF values for each native compound and isotopically labeled compound for all the initial calibration standards that were analyzed. The RSD must be ≤ 20% to establish instrument linearity.

$$mean\ RR\ or\ RF = \frac{\sum_{i=1}^{n}(RR\ or\ RF)_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(RR\ or\ RF_i - \text{mean RR or RF})^2}{n}}$$

$$RSD = \frac{SD}{mean}\ x\ 100$$

where:

$RR\ or\ RF_i$ = RR or RF for calibration standard *i*

n = Number of calibration standards

Option 2: Calculate the relative standard error (RSE) for each native compound and isotopically labeled compound for all the initial calibration standards that were analyzed. The RSE for all method analytes must be ≤ 20% to establish instrument linearity.

$$RSE = 100 \times \sqrt{\sum_{i=1}^{n} \frac{\left[\frac{x_i' - x_i}{x_i}\right]^2}{n-p}}$$

where,

x_i = Nominal concentration (true value) of each calibration standard

x'_i = Measured concentration of each calibration standard

n = Number of standard levels in the curve

p = Type of curve (2 = linear, 3 = quadratic)

In addition, although not required, it may be useful to compare the actual responses for each standard to the calibration model. Differences outside of a window of 70 – 130% of the modeled concentration may be cause for concern.

10.3.3.4 Non-extracted Internal Standard Area

Each time an initial calibration is performed, use the data from all the initial calibration standards used to meet the linearity test in Section 10.3.3.3 to calculate the mean area response for each of the NIS compounds, using the equation below.

$$Mean\ Area_{NIS_i} = \frac{\sum Area_{NIS_i}}{n}$$

where:

$Area_{NISi}$ = Area counts for the *ith* NIS, where *i* ranges from 1 to 7, for the seven NIS compounds listed in Table 1

n = The number of ICAL standards (the default value is n = 6). If a different number of standards is used for the ICAL, for example, to increase the calibration range or by dropping a point at either end of the range to meet the linearity criterion, change 6 to match the actual number of standards used.

Record the mean areas for each NIS for use in evaluating results for sample analyses (see Section 14.9). There is no acceptance criterion associated with the mean NIS area data.

10.3.4 Initial calibration corrective actions

If the instrument sensitivity or the instrument linearity criteria for initial calibration are not met, inspect the system for problems and take corrective actions to achieve the criteria. This may require the preparation and analysis of fresh calibration standards or performing a new initial calibration. All initial calibration criteria must be met before any samples or required blanks are analyzed.

10.3.5 Bile salts interference check

The laboratory must analyze a bile salt interference check standard (see Section 7.5) after the initial calibration as a check on the chromatographic conditions, **regardless of the sample matrix to be analyzed**. If an interference is present, the chromatographic conditions must be modified to eliminate the interference from the bile salts (e.g., changing the conditions such that the retention time of the bile salts fall outside the retention time window for any of the linear or branched PFOS isomers in the standard described in Section 7.3.3 by at least one minute), and the initial calibration repeated.

11.0 Sample Preparation and Extraction

For aqueous samples that contain particles and solid samples, percent solids are determined using the procedures in Section 11.1. This section describes the sample preparation procedures for aqueous samples with < 50 mg solids in the sample volume to be extracted (Section 11.2), solid (soil, sediment or biosolid) samples (Section 11.3) and tissue samples (Section 11.4).

Absent of source-specific knowledge of the PFAS levels in samples or project-specific requirements, the laboratory must pre-screen all samples prior to performing the quantitative analysis (see Appendix A). For aqueous samples, use the secondary container provided for percent solids to perform the pre-screening. If high levels of PFAS are present in the sample, a lower volume may be required for analysis.

Note: *The laboratory may subsample the aqueous samples as described in Appendix B; however, subsampling must meet project-specific requirements. The laboratory must notify the client before proceeding with subsampling, in the event that a more appropriate size sample can be collected and sent to the laboratory. Once the laboratory becomes familiar with the levels of PFAS in the samples for their clients, the samples should be collected in the appropriate sample container size to avoid subsampling. The sample data report must state when subsampling has been employed.*

Do not use any fluoropolymer articles or task wipes in these extraction procedures. Use only HDPE or polypropylene wash bottles and centrifuge tubes. Reagents and solvents for cleaning syringes may be kept in glass containers.

11.1 Determination of solids contents of samples

Two types of solids determinations are described in this method. The first is the determination of the total suspended solids (TSS) content of aqueous samples. Because aqueous samples are processed with SPE cartridges that can be clogged by suspended solids in the sample, the method recommends a limit of 50 mg of solids in the total volume of sample that is processed by SPE.

The second type of solids determination is the percent solids (% solids) of soil, sediment, and biosolids samples. The percent solids is used to report results for these sample matrices as dry-weight concentrations. (Tissue samples do not require any solids determination.)

Note: *The earlier drafts of Method 1633 described the determination of percent solids for both aqueous and solid matrices, in an attempt to "simplify" the procedures across matrix types. However, in practice, the use of TSS for aqueous matrices is a more straightforward way to examine the risk of clogging the SPE and the results do not need to be as accurate as the percent solids data used for reporting dry-weight concentrations of the other matrices.*

11.1.1 Determination of total suspended solids (TSS) in aqueous matrices

11.1.1.1 Desiccate and weigh a glass fiber filter (Section 6.4.3) in milligrams (mg) to two significant figures.

11.1.1.2 Filter 10.0 ± 0.02 mL of well-mixed sample through the filter. This volume is sufficient for the purposes of assessing the risk of clogging the SPE cartridge.

11.1.1.3 Dry the filter a minimum of 1 hour at 103 - 105 ºC and cool in a desiccator.

11.1.1.4 Calculate TSS as follows:

$$TSS\ (mg/L) = \frac{weight\ of\ sample\ aliquot\ after\ drying\ (mg) - weight\ of\ filter\ (mg)}{0.01\ L}$$

11.1.1.5 Multiply the TSS by the volume of the sample aliquot to be extracted, in liters, to obtain the milligrams of solids in the sample. If the sample volume contains more than 50 mg of TSS, at a minimum, the analyst should prepare a second SPE cartridge (see the Note in Section 12.1.4) prior to start the extraction in the event of clogging. Laboratories may develop other strategies for minimizing the disruptions due to SPE clogging and slow extractions.

11.1.1.6 In the absence of client-specific requirements, an alternative to determining the TSS may be to identify samples likely to contain more than 50 mg of solids by visual comparison to examples maintained in the laboratory. More specifically, a trained analyst should be able to distinguish samples with very low TSS and focus the TSS determinations on only those samples that might present a risk of clogging. However, given the translucent nature of HDPE containers, this may require pouring a small volume of sample from the container designated for the solids determination to a clear glass vessel. If this is done, that volume should be discarded after the assessment.

11.1.1.7 Regardless of the approach used, the laboratory must maintain records of the manner in which the solids content of each aqueous sample was assessed.

11.1.2 Determination of percent solids in soils, sediments, and biosolids

11.1.2.1 Weigh 5 to 10 g of sample to three significant figures in a tared beaker.

11.1.2.2 Dry a minimum of 12 hours at 110 ± 5 ºC, and cool in a desiccator and weigh the beaker.

11.1.2.3 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ after\ drying\ (g)}{weight\ of\ sample\ aliquot\ before\ drying\ (g)}\ x\ 100$$

11.2 Aqueous sample processing

This method was validated with aqueous samples containing no more than 50 mg of suspended solids per sample. The procedure requires the preparation of the entire sample and samples containing large amounts of suspended solids are likely to clog the SPE media, dramatically slowing or precluding sample extraction. Smaller sample volumes may be analyzed for samples containing solids greater than that specified for this method, or when unavoidable due to high levels of PFAS; however, subsampling should be avoided whenever possible.

The nominal sample size for wastewater, surface water, and groundwater and their associated QC samples is 500 mL; however, sample size may be increased up to 1,000 mL if required for a specific project. The sample is to be analyzed in its entirety and must not be filtered. Leachate samples and their associated QC samples are analyzed using a 100-mL sample volume. Therefore, leachates must not be included in the same sample preparation batch as other aqueous samples that are analyzed using 500-mL sample volumes.

11.2.1 Homogenize the sample by inverting the sample 3 – 4 times and allowing the sample to settle. Do not filter the sample. The standard procedure is to analyze the entire sample, plus a basic methanol rinse of the container.

11.2.2 The volume of the aqueous sample analyzed is determined by weighing the full sample bottle and then the empty sample bottle (see Section 12.2). Weigh each sample bottle (with the lid) to 0.1 g.

11.2.3 Prepare a method blank and two OPRs using PFAS-free water in HDPE bottles. Select a volume of water that is typical of the samples in the batch (nominally 500 mL). Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample bottles designated for use as MS/MSD samples with native standard solution (Section 7.3.3) at concentrations roughly 3 to 5 times the background concentration determined during screening of the unspiked sample, but not to exceed the calibration range. This may require multiple spiking solutions. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.2.4 Spike an aliquot of EIS solution (Section 7.3.1) directly into the sample in the original bottle (or subsampled bottle) as well as to the bottles prepared for the QC samples. Mix by swirling the sample container. If centrifugation is used to prevent samples with high TSS from clogging the SPE, the EISs must be spiked into the original sample container prior to centrifugation.

11.2.5 Using a PFAS-free pipette or other device, transfer a few drops of the sample to pH paper and check that the pH is 6.5 ± 0.5. If necessary, adjust pH with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6.2]). The sample is now ready for solid-phase extraction (SPE) and cleanup (Section 12.0).

11.3 Solid sample processing (excluding tissues)

Use a stainless spoon to mix the sample in its original jar. If it is impractical to mix the sample within its container, transfer the sample to a larger container. Remove rocks, invertebrates, and foreign objects. Vegetation can either be removed from the sample before homogenization or cut into small pieces and included in the sample, based on project requirements. Mix the sample thoroughly, stirring from the bottom to the top and in a circular motion along the sides of the jar, breaking particles to less than 1 mm by pressing against the side of the container. The homogenized sample should be even in colour and have no separate layers. Store the homogenized material in its original container or in multiple smaller containers. Determine the percent solids as per Section 11.1.2.

Note: *The maximum sample weight for sediment or soil is 5 g dry weight. The maximum sample weight for biosolids is 0.5 g dry weight.*

Small amounts of the reagent water used for aqueous method blanks (10% of sample weight or less) can be added to unusually dry samples to facilitate extraction. This is an option, not a requirement, and if used, the solid method blank associated with the samples must contain similar amounts of added water.

11.3.1 Weigh out an aliquot of solid sample, not dried (aliquot should provide 5 g dry weight for soil and sediment or 0.5 g dry-weight for biosolids) into a 50-mL polypropylene centrifuge tube. Because biosolids samples are analyzed with a 0.5-g sample, they must not be

included in the same sample preparation batch as solid samples analyzed with nominal 5-g sample masses.

11.3.2 Prepare batch QC samples using 5 g of reference solid (Section 7.2.2) wetted with 2.5 g of reagent water for the method blank and two OPRs (use 0.5 g of reference solid with 0.25 g of reagent water for biosolid sample batches). The addition of reagent water to the sand provides a matrix closer in composition to real-world samples. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated for MS/MSD samples with native standard solution (Section 7.3.3) at concentrations roughly 3 to 5 times the background concentration determined during screening of the unspiked sample, but not to exceed the calibration range. This may require multiple spiking solutions. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.3.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each centrifuge tube containing the aliquoted field and QC samples. Vortex the sample to disperse the standard and allow to equilibrate for at least 30 minutes.

11.3.4 Add 10 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and transfer the supernatant to a clean 50-mL polypropylene centrifuge tube.

11.3.5 Add 15 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining solid sample in each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the second extraction into the centrifuge tube with the supernatant from the first extraction.

11.3.6 Add another 5 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining sample in each centrifuge tube. Shake by hand to disperse, centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the third extraction into the centrifuge tube with supernatant from the first and second extractions.

11.3.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking for 5 minutes and no more, and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.3.8 The laboratory has the option to dilute the extract to approximately 35 mL with reagent water. (Some laboratories may prefer not to add any additional water, therefore, this dilution is optional.) A separate concentrator tube marked at the 35-mL level may be kept for a visual reference to get the approximate volume. Samples containing more than 50% water may yield extracts that are greater than 35 mL in volume; therefore, do not add water to these. Determine the water content in the sample as follows (percent moisture is determined from the % solids):

$$Water\ Content\ in\ Sample = \frac{Sample\ Weight\ (g) \times Moisture\ (\%)}{100} + any\ water\ added\ in\ 11.3.2\ and\ 11.3.8$$

11.3.9 Concentrate each extract at approximately 55 ºC with a N_2 flow of approximately 1.2 L/min to a final volume that is based on the water content of the sample (*see table below*). Allow extracts to concentrate for 25 minutes, then mix (by vortex if the volume is < 20 mL or using a glass pipette if the volume is > 20 mL). Continue concentrating and mixing every 10 minutes until the extract has been reduced to the required volume as specified in the table below. If the extract volume appears to stop dropping, the concentration must be stopped and the volume at which it was stopped recorded. The concentrated extract must still contain some methanol, about 5-10 mL. The pre-cleanup extract in 11.3.10 should contain no more than 20% methanol. The laboratory has flexibility to modify the volumes used to achieve this goal. Some laboratories may prefer not to add water in Section 11.3.8. The following table provides guidance to help determine the final extract volume, based on the water content of the original solid sample.

Water Content in Sample*	Concentrated Final Volume
< 5 g	7 mL
5 - 8 g	8 mL
8 - 9 g	9 mL
9 - 10 g	10 mL

* Based on the % solids result determined in Section 11.1.2.3, and including any water added to the sample in Sections 11.3.2 or the extract in Section 11.3.8.

A good rule of thumb is to make the "Concentrated Final Volume" 7 - 10 mL above the "Water Content in Sample" value.

Note: *Slowly concentrating extracts, in 1-mL increments, is necessary to prevent excessive concentration and the loss of neutral compounds (methyl and ethyl FOSEs and FOSAs) and other more volatile compounds. The extract must be concentrated to remove the methanol as excess methanol present during SPE clean-up results in poor recovery of C_{13} and C_{14} carboxylic acids and C_{10} and C_{12} sulfonates.*

If all of the methanol is evaporated, the aforementioned neutral compounds are likely to have poor recovery, if too much methanol is in the final concentrated extract, then the aforementioned longer-chain compounds are likely to have poor recovery.

11.3.10 Add 40 - 50 mL of reagent water to the extract and vortex. Check that the pH is 6.5 ±0.5 and adjust as necessary with 50% formic acid (Section 7.1.13.4) or 30% ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6]). The extracts are ready for SPE and cleanup (Section 12.0).

11.4. Tissue sample processing

Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish with the skin on, whole fish with the skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the samples must be prepared and homogenized.

If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, cover the benchtop with clean aluminum foil and use clean processing equipment (e.g., knives, scalpels, tweezers) to dissect each sample to prevent cross-contamination. Samples should be handled in a semi-thawed state for compositing and/or homogenization. All tissue comprising a sample is collected in a tared stainless-steel bowl during grinding or maceration, the total tissue mass weighed, and then mixed using a stainless-steel

spoon. If not aliquoted immediately, homogenized samples must be stored in clean HDPE containers and stored frozen for subsequent use.

If using a grinder, chilling the grinder briefly with a few pellets of dry ice may keep the tissue from sticking to the equipment. Pellets of dry ice also may be added to the tissue as it enters the grinder. After the entire sample has been processed, mix the ground tissue with a spoon, transfer back to the grinder, and repeat the grinding at least two more times until the homogenize tissue has a consistent texture and color. Between samples, disassemble the grinder or maceration device, remove any remaining tissue, and wash all parts with PFAS-free detergents, rinse with tap water, then reagent water, and finally methanol. Do not bake the grinder parts.

Once during the preparation of each batch of tissue samples (up to 20), prepare an equipment blank by pouring 500 mL of reagent water through the reassembled grinder and collecting the rinsate in a 500-mL HDPE container. Process that rinsate as an aqueous sample, but record the result in nanograms (ng) of each analyte. Barring other project-specific requirements, assess the levels of any PFAS in the rinsate by assuming that the entire mass of the analyte in the rinsate was transferred to the smallest mass of any bulk tissue sample that was collected during the grinding process (not the 2-g aliquot taken for analysis below). For example, if the smallest fish sample in the batch yields 500 g of ground tissue, divide the mass of each PFAS analyte in the rinsate by 500, and compare those amounts to the MDLs for tissue samples.

11.4.1 For each sample, weigh a 2-g aliquot of homogenized tissue into a 15-mL polypropylene centrifuge tube. Reseal the container with the remaining homogenized portion of the sample and return it to frozen storage in the event that it needs to be used for reanalysis.

Note: *The default sample weight for tissue is 2 g wet weight; however, a 1-g sample may be used. Higher sample weights are not recommended for this method.*

11.4.2 Prepare the batch QC samples using 2 g of reference tissue matrix (Section 7.2.3) for the method blank and two OPRs. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated as MS/MSD samples with native standard solution (Section 7.3.3) at concentrations roughly 3 to 5 times the background concentration determined during screening of the unspiked sample, but not to exceed the calibration range. This may require multiple spiking solutions. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.4.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each field and QC sample. Vortex and allow to equilibrate for at least 30 minutes.

11.4.4 Add 10 mL of 0.05M KOH in methanol (Section 7.1.8) to each sample. Vortex to disperse the tissue then place tubes on a variable speed mixing table set at low speed to extract for at least 16 hours. Avoid violent shaking of the samples. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant in a 50-mL polypropylene centrifuge tube.

11.4.5 Add 10 mL of acetonitrile to remaining tissue in the 15-mL centrifuge tube, vortex to mix and disperse the tissue. Sonicate for 30 minutes. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the initial extract.

11.4.6 Add 5 mL of 0.05M KOH in methanol (Section 7.1.8) to the remaining sample in each centrifuge tube. Vortex to disperse the tissue and hand mix briefly. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the first two extracts.

11.4.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking over a period of 5 minutes and no more, then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.4.8 Add 1 mL of reagent water to each evaporation/concentrator tube, set the evaporator/concentrator to 55 ºC with a N_2 flow of 1.2 L/min and concentrate the extract to 2.5 mL (only ~1 mL of the methanol should remain).

11.4.9 Add reagent water to each evaporation/concentrator tube to dilute the extracts to 50 mL. Check that the pH = 6.5 ± 0.5 and adjust as needed with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [7.1.6.2]). The extracts are ready for SPE and cleanup (Section 12.0).

12.0 Extraction, Cleanup, and Concentration

Samples of all matrices (and the associated batch QC) must undergo SPE and carbon cleanup to remove interferences (Section 12.1). Sample elution as well as any further extract treatment is matrix specific and may be found in Sections 12.2 through 12.4.

Note: *Carbon cleanup is required. Carbon cleanup may remove analytes if the sample has a very low organic carbon content (this is unusual for non-drinking water environmental samples). This will be apparent if the isotope dilution standard recoveries are significantly higher on the reanalysis. If the laboratory can demonstrate that the carbon cleanup is detrimental to the analysis of samples from a particular source (by comparing results when skipping the carbon cleanup during reanalysis), then the carbon cleanup may be skipped for samples from that specific source, with client approval.*

12.1 All sample matrices

12.1.1 Pack clean silanized glass wool to half the height of the WAX SPE cartridge barrel (Section 6.7.1).

12.1.2 Set up the vacuum manifold with one WAX SPE cartridge plus a reservoir and reservoir adaptor for each cartridge for each sample and QC aliquot.

12.1.3 Pre-condition the cartridges by washing them with 15 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2) followed by 5 mL of 0.3M formic acid (Section 7.1.13.2) (do not use the vacuum for this step). Do not allow the WAX SPE to go dry. Discard the wash solvents.

12.1.4 Pour the sample into the reservoir (do not use a pipette), taking care to avoid splashing while loading. Adjust the vacuum and pass the sample through the cartridge at 5 mL/min. Retain the empty sample bottle and allow it to air dry for later rinsing (Section 12.2.2). Discard eluate.

Note: *For aqueous samples, in the event the SPE cartridge clogs during sample loading, place a second pre-conditioned cartridge and continue loading the remaining sample aliquot using the same reservoir. Proceed to Section 12.1.5.*

12.1.5 Rinse the walls of the reservoir with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol (Section 7.1.13.5) and pass those rinses through the cartridge using vacuum. Dry the cartridge by pulling air through for 15 seconds. Discard the rinse solution. Continue to the elution steps based on the matrix (see Section 12.2 – Aqueous, Section 12.3 – Solids, and Section 12.4 – Tissue).

12.2 Elution of aqueous samples

Note: *If two cartridges were used, perform Sections 12.2.1 through 12.2.3 with each cartridge. Filter the eluates through a 25-mm, 0.2-µm syringe filter. Combine both sets of filtered eluates into a clean tube, add the NIS solution, and vortex to mix. Transfer 350 µL of the filtered extract into a 1-mL polypropylene microvial and mark the level. Add another 350-µL portion and using a gentle stream of nitrogen (water bath at 40 ºC), concentrate to the 350-µL mark and submit for LC-MS/MS analysis. This concentration step is only applicable to situations where two SPE cartridges were eluted, each with 5 mL of elution solvent.*

12.2.1 Place clean collection tubes (13 x 100 mm polypropylene) inside the manifold, ensuring that the extract delivery needles do not touch the walls of the tubes. DO NOT add NIS to these collection tubes.

12.2.2 Rinse the inside of the sample bottle with 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the SPE reservoir, washing the walls of the reservoir. Use vacuum to pull the elution solvent through the cartridge and into the collection tubes.

Note: *Air dry the empty sample bottle after the rinse is transferred. Weigh the empty bottle with the cap on and subtract from the weight with the sample determined in Section 11.2.2.*

12.2.3 Add 25 µL of concentrated acetic acid to each sample eluted in the collection tubes and vortex to mix. Add 10 mg of carbon (Section 7.1.17) to each sample and batch QC extract, using a 10-mg scoop. Hand-shake occasionally for 5 minutes and no more. It is important to minimize the time the sample extract is in contact with the carbon. Immediately vortex (30 seconds) and centrifuge at 2800 rpm for 10 minutes (other rotational speeds may be used for centrifuges other than the one described in Section 6.5.1).

12.2.4 Add NIS solution (Section 7.3.2) to a clean collection tube. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant the sample supernatant into the syringe barrel. Replace the plunger and filter the entire extract into the new collection tube containing the NIS. Vortex to mix and transfer a portion of the extract into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 6 °C.

12.3 Elution of solid samples

12.3.1 Add NIS solution (Section 7.3.2) to a clean collection tube (13 x 100 mm polypropylene) for each sample and QC aliquot and place them into the manifold rack, ensuring the extract delivery needles are not touching the walls of the tubes.

12.3.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.3.3 Add 25 µL of concentrated acetic acid to each sample extract in its collection tube and swirl to mix. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5 mL polypropylene syringe. Take the plunger out and carefully decant ~1 mL of sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 6 °C.

12.4 Elution of tissue samples

12.4.1 Add NIS solution (Section 7.3.2) to clean collection tubes (13 x 100 mm, polypropylene) for each sample and QC aliquot. Place the tubes into the manifold rack and ensure the extract delivery needles are not touching the walls of the tubes.

12.4.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.4.3 Add 25 µL of concentrated acetic acid to each sample extract. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant an aliquot (~1 mL) of the sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 6 °C.

13.0 Instrumental Analysis

Analysis of sample extracts for PFAS by LC-MS/MS is performed on an ultrahigh performance liquid chromatograph coupled to a triple quadrupole mass spectrometer, running manufacturer's software. The mass spectrometer is run with unit mass resolution in the multiple reaction monitoring (MRM) mode.

13.1 Perform mass calibration (Section 10.1), establish the operating conditions (Section 10.2), and perform an initial calibration (Section 10.3) at the frequencies described in those sections prior to analyzing samples.

13.2 Only after all performance criteria in Sections 10.1, 10.2, and 10.3 are met may blanks, MDLs, IPRs/OPRs, and samples be analyzed.

13.3 After a successful initial calibration has been completed, the analytical sequence for a batch of samples analyzed during the same time period is as follows. The volume injected for samples and QC samples must be identical to the volume used for calibration (Section 10.2.3).

Standards and sample extracts **must** be brought to room temperature and vortexed prior to aliquoting into an instrument vial in order to ensure homogeneity of the extract.

1. Instrument Blank
2. Instrument Sensitivity Check (see Section 10.3.3.1)
3. Calibration Verification Standard
4. Qualitative Identification Standards
5. Instrument Blank
6. Method Blank
7. Low-level OPR (LLOPR)
8. OPR
9. Bile salt interference check standard (Section 7.5)
10. Injections of sample extracts, diluted extracts, and QC sample extracts (10 or fewer field sample extracts)
11. Calibration Verification Standard
12. Instrument Blank
13. Injections (10 or fewer field sample extracts)
14. Calibration Verification Standard
15. Instrument Blank

If the results are acceptable, the closing calibration verification solution (#14 above) may be used as the opening solution for the next analytical sequence.

13.4 If the response exceeds the calibration range for any analyte, the sample extract is diluted as per Section 15.3 to bring all target responses within the calibration range.

Note: *If the analytes that exceed the calibration range in the original analysis are known to not be of concern for the specific project (e.g., are not listed in a discharge permit), then the laboratory may consult with the client regarding the possibility of reporting sample results over the calibration range from the undiluted analysis, provided that they are clearly identified as such and appropriately qualified.*

14.0 Performance Tests during Routine Operations

The following performance tests must be successfully completed as part of each routine instrumental analysis shift described in Section 13.3 above (also see Table 9).

14.1 Instrument sensitivity check

The signal-to-noise ratio of the ISC standard (Section 7.3.4) must be greater than or equal to 3:1 for the quantitation and confirmation ions that exist, and must meet the ion ratio requirements in Section 15.1.3. If the analyte has no confirmation ions, then a 10:1 signal to noise ratio is required. If the requirements cannot be met, the problem must be corrected before analyses can proceed. In addition, the measured concentration of each native target analyte in the ISC must fall within ± 30% of its nominal concentration. If that requirement cannot be met for any target analyte relevant to a project, analysis must be halted and the sensitivity of the LC-MS/MS system adjusted before analysis of field samples.

14.2 Bile salt interference check

The retention time of the bile salts in the standard in Section 7.5 must fall at least one minute outside the retention time window for any of the linear or branched PFOS isomers in the standard described in Section 7.3.3. If this requirement is not met, the chromatographic conditions must be adjusted to meet the requirement and the initial calibration must be repeated before any field sample are analyzed.

14.3 Calibration verification (CV)

After a passing instrument sensitivity check (Section 14.1) and a successful initial calibration (Section 10.3.3.3) is achieved, prior to the analysis of any samples, analyze a mid-level calibration standard (Section 7.3.4).

14.3.1 The calibration is verified by analyzing a CV standard at the beginning of each analytical sequence, every ten samples or less, and at the end of the analytical sequence.

14.3.2 Calculate concentration for each native and isotopically labeled compound in the CV using the equation in Section 15.2.

14.3.3 The recovery of native compounds for the CVs must be within 70 - 130% unless the analyte is not of concern for a given project.

14.3.4 If the CV criterion in Section 14.3.3 is not met, recalibrate the LC-MS/MS instrument according to Section 10.3 and reanalyze any extracts that were analyzed between the last passing CV and the one that failed with the following exception. If an analyte in the CV failed because of high recovery, but that analyte was not detected in a sample extract, then that sample extract need not be reanalyzed.

14.3.5 Ion abundance ratios

Using the data from the CV standard, compute the ion abundance ratio for each target analyte listed with a confirmation ion mass in Table 7, using the equation below. These ion abundance ratios will be used a part of the qualitative identification criteria in Section 15.1.

$$IAR = \frac{Area_{Q1}}{Area_{Q2}}$$

where:

IAR = Ion abundance ratio

$Area_{Q1}$ = The measured area of the Q1 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration, as described in Section 15.1.3

$Area_{Q2}$ = The measured area of the Q2 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration, as described in Section 15.1.3

Note: *Some of the native analytes in Table 7 do not produce confirmation ions, or produce confirmation ions with very low relative abundances; therefore, for those analytes, the IAR does not apply.*

Pending completion of the multi-laboratory validation study, construct an acceptance window for the IAR of each target analyte as 50% to 150% of the IAR in the mid-point calibration standard or daily CV standard as applicable per section 15.1.3.

14.4 Retention times and resolution

14.4.1 For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

14.4.2 The retention times of each native and isotopically labeled compound must be within ± 0.4 minutes of the ICAL or CV used to establish the RT windows for the samples and batch QC.

14.5 Ongoing precision and recovery (OPR)

14.5.1 After completing the first 6 steps in the analytical sequence described in Section 13.3,analyze the extracts of the low-level OPR (LLOPR) and the mid-level OPR) (Sections 11.3.3, 11.3.2, and 11.4.2) prior to analysis of samples from the same batch to ensure the analytical process is under control.

14.5.2 Compute the percent recovery of the native compounds by the appropriate quantification method depending on the compound (Section 10.3). Compute the percent recovery of each isotopically labeled compound by the non-extracted internal standard method (Sections 1.2 and 10.3).

$$Recovery\ (\%) = \frac{Concentration\ found\ (ng/mL)}{Concentration\ spiked\ (ng/mL)}\ x\ 100$$

14.5.3 For the native compounds and EISs, compare the recovery to the OPR and LLOPR limits given in Table 5. Aqueous OPR and LLOPR results must meet the acceptance criteria in that table. Pending completion on the multi-laboratory validation study and development of formal acceptance criteria, OPR results for other matrices generally should fall within the single-laboratory study ranges shown in Table 5A. Minor deviations (e.g., less than 10% lower or higher than the single-laboratory study range) are acceptable. Major deviations for native PFAS analytes in solid and tissue matrices require corrective actions.

For wastewater matrices, if all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and wastewater samples may proceed. If, however, any individual concentration falls outside of the given range, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch, including any QC samples, and repeat the ongoing precision and recovery test.

14.6 Instrument blank – At the beginning of the analytical sequence and after the analysis of high concentration samples (e.g., highest calibration standard, CV), analyze an instrument blank to ensure no instrument contamination has occurred. The instrument blank should not contain any target analyte that would yield a response equivalent to the mass of the analyte that would be present in a whole-volume sample at the analyte's MDL. If an analyte is present at such levels, analyze one or more additional instrument blanks until the response of the analyte is no longer detectable, or perform additional troubleshooting steps to identify and minimize other potential sources of PFAS contamination.

14.7 Method blank – After the analysis of the solvent blank and prior to the analysis of samples, analyze a method blank (Section 9.5).

14.8 Analyze a qualitative identification standard (Section 7.3.5) containing all available isomers (branched and linear) once daily, at the beginning of the analytical sequence, to confirm the retention time of each linear and known branched isomer or isomer group.

14.9 Instrument sensitivity (optional)

This step is recommended as a follow-up step if the ISC 14.1 does not meet the criteria in Section 14.1. Calculate the ratio of the NIS peak areas from the QC and field samples relative to the mean area of the corresponding NIS in the most recent initial calibration to check for possible bad injections of NIS solution or loss of instrument sensitivity.

$$Area\ Ratio_{NIS_i}(\%) = 100 \times \frac{Area\ of\ NIS_i\ in\ the\ Sample}{Mean\ Area_{NIS_i}}$$

where:

Area of NIS_i in the Sample = Observed area counts for NIS_i in the sample

Mean $Area_{NISi}$ = The mean area counts for the corresponding NIS from the most recent initial calibration, calculated as described in Section 10.3.3.4

i = Indicates each of the seven NIS compounds listed in Table 1

The NIS areas in the field samples and QC samples must be within 40 to 200% of the area of the calibration verification standard run at the beginning of the analytical sequence. If the areas are low for all the field samples and QC samples in the batch, it suggests a loss of instrument sensitivity, while low areas in only some field or QC samples suggests a possible bad injection.

15.0 Data Analysis and Calculations

15.1 Qualitative determination and peak identification

A native or isotopically labeled compound is identified in a standard, blank, sample, or QC sample when all of the criteria in Sections 15.1.1 through 15.1.4 are met.

15.1.1 For target analytes or EISs to be identified, peak responses of the quantitation and confirmation ions must be at least three times the background noise level (S/N 3:1). The quantitation ion must have a S/N $\geq$ 10:1 if there is no confirmation ion . If the S/N ratio is not met due to high background noise, the laboratory must correct the issue (e.g., perform instrument troubleshooting and any necessary maintenance, such as cleaning the ion source, replacing the LC column, or if needed, repeat the cleanup steps to remove background due to the sample matrix). If the S/N ratio is not met but the background is low, then the analyte is to be considered a non-detect.

15.1.2 Target analyte, EIS analyte, and NIS analyte RTs must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. The retention time window used must be of sufficient width to detect earlier-eluting branched isomers. For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

15.1.3 The laboratory must follow the identification requirements specified by the client for the project. In the event there are no project-specific requirements, the following general requirements apply. For concentrations at or above the method LOQ, the IAR must fall within ± 50% of the IAR observed in the mid-point initial calibration standard. If project-specific requirements involve reporting sample concentrations below the LOQ or ML, these peaks should also meet the IAR criterion to be reported (see Section 14.3.5).

The total response of all isomers (branched and linear) in the quantitative standards should be used to define the IAR. In samples, the total response should include only the branched isomer peaks that have been identified in either the quantitative or qualitative standard (see Section 7.3 regarding records of traceability of all standards). If standards (either quantitative or qualitative) are not available for purchase, only the linear isomer can be identified and quantitated in samples. The ratio requirement does not apply for PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA because suitable (not detectable or inadequate S/N) secondary transitions (Q2) are unavailable.

15.1.4 If the field sample result does not all meet the criteria stated in Sections 15.1.1 through 15.1.3, and all sample preparation avenues (e.g., extract cleanup, sample dilution, etc.) have been exhausted, the result may only be reported with a data qualifier alerting the data user that the result could not be confirmed because it did not meet the method-required criteria and therefore should be considered an estimated value. If the criteria listed above are not met for the standards, the laboratory must stop analysis of samples and correct the issue.

15.2 Quantitative determination

Concentrations of the target analytes are determined with respect to the extracted internal standard (EIS) which is added to the sample prior to extraction. The EIS is quantitated with respect to a non-extracted internal standard (NIS), as shown in Table 7, using the response ratios or response factors from the most recent multi-level initial calibration (Section 10.3). Other equations may be used if the laboratory demonstrates that those equations produce the same numerical result as produced by the equations below.

For the native analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_n\ M_{EIS}}{Area_{EIS}(\overline{RR}\ or\ \overline{RF})} \times \frac{1}{W_S}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS
$Area_{EIS}$ = The measured area at the Q1 m/z for the EIS. *See note below.*
M_{EIS} = The mass of the EIS added (ng)
$\overline{RR}$ = Average response ratio used to quantify target compounds by the isotope dilution method
$\overline{RF}$ = Average response factor used to quantify target compounds by the extracted internal standard method
W_S = Sample volume (L) or weight (g)

Note: *For better accuracy, EPA recommends that PFTrDA be quantified using the average of the areas of labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.*

And for the EIS analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_{EIS}\ M_{NIS}}{Area_{NIS}\overline{RF_s}} \times \frac{1}{W_S}$$

where:

$Area_{EIS}$ = The measured area at the Q1 m/z for the EIS
$Area_{NIS}$ = The measured area of the Q1 m/z for the NIS
M_{NIS} = The mass of the NIS added (ng)
W_S = Sample volume (L) or weight (g)
$\overline{RF_s}$ = Average response factor used to quantify the EIS by the non-extracted internal standard method

Results for native compounds are recovery corrected by the method of quantification. Extracted internal standard (EIS) recoveries are determined similarly against the non-extracted internal standard (NIS) and are used as general indicators of overall analytical quality.

The instrument measures the target analytes as either their anions or neutral forms. **The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms,** using the following equation to convert the concentrations:

$$C_{Acid} = C_{Anion} \times \frac{MW_{Acid}}{MW_{Anion}}$$

where:

C_{Anion} = The analyte concentration in anion form
MW_{Acid} = The molecular weight of the acid form
MW_{Anion} = The molecular weight of the anion form

15.3 Sample dilutions

15.3.1 If the Q1 area for any compound exceeds the calibration range of the system, dilute a subsample of the sample extract with the methanolic ammonium hydroxide and acetic acid solution in Section 7.1.9 and analyze the diluted extract. If the responses for each EIS in the diluted extract meet the S/N and retention time requirements in Sections 15.1.1 and 15.1.2, and the EIS recoveries from the analysis of the diluted extract are greater than 5%, then the compounds associated with those EISs may be quantified using the EIS response. Therefore, use the EIS recoveries from the original analysis to select the dilution factor, with the objective of keeping the EIS recoveries in the dilution above that 5% lower limit (i.e., if the EIS recovery of the affected analyte in the undiluted analysis is 50%, then the sample cannot be diluted more than 10:1; if the EIS recovery of the affected analyte in the undiluted analysis is 30%, then the sample cannot be diluted more than 6:1). Adjust the compound concentrations, detection limits, and minimum levels to account for the dilution.

If the EIS responses in the diluted extract do not meet those S/N and retention time requirements, then the compound cannot be measured reliably by isotope dilution in the diluted extract. In such cases, the laboratory must take a smaller aliquot of any affected aqueous sample and dilute it to 500 mL with reagent water and analyze the diluted aqueous sample, or analyze a smaller aliquot of soil, biosolid, sediment, or tissue sample. Adjust the calibration ranges, detection limits, and minimum levels to account for the dilution.

If a dilution results in a EIS recovery less than 5%, then the laboratory must prepare and analyze a diluted aqueous sample or a smaller aliquot of a solid sample.

15.3.2 If the recovery of any EIS in a wastewater sample is outside of the acceptance limits in Table 8, a diluted aqueous sample must be analyzed (Section 15.3.1). If the recovery of any EIS in the diluted sample is below 5%, the method does not apply to the sample being analyzed and the result may not be reported or used for permitting or regulatory compliance purposes. In this case, an alternative column could be employed to resolve the interference.

If all cleanup procedures in this method and an alternative column have been employed and EIS recovery remains outside of the acceptance range, extraction and/or cleanup procedures that are beyond this scope of this method may be needed to analyze the sample.

Table 8A includes example performance data for solids and tissues from the single-laboratory validation study and are provided for illustrative purposes (e.g., those figures are not required acceptance criteria). EIS recoveries in solids and tissues that fall well outside of the ranges in Table 8A are a potential cause for concern and laboratories should take similar steps to those described for wastewater samples to improve EIS recoveries, including preparing and analyzing a smaller sample aliquot.

15.4 Reporting of analytical results (acid/neutral forms)

The data reporting practices described here are focused on NPDES monitoring needs and may not be relevant to other uses of the method. For analytes reported in their acid form, use the equations in Section 15.2 and the analyte names Table 1. For analytes reported in their anion form, see Table 2 for the appropriate names and CAS Registry Numbers.

15.4.1 Report results for aqueous samples in ng/L. Report results for solid samples in ng/g, on a dry-weight basis, and report the percent solids for each sample separately. Report results for tissue samples in ng/g, on a wet-weight basis. Other units may be used if required in a permit or for a project. Report all QC data with the sample results.

15.4.2 Reporting level

Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria are reported down to the concentration of the ML established by the laboratory through calibration of the instrument (see the glossary for the derivation of the ML). EPA considers the terms "reporting limit," "quantitation limit," "limit of quantitation," and "minimum level" to be synonymous.

15.4.2.1 Report a result for each analyte in each field sample or QC standard at or above the ML to 3 significant figures. Report a result for each analyte found in each field sample or QC standard below the ML as "<ML," where ML is the concentration of the analyte at the ML, or as required by the regulatory/control authority or permit.

15.4.2.2 Report a result for each analyte in a blank at or above the MDL to 2 significant figures. Report a result for each analyte found in a blank below the MDL as "<MDL," where MDL is the concentration of the analyte at the MDL, or as required by the regulatory/control authority or permit.

15.4.2.3 Report a result for an analyte found in a sample or extract that has been diluted at the least dilute level at which the area at the quantitation m/z is within the calibration range (e.g., above the ML for the analyte and below the highest calibration standard) and with isotopically labeled compound recoveries within their respective QC acceptance criteria. This may require reporting results for some analytes from different analyses.

15.4.2.4 Report recoveries of all associated EIS compounds for all field samples and QC standards. If a sample extract was diluted and analyzed, report the EIS recoveries from both the original analysis and the analysis of the dilution.

15.4.3 Results from tests performed with an analytical system that is not in control (i.e., that does not meet acceptance criteria for any QC tests in this method) must be documented and reported (e.g., as a qualifier on results), unless the failure is not required to be reported as determined by the regulatory/control authority. Results associated with a QC failure cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results. If the holding time would be exceeded for a reanalysis of the sample, the regulatory/control authority should be consulted for disposition.

16.0 Method Performance

Routine method performance is validated through analysis of matrix-specific reference samples, including IPRs, MDLs, and certified reference materials. Ongoing method performance is monitored through QC samples analyzed alongside samples. The parameters monitored include percent recovery of isotopically labeled compounds, blank concentrations, and native compound recoveries.

This method is being validated, and performance specifications will be developed using data from DoD's interlaboratory validation study (Reference 10). Wastewater data from that study were used to develop the QC acceptance criteria in Table 5 (IPR/OPR/LLOPR) and Table 8 (EIS recoveries). Table 6 provides the pooled MDL results from aqueous matrices portion of the multi-laboratory validation study.

For solid and tissue matrices, Table 5A and 8A summarize the results from the single-laboratory validation study, which should be used as guidance in assessing the results for solid and tissue matrices until EPA develops formal QC acceptance criteria. Table 6 provides examples of the MDL and ML results from the single-laboratory validation study for solids and tissues.

17.0 Pollution Prevention

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operations. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to minimize waste generation. When wastes cannot be reduced feasibly at the source, EPA recommends recycling as the next best option.

17.2 The compounds in this method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

17.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 7).

18.0 Waste Management

18.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly regarding management of hazardous waste , and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations.

Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (Reference 8).

18.2 Samples at pH < 2 or pH > 12.5, are hazardous and must be handled and disposed of as hazardous waste or neutralized and disposed of in accordance with all federal, state, and local regulations

18.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better-Laboratory Chemical Management for Waste Reduction*, (Reference 9).

19.0 References

1. "Working with Carcinogens," Department of Health, Education, & Welfare, Public Health Service, Centers for Disease Control, NIOSH, Publication 77-206, August 1977, NTIS PB-277256.

2. "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

3. "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

4. "Standard Methods for the Examination of Water and Wastewater," 18th edition and later revisions, American Public Health Association, 1015 15th St, NW, Washington, DC 20005, 1-35: Section 1090 (Safety), 1992.

5. "Standard Practice for Sampling Water," ASTM Annual Book of Standards, ASTM, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

6. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA EMSL, Cincinnati, OH 45268, EPA 600/4-79-019, April 1979.

7. "Less is Better: Laboratory Chemical Management for Waste Reduction," American Chemical Society, 1993. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

8. "Environmental Management Guide for Small Laboratories," USEPA, Small Business Division, Washington DC, EPA 233-B-00-001, May 2000.

9. "The Waste Management Manual for Laboratory Personnel," American Chemical Society, 1990. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

10. Willey, J., R. Anderson, A. Hanley, M. Mills, C. Hamilton, T. Thompson, and A. Leeson. 2021. "Report on the Single-Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS," Strategic Environmental Research and Development Program (SERDP) Project ER19-1409.

11. *DoD interlaboratory study reference will be added here.*

12. DoD QSM (US Department of Defense Quality Systems Manual for Environmental Laboratories, version 5.3, 2019).

13. Woudneh, Million B., Bharat Chandramouli, Coreen Hamilton, Richard Grace, 2019, "Effects of Sample Storage on the Quantitative Determination of 29 PFAS: Observation of Analyte Interconversions during Storage," Environmental Science and Technology 53(21): 12576-12585.

14. ISO 17034:2016, *General requirements for the competence of reference material producers*, ISO, Geneva, Switzerland, 2016.

20.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Perfluoroalkyl carboxylic acids		
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	376-06-7
Perfluoroalkyl sulfonic acids		
Acid Form		
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluoropentansulfonic acid	PFPeS	2706-91-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanesulfonic acid	PFNS	68259-12-1
Perfluorodecanesulfonic acid	PFDS	335-77-3
Perfluorododecanesulfonic acid	PFDoS	79780-39-5
Fluorotelomer sulfonic acids		
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorooctane sulfonamides *		
Perfluorooctanesulfonamide	PFOSA	754-91-6
N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2
Perfluorooctane sulfonamidoacetic acids *		
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
Perfluorooctane sulfonamide ethanols *		
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2
Per- and Polyfluoroether carboxylic acids		
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
EIS Compounds		
Perfluoro-n-[$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	NA
Perfluoro-n-[$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	$^{13}C_2$-PFTeDA	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid	$^{13}C_3$-PFBS	
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid	$^{13}C_3$-PFHxS	
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid	$^{13}C_8$-PFOS	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	$^{13}C_8$-PFOSA	
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D_3-NMeFOSAA	
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D_5-NEtFOSAA	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonic acid	$^{13}C_2$-4:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]octane sulfonic acid	$^{13}C_2$-6:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]decane sulfonic acid	$^{13}C_2$-8:2FTS	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	
N-methyl-d_7-perfluorooctanesulfonamidoethanol	D_7-NMeFOSE	
N-ethyl-d_9-perfluorooctanesulfonamidoethanol	D_9-NEtFOSE	
N-ethyl-d_5-perfluoro-1-octanesulfonamide	D_5-NEtFOSA	
N-methyl-d_3-perfluoro-1-octanesulfonamide	D_3-NMeFOSA	
NIS Compounds		
Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	NA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid	$^{13}C_4$-PFOS	
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	$^{13}C_5$-PFNA	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid	$^{18}O_2$-PFHxS	

[1] The target analyte names are for the acid and neutral forms of the analytes. See Table 2 for the names and CASRN of the corresponding anion forms, where applicable.

NA Not assigned a CASRN

* Analytes in this class may not perform as well as others (see Section 1.6)

Table 2. Cross-reference of Abbreviations, Analyte Names, CAS Numbers for the Acid and Anion Forms of the Perfluoroalkyl carboxylates and Perfluoroalkyl sulfonates

Perfluoroalkyl carboxylic acids/anions				
Abbreviation	**Acid Name**	**CASRN**	**Anion Name**	**CASRN**
PFBA	Perfluorobutanoic acid	375-22-4	Perfluorobutanoate	45048-62-2
PFPeA	Perfluoropentanoic acid	2706-90-3	Perfluoropentanoate	45167-47-3
PFHxA	Perfluorohexanoic acid	307-24-4	Perfluorohexanoate	92612-52-7
PFHpA	Perfluoroheptanoic acid	375-85-9	Perflluoroheptanoate	120885-29-2
PFOA	Perfluorooctanoic acid	335-67-1	Pefluorooctanoate	45285-51-6
PFNA	Perfluorononanoic acid	375-95-1	Perfluorononanoate	72007-68-2
PFDA	Perfluorodecanoic acid	335-76-2	Perfluorodecanoate	73829-36-4
PFUnA	Perfluoroundecanoic acid	2058-94-8	Perfluoroundecanoate	196859-54-8
PFDoA	Perfluorododecanoic acid	307-55-1	Perfluorododecanoate	171978-95-3
PFTrDA	Perfluorotridecanoic acid	72629-94-8	Perfluorotridecanoate	862374-87-6
PFTeDA	Perfluorotetradecanoic acid	376-06-7	Perfluorotetradecanoate	365971-87-5
Perfluoroalkyl sulfonic acids/anions				
PFBS	Perfluorobutanesulfonic acid	375-73-5	Perfluorobutane sulfonate	45187-15-3
PFPeS	Perfluoropentansulfonic acid	2706-91-4	Perfluoropentane sulfonate	175905-36-9
PFHxS	Perfluorohexanesulfonic acid	355-46-4	Perfluorohexane sulfonate	108427-53-8
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	Perfluoroheptane sulfonate	146689-46-5
PFOS	Perfluorooctanesulfonic acid	1763-23-1	Perfluorooctane sulfonate	45298-90-6
PFNS	Perfluorononanesulfonic acid	68259-12-1	Perfluorononane sulfonate	474511-07-4
PFDS	Perfluorodecanesulfonic acid	335-77-3	Perfluorodecane sulfonate	126105-34-8
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	Perfluorododecane sulfonate	343629-43-6

Table 3. Nominal Masses of Spike Added to Samples or Extracts

Analyte	Amount Added (ng)
Extracted Internal Standards	
$^{13}C_4$-PFBA	40
$^{13}C_5$-PFPeA	20
$^{13}C_5$-PFHxA	10
$^{13}C_4$-PFHpA	10
$^{13}C_8$-PFOA	10
$^{13}C_9$-PFNA	5
$^{13}C_6$-PFDA	5
$^{13}C_7$-PFUnA	5
$^{13}C_2$-PFDoA	5
$^{13}C_2$-PFTeDA	5
$^{13}C_3$-PFBS	10
$^{13}C_3$-PFHxS	10
$^{13}C_8$-PFOS	10
$^{13}C_2$-4:2FTS	20
$^{13}C_2$-6:2FTS	20
$^{13}C_2$-8:2FTS	20
$^{13}C_8$-PFOSA	10
D_3-NMeFOSA	10
D_5-NEtFOSA	10
D_3-NMeFOSAA	20
D_5-NEtFOSAA	20
D_7-NMeFOSE	100
D_9-NEtFOSE	100
$^{13}C_3$-HFPO-DA	40
Non-extracted Internal Standards	
$^{13}C_3$-PFBA	20
$^{13}C_2$-PFHxA	10
$^{13}C_4$-PFOA	10
$^{13}C_5$-PFNA	5
$^{13}C_2$-PFDA	5
$^{18}O_2$-PFHxS	10
$^{13}C_4$-PFOS	10

Table 4. Calibration Solutions (ng/mL) Used in the Method Validation Studies

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Perfluoroalkyl carboxylic acids							
PFBA	0.8	2	5	10	20	50	250
PFPeA	0.4	1	2.5	5	10	25	125
PFHxA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFUnA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTrDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTeDA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluoroalkyl sulfonic acids							
PFBS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFPeS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHxS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoS	0.2	0.5	1.25	2.5	5	12.5	62.5
Fluorotelomer sulfonic acids							
4:2FTS	0.8	2	5	10	20	50	NA
6:2FTS	0.8	2	5	10	20	50	NA
8:2FTS	0.8	2	5	10	20	50	NA
Perfluorooctane sulfonamides							
PFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NMeFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamidoacetic acids							
NMeFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamide ethanols							
NMeFOSE	2	5	12.5	25	50	125	625
NEtFOSE	2	5	12.5	25	50	125	625
Per- and polyfluoroether carboxylic acids							
HFPO-DA	0.8	2	5	10	20	50	250
ADONA	0.8	2	5	10	20	50	250
PFMPA	0.4	1	2.5	5	10	25	125
PFMBA	0.4	1	2.5	5	10	25	125
NFDHA	0.4	1	2.5	5	10	25	125
Ether sulfonic acids							
9Cl-PF3ONS	0.8	2	5	10	20	50	250
11Cl-PF3OUdS	0.8	2	5	10	20	50	250
PFEESA	0.4	1	2.5	5	10	25	125

Table 4. Calibration Solutions (ng/mL) Used in the Method Validation Studies

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Fluorotelomer carboxylic acids							
3:3FTCA	1.0	2.5	6.26	12.5	25	62.4	312
5:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
7:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
Extracted Internal Standard (EIS) Analytes							
$^{13}C_4$-PFBA	10	10	10	10	10	10	10
$^{13}C_5$-PFPeA	5	5	5	5	5	5	5
$^{13}C_5$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFHpA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_9$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_6$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_7$-PFUnA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDoA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFTeDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_3$-PFBS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_3$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_2$-4:2FTS	5	5	5	5	5	5	5
$^{13}C_2$-6:2FTS	5	5	5	5	5	5	5
$^{13}C_2$-8:2FTS	5	5	5	5	5	5	5
$^{13}C_8$-PFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_5-NEtFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSAA	5	5	5	5	5	5	5
D_5-NEtFOSAA	5	5	5	5	5	5	5
D_7-NMeFOSE	25	25	25	25	25	25	25
D_9-NEtFOSE	25	25	25	25	25	25	25
$^{13}C_3$-HFPO-DA	10	10	10	10	10	10	10
Non-extracted Internal Standard (NIS) Analytes							
$^{13}C_3$-PFBA	5	5	5	5	5	5	5
$^{13}C_2$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_5$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{18}O_2$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] This calibration point is used as the calibration verification (CV)

[2] A minimum of six contiguous calibrations standards are required for linear models and a minimum of seven calibration standards are required for second-order models.

Table 5. IPR/OPR/LLOPR Acceptance Limits for Wastewater Samples

	Aqueous Matrix [1, 2]			
	IPR			
Compounds	Recovery (%)	RSD (%)	OPR Recovery (%)	LLOPR Recovery (%)
PFBA	60 – 147	20	58 – 148	44 - 157
PFPeA	56 – 150	20	54 – 152	57 - 148
PFHxA	59 – 148	25	55 – 152	62 - 149
PFHpA	60 – 149	25	54 – 154	56 - 150
PFOA	55 – 158	25	52 – 161	57 - 161
PFNA	64 – 144	25	59 – 149	53 - 157
PFDA	57 – 142	25	52 – 147	43 - 158
PFUnA	54 – 153	30	48 – 159	50 - 155
PFDoA	73 – 133	25	64 – 142	60 - 141
PFTrDA	52 – 145	25	49 – 148	52 - 140
PFTeDA	49 - 158	25	47 – 161	52 - 156
PFBS	66 – 141	20	62 – 144	63 - 145
PFPeS	66 – 144	25	59 – 151	58 - 144
PFHxS	62 – 141	25	57 – 146	44 - 158
PFHpS	59 – 148	25	55 – 152	51 - 150
PFOS	61 – 145	20	58 – 149	43 - 162
PFNS	57 – 143	25	52 – 148	46 - 151
PFDS	56 – 142	25	51 – 147	50 - 144
PFDoS	41 – 140	30	36 – 145	30 - 138
4:2FTS	77 – 135	25	67 – 146	52 - 158
6:2FTS	75 – 137	30	61 – 151	48 - 158
8:2FTS	79 – 136	30	63 – 152	46 - 165
PFOSA	65 – 144	20	61 – 148	47 - 163
NMeFOSA	76 – 132	25	63 – 145	54 - 155
NEtFOSA	75 – 129	25	65 – 139	49 - 156
NMeFOSAA	69 – 134	25	58 – 144	32 - 160
NEtFOSAA	65 – 140	25	59 – 146	51 - 154
NMeFOSE	79 – 129	20	71 – 136	56 - 151
NEtFOSE	79 – 126	25	69 – 137	60 - 147
HFPO-DA	72 – 135	25	63 – 144	58 - 154
ADONA	75 – 138	20	68 – 146	61 - 148
PFMPA	55 – 141	25	51 – 145	48 - 150
PFMBA	59 – 145	20	55 – 148	49 - 154
NFDHA	63 – 146	35	48 – 161	47 - 160
9Cl-PF3ONS	72 – 140	30	56 – 156	44 - 167
11Cl-PF3OUdS	61 – 140	35	46 – 156	36 - 158
PFEESA	57 – 149	20	56 – 151	56 - 144
3:3FTCA	66 – 126	20	62 – 129	32 - 161
5:3FTCA	68 – 130	20	63 – 134	39 - 156
7:3FTCA	55 – 133	25	50 – 138	36 - 149
$^{13}C_4$-PFBA	10 – 130	30	10-130	10-130
$^{13}C_5$-PFPeA	35 – 150	30	40 -150	40 -150
$^{13}C_5$-PFHxA	55 – 150	30	40 -150	40 -150
$^{13}C_4$-PFHpA	55 – 150	30	40 -150	40 -150
$^{13}C_8$-PFOA	60 – 140	30	30-140	30-140
$^{13}C_9$-PFNA	55 – 140	30	30-140	30-140
$^{13}C_6$-PFDA	50 – 140	30	20-140	20-140

Table 5. IPR/OPR/LLOPR Acceptance Limits for Wastewater Samples

	Aqueous Matrix [1, 2]			
	IPR			
Compounds	Recovery (%)	RSD (%)	OPR Recovery (%)	LLOPR Recovery (%)
$^{13}C_7$-PFUnA	30 – 140	30	20-140	20-140
$^{13}C_2$-PFDoA	10 – 150	30	10-150	10-150
$^{13}C_2$-PFTeDA	10 – 130	30	10-130	10-130
$^{13}C_3$-PFBS	55 – 150	30	25-150	25-150
$^{13}C_3$-PFHxS	55 – 150	30	25-150	25-150
$^{13}C_8$-PFOS	45 – 140	30	20-140	20-140
$^{13}C_2$-4:2FTS	60 – 200	30	25-200	25-200
$^{13}C_2$-6:2FTS	60 – 200	30	25-200	25-200
$^{13}C_2$-8:2FTS	50 – 200	30	25-200	25-200
$^{13}C_8$-PFOSA	30 – 130	30	10-130	10-130
D_3-NMeFOSA	15 – 130	30	10-130	10-130
D_5-NEtFOSA	10 – 130	30	10-130	10-130
D_3-NMeFOSAA	45 – 200	30	10-200	10-200
D_5-NEtFOSAA	10 – 200	30	10-200	10-200
D_7-NMeFOSE	10 – 150	30	10-150	10-150
D_9-NEtFOSE	10 – 150	30	10-150	10-150
$^{13}C_3$-HFPO-DA	25 – 160	30	25-160	25-160

[1] The recovery limits apply to the target analyte results for IPR, OPR, and LLOPR samples for wastewater matrices. Data for this matrix type are derived from the multi-laboratory validation study and are therefore the limits required for this method.

[2] The recovery limits for the EIS compounds were derived by EPA from the wastewater sample data from multi-laboratory validation study. To simplify laboratory operations, EPA has applied the same EIS recovery limits used for field sample analyses to the EIS recoveries in the IPR, OPR, and LLOPR samples.

Table 5A Example Performance Data for Solids and Tissues

Compounds	Solid Matrix[1]			Tissue Matrix[1]		
	IPR		OPR Recovery (%)	IPR		OPR Recovery (%)
	Recovery (%)	RSD[2] (%)		Recovery (%)	RSD[2] (%)	
PFBA	95 – 99	5	92 – 108	89 – 104	5	90 – 110
PFPeA	92 – 105	5	94 – 115	80 – 98	5	96 – 114
PFHxA	93 – 101	5	89 – 107	72 – 110	10	90 – 111
PFHpA	94 – 102	5	89 – 107	87 – 102	5	87 – 118
PFOA	92 – 100	5	90 – 106	78 – 85	5	82 – 114
PFNA	91 – 102	5	88 – 112	85 – 110	6	87 – 119
PFDA	97 – 103	5	89 – 118	76 – 115	10	84 – 112
PFUnA	91 – 107	5	92 – 111	83 – 102	5	91 – 117
PFDoA	73 – 120	12	88 – 119	83 – 105	6	77 – 141
PFTrDA	91 – 112	5	89 – 125	92 – 114	5	106 – 133
PFTeDA	94 – 104	5	92 – 110	76 – 103	7	91 – 111
PFBS	91 – 103	5	91 – 111	69 – 105	10	89 – 117
PFPeS	87 – 103	5	89 – 112	77 – 96	5	89 – 112
PFHxS	98 – 106	5	96 – 113	81 – 101	5	91 – 123
PFHpS	87 – 104	5	88 – 104	77 – 108	8	86 – 108
PFOS	95 – 108	5	94 – 115	98 – 112	6	97 – 124
PFNS	98 – 111	5	76 – 117	65 – 88	8	85 – 114
PFDS	83 – 102	5	84 – 107	82 – 94	5	78 – 110
PFDoS	76 – 99	7	77 – 100	73 – 96	7	29 – 108
4:2FTS	98 – 100	5	87 – 113	66 – 126	16	90 – 103
6:2FTS	94 – 123	7	60 – 166	77 – 105	8	92 – 119
8:2FTS	109 – 128	5	104 – 127	66 – 148	19	102 – 136
PFOSA	92 – 106	5	94 – 114	92 – 116	6	96 – 121
NMeFOSA	87 – 104	5	91 – 117	81 – 100	6	86 – 117
NEtFOSA	98 – 102	5	96 – 115	74 – 114	11	90 – 127
NMeFOSAA	91 – 107	5	90 – 113	89 – 136	10	93 – 117
NEtFOSAA	102 – 108	5	87 – 117	53 – 115	18	90 – 117
NMeFOSE	98 – 103	5	94 – 112	71 – 292	30	118 – 344
NEtFOSE	97 – 104	5	96 – 115	97 – 133	8	61 – 159
HFPO-DA	83 – 105	6	80 – 120	73 – 100	8	86 – 114
ADONA	85 – 96	5	76 – 124	82 – 95	5	86 – 132
PFMPA	91 – 98	5	85 – 117	78 – 93	5	86 – 109
PFMBA	88 – 97	5	85 – 120	74 – 104	8	84 – 117
NFDHA	53 – 103	16	58 – 136	49 – 86	14	56 – 115
9Cl-PF3ONS	84 – 100	5	79 – 131	69 – 98	9	95 – 126
11Cl-PF3OUdS	84 – 96	5	77 – 127	85 – 100	5	94 – 138
PFEESA	80 – 93	5	89 – 109	68 – 99	9	88 – 107
3:3FTCA	86 – 98	5	76 – 116	66 – 94	9	41 – 126
5:3FTCA	83 – 94	5	80 – 101	95 – 131	8	78 – 199
7:3FTCA	90 – 106	5	75 – 104	84 – 111	7	99 – 139
$^{13}C_4$-PFBA	92 – 99	5	95 – 109	93 – 97	5	95 – 105
$^{13}C_5$-PFPeA	86 – 106	5	80 – 110	85 – 108	6	89 – 103
$^{13}C_5$-PFHxA	83 – 101	5	92 – 106	79 – 111	9	88 – 98
$^{13}C_4$-PFHpA	87 – 102	5	90 – 100	88 – 93	5	80 – 102
$^{13}C_8$-PFOA	89 – 101	5	92 – 104	91 – 98	5	86 – 102
$^{13}C_9$-PFNA	86 – 101	5	90 – 106	91 – 104	5	89 – 101
$^{13}C_6$-PFDA	79 – 101	6	86 – 109	89 – 104	5	90 – 104

Table 5A Example Performance Data for Solids and Tissues

Compounds	Solid Matrix[1]			Tissue Matrix[1]		
	IPR		OPR Recovery (%)	IPR		OPR Recovery (%)
	Recovery (%)	RSD[2] (%)		Recovery (%)	RSD[2] (%)	
$^{13}C_7$-PFUnA	84 – 104	5	91 – 116	84 – 118	8	88 – 109
$^{13}C_2$-PFDoA	70 – 93	7	73 – 106	95 – 125	7	70 – 108
$^{13}C_2$-PFTeDA	83 – 88	5	74 – 107	81 – 114	9	10 – 110
$^{13}C_3$-PFBS	97 – 105	5	96 – 109	87 – 114	7	95 – 106
$^{13}C_3$-PFHxS	92 – 97	5	92 – 106	92 – 97	5	91 – 103
$^{13}C_8$-PFOS	87 – 107	5	95 – 109	87 – 93	5	95 – 103
$^{13}C_2$-4:2FTS	132 – 135	5	123 – 145	106 – 221	18	155 – 291
$^{13}C_2$-6:2FTS	118 – 129	5	104 – 138	87 – 135	11	117 – 149
$^{13}C_2$-8:2FTS	96 – 122	6	93 – 123	179 – 299	13	79 – 304
$^{13}C_8$-PFOSA	69 – 86	5	66 – 100	104 – 153	9	88 – 120
D_3-NMeFOSA	47 – 59	5	25 – 64	20 – 58	25	3 – 34
D_5-NEtFOSA	43 – 51	5	18 – 58	30 – 56	15	0 – 56**
D_3-NMeFOSAA	98 – 107	5	86 – 109	102 – 187	15	144 – 196
D_5-NEtFOSAA	98 – 104	5	85 – 109	178 – 216	5	175 – 223
D_7-NMeFOSE	50 – 61	5	35 – 76	3 – 5	12	0 – 8**
D_9-NEtFOSE	46 – 57	5	32 – 72	8 – 33	30	0 – 33**
$^{13}C_3$-HFPO-DA	98 – 108	5	83 – 125	87 – 106	5	81 – 106

[1] The data for these matrices were derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated to reflect the interlaboratory study results in a subsequent revision. Therefore, these criteria will change after interlaboratory validation. Laboratories may use these data as guidance is assessing their IPR and OPR results for solids and tissues.

[2] RSD values from the single-laboratory validation study that were les than 5% have all been raised to 5% for the purposes of this draft of the method.

** Statistically derived lower acceptance limits below 0% were set to 0% for the purposes of this table.

Table 6. Pooled MDL and ML Values for Aqueous Matrices and Example Solid and Tissue MDL and ML Values*

	Aqueous (ng/L)[1]		Solid (ng/g)[2]		Tissue (ng/g)[2]	
Compound	**Pooled MDLs**	**ML**	**MDLs**	**ML**	**MDLs**	**ML**
PFBA	0.80	2.0	0.40	0.8	0.59	2.0
PFPeA	0.53	2.0	0.02	0.4	0.08	1.0
PFHxA	0.48	2.0	0.02	0.2	0.10	0.5
PFHpA	0.39	2.0	0.03	0.2	0.09	0.5
PFOA	0.55	2.0	0.04	0.2	0.09	0.5
PFNA	0.46	2.0	0.09	0.2	0.16	0.5
PFDA	0.53	2.0	0.03	0.2	0.12	0.5
PFUnA	0.44	2.0	0.03	0.2	0.15	0.5
PFDoA	0.37	2.0	0.06	0.2	0.13	0.5
PFTrDA	0.46	2.0	0.04	0.2	0.09	0.5
PFTeDA	0.51	2.0	0.03	0.2	0.19	0.5
PFBS	0.37	2.0	0.01	0.2	0.07	0.5
PFPeS	0.53	2.0	0.02	0.2	0.03	0.5
PFHxS	0.56	2.0	0.02	0.2	0.08	0.5
PFHpS	0.87	2.0	0.06	0.2	0.04	0.5
PFOS	0.64	2.0	0.07	0.2	0.29	0.5
PFNS	0.49	2.0	0.05	0.2	0.11	0.5
PFDS	0.90	2.0	0.04	0.2	0.10	0.5
PFDoS	0.64	2.0	0.04	0.2	0.18	0.5
4:2FTS	1.74	5.0	0.28	0.8	0.74	2.0
6:2FTS	2.52	10	0.12	0.8	1.15	2.0
8:2FTS	2.58	10	0.23	0.8	0.37	2.0
PFOSA	0.32	2.0	0.07	0.2	0.09	0.5
NMeFOSA	0.41	2.0	0.05	0.2	0.16	0.5
NEtFOSA	0.43	2.0	0.04	0.2	0.17	0.5
NMeFOSAA	1.04	2.0	0.03	0.2	0.09	0.5
NEtFOSAA	0.80	2.0	0.04	0.2	0.14	0.5
NMeFOSE	3.93	10	0.20	2.0	9.98	5.0
NEtFOSE	5.13	20	0.25	2.0	1.50	5.0
HFPO-DA	1.54	5.0	0.14	0.8	0.16	2.0
ADONA	1.47	5.0	0.06	0.8	0.08	2.0
PFEESA	0.79	2.0	0.02	0.4	0.05	1.0
PFMPA	0.54	2.0	0.03	0.4	0.07	1.0
PFMBA	0.53	2.0	0.03	0.4	0.07	1.0
NFDHA	1.92	5.0	0.08	0.4	0.29	1.0
9Cl-PF3ONS	1.42	5.0	0.04	0.8	0.15	2.0
11Cl-PF3OUdS	1.78	5.0	0.07	0.8	0.31	2.0
3:3FTCA	2.54	10	0.06	1.0	0.25	2.5
5:3FTCA	9.92	20	0.36	5.0	1.54	12.5
7:3FTCA	9.14	20	0.31	5.0	0.85	12.5

* A standard containing a mixture of branched and linear isomer of suitable quality to be used for quantitation is currently available and required to be used for all calibration, calibration verifications, and QC samples. If more become commercially available for other target analytes, they must be utilized in the same manner.

[1] The pooled MDL and ML for aqueous matrices data are derived from the multi-laboratory validation study using data from eight laboratories for a total of 24 individual MDL studies and are therefore the limits required for this method.

[2] The MDL and ML values for solid and tissue matrices are example data from the single-laboratory validation study and are only provided as examples for this draft method.

Table 7. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
Target Analytes						
PFBA	1.96	212.8	168.9	NA	NA	$^{13}C_4$-PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	$^{13}C_5$-PFPeA
PFHxA	4.81	313.0	269.0	118.9	13	$^{13}C_5$-PFHxA
PFHpA	5.32	363.1	319.0	169.0	3.5	$^{13}C_4$-PFHpA
PFOA	6.16	413.0	369.0	169.0	3.0	$^{13}C_8$-PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	$^{13}C_9$-PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	$^{13}C_6$-PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	$^{13}C_7$-PFUnA
PFDoA	8.13	613.1	569.0	319.0	10	$^{13}C_2$-PFDoA
PFTrDA[2]	8.53	663.0	619.0	168.9	6.7	avg.$^{13}C_2$-PFTeDA and$^{13}C_2$-PFDoA
PFTeDA	8.96	713.1	669.0	168.9	6.0	$^{13}C_2$-PFTeDA
PFBS	4.79	298.7	79.9	98.8	2.1	$^{13}C_3$-PFBS
PFPeS	5.38	349.1	79.9	98.9	1.8	$^{13}C_3$-PFHxS
PFHxS	6.31	398.7	79.9	98.9	1.9	$^{13}C_3$-PFHxS
PFHpS	7.11	449.0	79.9	98.8	1.7	$^{13}C_8$-PFOS
PFOS	7.59	498.9	79.9	98.8	2.3	$^{13}C_8$-PFOS
PFNS	7.92	548.8	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDoS	9.14	699.1	79.9	98.8	1.9	$^{13}C_8$-PFOS
4:2FTS	4.67	327.1	307.0	80.9	1.7	$^{13}C_2$-4:2FTS
6:2FTS	5.81	427.1	407.0	80.9	1.9	$^{13}C_2$-6:2FTS
8:2FTS	7.28	527.1	507.0	80.8	3.0	$^{13}C_2$-8:2FTS
PFOSA	8.41	498.1	77.9	478.0	47	$^{13}C_8$-PFOSA
NMeFOSA	9.70	511.9	219.0	169.0	0.66	D_3-NMeFOSA
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D_5-NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D_3-NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D_5-N-EtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	$^{13}C_3$-HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	$^{13}C_3$-HFPO-DA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3.0	$^{13}C_3$-HFPO-DA
3:3FTCA	3.89	241.0	177.0	117.0	1.70	$^{13}C_5$-PFPeA
5:3FTCA	5.14	341.0	237.1	217.0	1.16	$^{13}C_5$-PFHxA
7:3FTCA	6.76	441.0	316.9	336.9	0.69	$^{13}C_5$-PFHxA
PFEESA	5.08	314.8	134.9	82.9	9.22	$^{13}C_5$-PFHxA
PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	$^{13}C_5$-PFHxA
Extracted Internal Standards						
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA		$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	4.18	268.3	223.0	NA		$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.80	318.0	273.0	120.3		$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	5.32	367.1	322.0	NA		$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	6.99	472.1	427.0	NA		$^{13}C_5$-PFNA

Table 7. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
$^{13}C_6$-PFDA	7.47	519.1	474.1	NA		$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	7.81	570.0	525.1	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	8.13	615.1	570.0	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	8.96	715.2	670.0	NA		$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	4.78	302.1	79.9	98.9		$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	6.30	402.1	79.9	98.9		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	7.59	507.1	79.9	98.9		$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	4.67	329.1	80.9	309.0		$^{18}O_2$-PFHxS
$^{13}C_2$-6:2FTS	5.82	429.1	80.9	409.0		$^{18}O_2$-PFHxS
$^{13}C_2$-8:2FTS	7.28	529.1	80.9	509.0		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOSA	8.41	506.1	77.8	NA		$^{13}C_4$-PFOS
D_3-NMeFOSA	9.70	515.0	219.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSA	9.94	531.1	219.0	NA		$^{13}C_4$-PFOS
D_3-NMeFOSAA	7.51	573.2	419.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSAA	7.65	589.2	419.0	NA		$^{13}C_4$-PFOS
D_7-NMeFOSE	9.56	623.2	58.9	NA		$^{13}C_4$-PFOS
D_9-NEtFOSE	9.83	639.2	58.9	NA		$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	4.97	286.9	168.9	184.9		$^{13}C_2$-PFHxA
Non-Extracted Internal Standards						
$^{13}C_3$-PFBA	1.95	216.0	172.0	NA		
$^{13}C_2$-PFHxA	4.80	315.1	270.0	119.4		
$^{13}C_4$-PFOA	6.16	417.1	172.0	NA		
$^{13}C_5$-PFNA	6.99	468.0	423.0	NA		
$^{13}C_2$-PFDA	7.47	515.1	470.1	NA		
$^{18}O_2$-PFHxS	6.30	403.0	83.9	NA		
$^{13}C_4$-PFOS	7.59	502.8	79.9	98.9		

[1] Times shown are in decimal minute units. Example retention times are based on the instrument operating conditions and column specified in Section 10.2.

[2] For improved accuracy, PFTrDA is quantitated using the average areas of the labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.

NA= These analytes do not produce a confirmation ion mass.

Table 8. QC Acceptance Limits for EIS Recoveries in Wastewater Samples

EIS Compound	Recovery Range (%)
$^{13}C_4$-PFBA	10 – 130 *
$^{13}C_5$-PFPeA	35 - 150
^{13}C5-PFHxA	55 - 150
$^{13}C_4$-PFHpA	55 - 150
$^{13}C_8$-PFOA	60 - 140
$^{13}C_9$-PFNA	55 - 140
$^{13}C_6$-PFDA	50 - 140
$^{13}C_7$-PFUnA	30 - 140
$^{13}C_2$-PFDoA	10 - 150
$^{13}C_2$-PFTeDA	10 – 130 *
$^{13}C_3$-PFBS	55 - 150
$^{13}C_3$-PFHxS	55 - 150
$^{13}C_8$-PFOS	45 - 140
$^{13}C_2$-4:2FTS	60 – 200 *
$^{13}C_2$-6:2FTS	60 - 200 *
$^{13}C_2$-8:2FTS	50 – 200 *
$^{13}C_8$-PFOSA	30 – 130
D_3-NMeFOSA	15 – 130
D_5-NEtFOSA	10 – 130
D_3-NMeFOSAA	45 – 200 *
D_5-NEtFOSAA	10 – 200
D_7-NMeFOSE	10 – 150 *
D_9-NEtFOSE	10 – 150 *
$^{13}C_3$-HFPO-DA	25 - 160

* In the multi-laboratory validation study data for wastewater matrices, some laboratories had difficulties achieving EIS recoveries in this range.

Table 8A. Range of Recoveries for Extracted Internal Standards (EIS) in the Single-laboratory Validation Study for Solids and Tissues

	Solid Sample Recovery (%)		Tissue Sample Recovery (%)	
EIS Compound	**Min**	**Max**	**Min**	**Max**
$^{13}C_4$-PFBA	3	113	84	99
$^{13}C_5$-PFPeA	28	112	86	107
^{13}C5-PFHxA	79	110	92	95
$^{13}C_4$-PFHpA	73	111	80	93
$^{13}C_8$-PFOA	86	115	90	95
$^{13}C_9$-PFNA	87	110	90	98
$^{13}C_6$-PFDA	87	112	83	97
$^{13}C_7$-PFUnA	66	124	71	91
$^{13}C_2$-PFDoA	26	109	54	96
$^{13}C_2$-PFTeDA	18	110	31	102
$^{13}C_3$-PFBS	89	120	89	98
$^{13}C_3$-PFHxS	87	110	98	99
$^{13}C_8$-PFOS	79	113	92	103
$^{13}C_2$-4:2FTS	95	248	192	215
$^{13}C_2$-6:2FTS	76	127	145	230
$^{13}C_2$-8:2FTS	86	173	136	220
$^{13}C_8$-PFOSA	61	123	87	96
D_3-NMeFOSA	28	86	8	38
D_5-NEtFOSA	21	70	8	30
D_3-NMeFOSAA	52	142	106	139
D_5-NEtFOSAA	68	151	79	151
D_7-NMeFOSE	13	107	5	30
D_9-NEtFOSE	16	97	0	29
$^{13}C_3$-HFPO-DA	70	119	93	102

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

Table 9. Summary of Quality Control

Method Reference	Requirement	Specification and Frequency
Section 10.1	Mass Calibration	Annually and on as-needed basis
Section 10.1.7	Mass Calibration Verification	After mass calibration
Section 10.3	Initial Calibration (ICAL)	Minimum 6 calibration standards for linear model and 7 calibration standards for non-linear models.
Sections 10.2.2, 14.4	Retention Time (RT) window	After ICAL and at the beginning of analytical sequence
Sections 7.3.1, 9.4	Extracted Internal Standard (EIS) Analytes	All CAL standards, batch QC and field samples
Sections 7.3.2	Non-extracted Internal Standards (NIS)	All CAL standards, batch QC and field samples
Sections 7.3.4, 10.3.1, 13.3	Instrument Sensitivity Check (ISC)	Daily, prior to analysis
Section 14.3	Calibration Verification (CV)	At the beginning of the analytical sequence (except for sample analyzed immediately after an initial calibration) and every 10 field sample injections
Section 14.6	Instrument Blank	Daily prior to analysis and after high standards
Sections 9.1.3, 9.5, 14.7	Method Blank (MB)	One per preparation batch
Section 14.5	Ongoing Precision Recovery (OPR)	One per preparation batch
Section 11.0	Limit of Quantitation Verification (LLOPR)	One per preparation batch
Section 11.0	Matrix Spike (MS/MSD)	One per preparation batch (if required)

Table 10. Range of Recoveries for Non-Extracted Internal Standards in the Single-laboratory Validation Study, by Matrix

	Aqueous			Solid			Tissue		
	% Recovery		RSD	% Recovery		RSD	% Recovery		RSD
NIS Compounds	Min	Max	(%)	Min	Max	(%)	Min	Max	(%)
$^{13}C_3$-PFBA	60	91	10.3	54	89	6.4	51	82	7.0
$^{13}C_2$-PFHxA	43	94	18.6	52	90	7.4	41	80	19.3
$^{13}C_4$-PFOA	59	87	9.7	54	89	6.4	51	82	9.5
$^{13}C_5$-PFNA	64	87	7.5	59	94	7.1	52	88	11.2
$^{13}C_2$-PFDA	57	86	10.0	55	91	8.6	47	85	19.4
$^{18}O_2$-PFHxS	59	87	9.6	53	87	7.1	51	80	8.1
$^{13}C_4$-PFOS	60	82	7.5	58	86	7.0	52	85	10.3

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

21.0 Glossary

These definitions and purposes are specific to this method, but have been conformed to common usage to the extent possible.

21.1 Units of weight and measure and their abbreviations

21.1.1 Symbols

ºC	degrees Celsius
Da	Dalton (equivalent to "amu" below)
µg	microgram
µL	microliter
µm	micrometer
<	less than
≤	less than or equal
>	greater than
≥	greater than or equal
%	percent
±	plus or minus

21.1.2 Alphabetical abbreviations

amu	atomic mass unit (equivalent to Dalton)
cm	centimeter
g	gram
h	hour
L	liter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
cm	centimeter
m/z	mass-to-charge ratio
ng	nanogram
Q1	quantitation ion
Q2	confirmation ion
rpm	revolutions per minute
v/v	percent volume per volume

21.2 Definitions and acronyms (in alphabetical order)

Analyte – A PFAS compound included in this method. The analytes are listed in Table 1.

Calibration standard (CS) – A solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the LC-MS/MS instrument.

Calibration verification standard (CV) – The mid-point calibration standard (CS-4) that is used to verify calibration. See Table 4.

CFR – Code of Federal Regulations

Compound – One of many variants or configurations of a common chemical structure. Individual compounds are identified by the number of carbon atoms and functional group attached at the end of the chain.

Confirmation Ion – For the purpose of this method, the confirmation ion is produced by collisionally activated dissociation of a precursor ion to produce distinctive ions of smaller *m/z* than the precursor.

Class A glassware – Volumetric glassware that provides the highest accuracy. Class A volumetric glassware complies with the Class A tolerances defined in ASTM E694, must be permanently labeled as Class A, and is supplied with a serialized certificate of precision.

CWA – Clean Water Act

Extracted internal standard (EIS) – An isotopically labeled analog of a target analyte that is structurally identical to a native (unlabeled) analyte. The EISs are added to the sample at the beginning of the sample preparation process and are used to quantify the native target analytes.

Extracted internal standard (EIS) quantification – The process of determining the concentration of the native target analyte by its comparing response to the response of a structurally related isotopically labeled analog that was added to the sample at the beginning of the sample preparation process.

LC – Liquid chromatograph or liquid chromatography

Instrument sensitivity check – solution used to check the sensitivity of the instrument. The solution contains the native compounds at the concentration of the LOQ.

Internal standard – A labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of native PFAS compounds other than the compound of which it is a labeled analog. See Internal standard quantitation.

Internal standard quantitation – A means of determining the concentration of (1) a native compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Isotope dilution (ID) quantitation – A means of determining a native compound by reference to the same compound in which one or more atoms has been isotopically enriched. The labeled PFAS are spiked into each sample and allow identification and correction of the concentration of the native compounds in the analytical process.

Isotopically labeled compound – An analog of a target analyte in the method which has been synthesized with one or more atoms in the structure replaced by a stable (non-radioactive) isotope of that atom. Common stable isotopes used are ^{13}C (Carbon-13) or Deuterium (D or ^{2}H). These labeled compounds do not occur in nature, so they can be used for isotope dilution quantification or other method-specific purposes.

Limit of Quantitation (LOQ) – The smallest concentration that produces a quantitative result with known and recorded precision and bias. The LOQ shall be set at or above the concentration of the lowest initial calibration standard (the lowest calibration standard must fall within the linear range).

Low-level OPR (LLOPR) – A version of the ongoing precision and recovery standard that is spiked at twice the concentration of the laboratory's LOQ and used as a routine check of instrument sensitivity.

Method blank – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and labeled compounds that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method Detection Limit (MDL) – The minimum measured concentration of a substance that can be reported with 99% confidence that the measured analyte concentration is distinguishable from method blank results (40 CFR 136, Appendix B).

MESA – Mining Enforcement and Safety Administration

Minimum level of quantitation (ML) – The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. The ML represents the lowest concentration at which an analyte can be measured with a known level of confidence. It may be equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. Alternatively, the ML may be established by multiplying the MDL (pooled or unpooled, as appropriate) by 3.18 and rounding the result to the number nearest to 1, 2, or 5 x 10^n, where n is zero or an integer (see 68 FR 11770).

MS – Mass spectrometer or mass spectrometry

Matrix Spike/Matrix Spike Duplicate (MS/MSD) – Aliquots of field samples that have been fortified with a known concentration of target compounds, prior to sample preparation and extraction, and analyzed to measure the effect of matrix interferences. The use of MS/MSD samples is generally not required in isotope dilution methods because the labeled compounds added to every sample provide more performance data than spiking a single sample in each preparation batch.

Multiple reaction monitoring (MRM) – Also known as selected reaction monitoring (SRM). A type of mass spectrometry where a parent mass of the compound is fragmented through MS/MS and then specifically monitored for a single fragment ion.

Must – This action, activity, or procedural step is required.

NIOSH – The National Institute of Occupational Safety and Health

Non-extracted internal standard (NIS) –Labeled PFAS compounds spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the LC-MS/MS.

OPR – Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that

the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

PFAS – Per- and Polyfluoroalkyl substances –A group of man-made fluorinated compounds that are hydrophobic and lipophobic, manufactured and used in a variety of industries globally. These compounds are persistent in the environment as well as in the human body. This method analyzes for the PFAS listed in Table 1.

Precursor Ion – For the purpose of this method, the precursor ion is the deprotonated molecule ([M-H]-) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*. Also called a parent ion.

Product Ion – For the purpose of this method, a product ion is a charged fragment ion that is formed as the product of collisionally activated dissociation of a particular precursor ion. Also called a transition or transition ion.

Reagent water – Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation multiplied by 100 and divided by the mean. Also termed "coefficient of variation."

Relative Standard Error (RSE) – The standard error of the mean divided by the mean and multiplied by 100.

RF – Response factor. See Section 10.3.3.2.

RR – Relative response. See Section 10.3.3.2.

RT – Retention time**;** the time it takes for an analyte or labeled compound to elute off the HPLC/UPLC column

Should – This action, activity, or procedural step is suggested but not required.

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the mean height of the noise.

SPE – Solid-phase extraction; a technique in which an analyte is extracted from an aqueous solution or a solid/tissue extract by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, NIST, or a source that will attest to the purity and authenticity of the reference material.

Appendix A - Sample Pre-screening Instructions

Samples that are known or suspected to contain high levels of analytes may be pre-screened using the following procedure. These are example procedures using smaller sample aliquots spiked with EIS and NIS and no clean up procedures. Other pre-screening procedures may be used. The results of the pre-screening should be used by the analyst to assess the need for sample or extract dilutions necessary to keep the target analytes within the calibration range of the instrument. The results may also be used to reduce the risk of prevent gross contamination of the instrument when dealing with unfamiliar sources of samples.

Aqueous Samples

1. Weight out 10 (±0.1) g of sample into a 50-mL centrifuge tube.
2. Add 50 µL of EIS and NIS to the sample and vortex to mix.
3. Filter 1 mL of the sample through 0.2-µm membrane filter into a microvial. Sample is ready for instrumental analysis.

Solid and Tissue Samples

1. Weigh 1.0 (±0.1) g sample into 50-mL polypropylene centrifuge tubes.
2. Add 20 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Vortex and mix on a shaker table (or equivalent) for 10 min. Allow to settle and/or centrifuge to produce a clear extract.
3. Filter using a Single Step® filter vial:
   a. Add 20 µL of EIS to a clean Single Step® filter vial (chamber).
   b. Add 400 µL of clear extract from step 2 (e.g., by adding extract until it reaches the fill line), carefully vortex to mix.
   c. Use filter/plunger part and filter.
4. Transfer 30 µL of filtrate to a ~300-µL polypropylene micro-vial and dilute to 300 µL with 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Add NIS to the filtrate.
5. The extract is now a 10x dilution.
6. Sample is ready for instrumental analysis.

Calculate results using the equivalent sample weight computed as follows:

$$Equivalent\ Weight = Sample\ weight\ (g) \times \frac{0.4\ mL}{20\ mL}$$

Note that the EIS concentration in the diluted portion is 0.5x the level in the regular analysis of solid samples.

Appendix B - Aqueous Sample Subsampling Instructions

Warning: Because some target analytes may be stratified within the sample (e.g., AFFF-contaminated media, surfactants), or adhere to the walls of the sample container, subsampling may only be done on a project-specific basis. Subsampling has been shown to increase uncertainty in PFAS analysis, especially on foaming samples.

If a reduced sample size is required, transfer a weighed subsample using the following subsampling procedure to a 60-mL HDPE bottle and dilute to approximately 60 mL using reagent water. This container is now considered the "sample bottle."

1. Gently invert sample 3-4 times being careful to avoid foam formation and subsample immediately (do not let stand).

2. If foam forms and more than 5 mL is required – pour sample, avoiding any foam.

3. If foaming forms and a volume less than 5 mL is required – pipette from ½ cm below the foam.

4. If no foam forms – pour or pipette based on volume required.

Attachment 16

Appendix E, Table A Noncarcinogenic Health Effects

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
Inorganics					
7429-90-5	Aluminum	1.00E+00	PPRTV	Minimal neurotoxicity in the offspring of mice	Neurological
7440-36-0	Antimony	4.00E-04	IRIS	Longevity, blood glucose, and cholesterol	Whole body
7440-38-2	Arsenic	3.00E-04	IRIS	Hyperpigmentation, keratosis and possible vascular complications	Skin and Blood
7440-39-3	Barium	2.00E-01	IRIS	Nephropathy	Neurological
7440-41-7	Beryllium	2.00E-03	IRIS	Small intestine lesions	Gastrointestinal
7440-42-8	Boron	2.00E-01	IRIS	Decreased fetal weight (developmental)	Body Weight
7440-43-9	Cadmium	1.00E-04	ATSDR	No effects	Renal
16887-00-6	Chloride	**----**	**----**	**----**	**----**
7440-47-3	Chromium (total)	----	----	----	**----**
7440-48-4	Cobalt	3.00E-04	PPRTV	Decreased iodine uptake	Thyroid
7440-50-8	Copper	4.00E-02	HEAST	Irritation	Gastrointestinal
143-33-9	Cyanide	1.00E-03	IRIS	Decreased cauda epididymis weight in male F344/N rats	Testes
7681-49-4	Fluoride	5.00E-02	ATSDR	Increased prevalence of bone fractures	Skeletal
7439-89-6	Iron	7.00E-01	PPRTV	Adverse gastrointestinal effects	Gastrointestinal
7439-92-1	Lead	**----**	**----**	**----**	**----**
7439-93-2	Lithium	2.00E-03	PPRTV	Adverse effects (renal effects, neurological effects thyroid function, reproductive (male), developmental)	Several Organs and Systems
7439-96-5	Manganese	1.40E-01	IRIS	CNS effects	Nervous

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
7487-94-7	Mercury (mercuric chloride)	3.00E-04	IRIS	Autoimmune effects (autoimmune glomerulonephritis)	Immune, Urinary
7439-98-7	Molybdenum	5.00E-03	IRIS	Increased uric acid levels	Urinary
7440-02-0	Nickel	2.00E-02	IRIS	Decreased body and organ weights	Body Weight
14797-55-8	Nitrate as N	1.60E+00	IRIS	Early clinical signs of methemoglobinemia in excess of 10% (0-3 months old infants formula)	Blood
14797-73-0	Perchlorate	7.00E-04	IRIS	Radioactive iodide uptake inhibition (RAIU) in the thyroid	Thyroid
7440-14-4	Radium (combined 226+228)	----	----	----	----
7782-49-2	Selenium	5.00E-03	IRIS	Clinical selenosis	Whole Body
7440-22-4	Silver	5.00E-03	IRIS	Argyria	Skin
14808-79-8	Sulfate	----	----	----	----
	TDS (total dissolved solids)	----	----	----	----
7440-28-0	Thallium	1.00E-05	SCREEN	Histopathology	Skin
7440-62-2	Vanadium	7.00E-05	PPRTV	Kidney Histopathology	Kidney
7440-66-6	Zinc	3.00E-01	IRIS	Decreases in erythrocyte Cu, Zn-superoxide dismutase (ESOD) activity in healthy adult male and female volunteers	Blood
Organics					
83-32-9	Acenaphthene	6.00E-02	IRIS	Hepatotoxicity	Liver
67-64-1	Acetone	9.00E-01	IRIS	Neuropathy	Neurological
15972-60-8	Alachlor	1.00E-02	IRIS	Hemosiderosis, hemolytic anemia	Blood
116-06-3	Aldicarb	1.00E-03	IRIS	Sweating as clinical sign of AChe inhibition (other effect: Nausea, diarrhea, and other signs and	Whole Body

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
				symptoms. Clinical signs and symptoms of acetylcholinesterase inhibition including sweating, pinpoint pupils, leg weakness, and other effects.)	
120-12-7	Anthracene	3.00E-01	IRIS	No observed effects (assumed threshold for cellular necrosis)	Whole Body
1912-24-9	Atrazine	3.00E-03	ATSDR	Delayed estrus onset	Reproductive
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)	8.00E-03	ATSDR	Slight microscopic liver damage	Hepatic
71-43-2	Benzene	4.00E-03	IRIS	Decreased lymphocyte count	Blood
56-55-3	Benzo(a)anthracene	----	----	----	----
205-99-2	Benzo(b)fluoranthene	----	----	----	----
207-08-9	Benzo(k)fluoranthene	----	----	----	----
50-32-8	Benzo(a)pyrene	3.00E-04	IRIS	Neurobehavioral changes	Developmental
65-85-0	Benzoic acid	4.00E+00	IRIS	No observed effects (assumed threshold for cellular necrosis)	Whole Body
78-93-3	2-Butanone (methyl ethyl ketone)	6.00E-01	IRIS	Decreased pup body weight	Body Weight
1563-66-2	Carbofuran	5.00E-03	IRIS	Red blood cell and plasma cholinesterase inhibition, and testicular and uterine effects	Blood, Testes, and Uterus
75-15-0	Carbon disulfide	1.00E-01	IRIS	Fetal toxicity/malformations	Whole Body
56-23-5	Carbon tetrachloride	4.00E-03	IRIS	Elevated serum SDH activity	Blood
12789-03-6	Chlordane	5.00E-04	IRIS	Hepatic necrosis	Liver
108-90-7	Chlorobenzene	2.00E-02	IRIS	Histopathologic changes in liver	Liver
67-66-3	Chloroform	1.00E-02	IRIS	Moderate/marked fatty cyst formation in the liver and elevated SGPT	Liver

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
218-01-9	Chrysene	----	----	----	----
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)	1.00E-02	IRIS	Hematologic, hepatic and renal toxicity	Blood, Liver, and Kidney
75-99-0	Dalapon	3.00E-02	IRIS	Increased kidney body weight ratio	Kidney
53-70-3	Dibenzo(a,h)anthracene	----	----	----	----
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)	2.00E-04	PPRTV	Testicular effects	Testicle
1918-00-9	Dicamba	3.00E-02	IRIS	Maternal and fetal toxicity	Whole Body
95-50-1	*o*-Dichlorobenzene (1,2-dichlorobenzene)	9.00E-02	IRIS	No observed effects (assumed threshold for cellular necrosis)	Whole Body
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)	7.00E-02	ATSDR	Increased serum alkaline phosphatase and liver weight; hepatocellular hypertrophy	Hepatic
75-71-8	Dichlorodifluoromethane	2.00E-01	IRIS	Reduced body weight	Body Weight
75-34-3	1,1-Dichloroethane	2.00E-01	PPRTV	Renal injury	Kidney
107-06-2	1,2-Dichloroethane	6.00E-03	SCREEN	Greater than 10 percent increase in relative kidney weight	Kidney
75-35-4	1,1-Dichloroethylene	5.00E-02	IRIS	Liver toxicity (fatty change)	Liver
156-59-2	*cis*-1,2-Dichloroethylene	2.00E-03	IRIS	increased relative kidney weight in male rats	Kidney
156-60-5	*trans*-1,2-Dichloroethylene	2.00E-02	IRIS	Decrease in number of antibody forming cells (AFCs) agianst sheep red blood cels (sRBCs) in male mice	Red Blood Cells
75-09-2	Dichloromethane (methylene chloride)	6.00E-03	IRIS	hepatic effects (hepatic vacuolation, liver foci)	Liver

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
78-87-5	1,2-Dichloropropane	4.00E-02	PPRTV	Delayed skeletal ossification of skull bones	Skeletal
117-81-7	Di(2-ethylhexyl)phthalate	2.00E-02	IRIS	Increased relative liver weight	Liver
84-66-2	Diethyl phthalate	8.00E-01	IRIS	Decreased growth rate, food consumption and altered organ weights	Whole Body
84-74-2	Di-*n*-butyl phthalate	1.00E-01	IRIS	Increased mortality	Whole Body
99-65-0	1,3-Dinitrobenzene	1.00E-04	IRIS	Increased splenic weight	Spleen
121-14-2	2,4-Dinitrotoluene	2.00E-03	IRIS	Neurotoxicity, Heinz bodies and biliary tract hyperplasia	Nervous, Hepatic, Hematologic
606-20-2	2,6-Dinitrotoluene	3.00E-04	SCREEN	Increased incidence of spenic extramedullary hematopoiesis	Spleen
88-85-7	Dinoseb	1.00E-03	IRIS	Decreased fetal weight	Body Weight
123-91-1	1,4-Dioxane (*p*-dioxane)	3.00E-02	IRIS	Liver and kidney toxicity	Liver and Kidney
145-73-3	Endothall	2.00E-02	IRIS	Increased absolute and relative weights of stomach and small intestine	Stomach and Small Intestine
72-20-8	Endrin	3.00E-04	IRIS	Mild histological lesions in liver, occasional convulsions	Liver
100-41-4	Ethylbenzene	5.00E-02	PPRTV	Centrilobular hepatocyte hypertrophy	Liver
106-93-4	Ethylene dibromide (1,2-dibromoethane)	9.00E-03	IRIS	Testicular atrophy, liver peliosis, and adrenal cortical degeneration	Testes, Liver, and Adrenal Gland
206-44-0	Fluoranthene	4.00E-02	IRIS	Nephropathy, increased liver weights, hematological alterations, and clinical effects	Whole Body

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
86-73-7	Fluorene	4.00E-02	IRIS	Decreased RBC, packed cell volume and hemoglobin	Blood
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)	1.00E-05	ATSDR	Changes in cell- and humoral-mediated immune system	Immune
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	3.00E-06	DWSHA	Adverse liver effects (parental females)	Liver
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	5.00E-02	IRIS	Hepatic lesions	Liver
76-44-8	Heptachlor	1.00E-04	ATSDR	Suppression of immune response to sheep RBC in offspring	Immune
1024-57-3	Heptachlor epoxide	1.30E-05	IRIS	Increased liver-to-body weight ratio in both males and females	Liver
77-47-4	Hexachlorocyclopentadiene	6.00E-03	IRIS	Chronic irritation	Gastrointestinal
193-39-5	Indeno(1,2,3-c,d)pyrene	----	----	----	----
98-82-8	Isopropylbenzene (cumene)	1.00E-01	IRIS	Increased average kidney weights in female rats	Kidney
93-65-2	MCPP (mecoprop)	1.00E-03	IRIS	Increased absolute and relative kidney weights	Kidney
1634-04-4	MTBE (methyl tertiary-butyl ether)	7.00E-02	IEPA	increased surem cholesterol; perisistent diarrhea	Liver, Gastrointestinal
72-43-5	Methoxychlor	5.00E-03	IRIS	Excessive loss of litters	Whole Body
90-12-0	1-Methylnaphthalene	7.00E-02	ATSDR	Increased incidence of pulmonary alveolar proteinosis	Respiratory
91-57-6	2-Methylnaphthalene	4.00E-03	IRIS	Pulmonary alveolar proteinosis	Lungs

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
95-48-7	2-Methylphenol (*o*-cresol)	5.00E-02	IRIS	Decreased body weights and neurotoxicity	Whole Body
91-20-3	Naphthalene	2.00E-02	IRIS	Decreased mean terminal body weight in males	Body Weight
98-95-3	Nitrobenzene	2.00E-03	IRIS	Increased methemoglobin levels	Blood
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)	----	----	----	----
375-73-5	PFBS (perfluorobutanesulfonic acid)	3.00E-04	PPRTV	Decreased serum total T4 in newborn (PND1) mice	Thyroid
355-46-4	PFHxS (perfluorohexanesulfonic acid)	2.00E-05	ATSDR	Hypertrophy and hyperplasia of thyroid follicular cells in males	Endocrine
375-95-1	PFNA (perfluorononanoic acid)	3.00E-06	ATSDR	Delayed postnatal development [eye opening, preputial separation and vaginal opening] and decreased body weight gain in males; decreased postnatal survival; full litter resorptions at 10 mg/kg/day	Developmental
335-67-1	PFOA (perfluorooctanoic acid)	3.00E-06	ATSDR	Altered femur and tibial bone morphology, decreased tibial mineral density; Increased locomotor activity in adult offspring	Developmental
1763-23-1	PFOS (perfluorooctanesulfonic acid)	2.00E-06	ATSDR	Delayed eye opening and transient decrease in F2 pup body weight (13%) on LDs 7-14 at greater or equal to 4 mg/kg/day; decreased pup	Developmental

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
				survival to postpartum day 21 at greater than or equal to 1.6 mg/kg/day	
87-86-5	Pentachlorophenol	5.00E-03	IRIS	hepatotoxicity	Liver
108-95-2	Phenol	3.00E-01	IRIS	Decreased maternal weight gain	Body Weight
1918-02-1	Picloram	7.00E-02	IRIS	Increased liver weights	Liver
129-00-0	Pyrene	3.00E-02	IRIS	Kidney effects (renal tubular pathology, decreased kidney weights)	Kidney
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	4.00E-03	IRIS	Convulsions in F344 rats	Nervous System
122-34-9	Simazine	5.00E-03	IRIS	Reduction in weight gains; hematological changes in females	Body Weight
100-42-5	Styrene	2.00E-01	IRIS	Red blood cell and liver effects	Blood and Liver
118-96-7	TNT (2,4,6-trinitrotoluene)	5.00E-04	IRIS	Liver effects	Liver
93-72-1	2,4,5-TP (silvex)	8.00E-03	IRIS	Histopathological changes in the liver	Liver
127-18-4	Tetrachloroethylene	6.00E-03	IRIS	Neurotoxicity (color vision) (reaction time, cognitive effects)	Nervous System
108-88-3	Toluene	8.00E-02	IRIS	Increased kidney weight	Kidney
8001-35-2	Toxaphene	9.00E-05	PPRTV	Cytoplasmic vacuolation	Thyroid
120-82-1	1,2,4-Trichlorobenzene	1.00E-02	IRIS	Increased adrenal weights; vacuolization of zona fasciculata in the cortex	Adrenal Gland
71-55-6	1,1,1-Trichloroethane	2.00E+00	IRIS	Reduced body weight	Whole Body
79-00-5	1,1,2-Trichloroethane	4.00E-03	IRIS	Clinical serum chemistry	Whole Body
79-01-6	Trichloroethylene	5.00E-04	IRIS	Increased fetal cardiac malformations in Sprague-Dawley rats	Heart
75-69-4	Trichlorofluoromethane	3.00E-01	IRIS	Survival and histopathology	Whole Body

CASRN	Constituent Name	Oral Reference Dose (RfD) mg/kg-day	Toxicity Source	RfD Critical Effect	RfD Target Organ
99-35-4	1,3,5-Trinitrobenzene	3.00E-02	IRIS	Methemoglobinemia and spleen-erythroid cell hyperplasia	Blood and Spleen
75-01-4	Vinyl chloride	3.00E-03	IRIS	Liver cell polymorphism	Liver
1330-20-7	Xylenes	2.00E-01	IRIS	Decreased body weight, increased mortality	Other

Primary Source:

U.S. EPA Regional Screening Levels (RSL) Calculator Oral Reference Dose Metadata (November 2022 updates).
Available at: https://www.epa.gov/risk/regional-screening-levels-rsls

Other Sources:

HFPO-DA ((hexafluoropropylene oxide dimer acid, GenX): U.S. EPA Office of Water Final Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). October 2021. Included as Attachment 1D 7, of the December 7, 2021, Initial Filing.

MTBE (methyl tertiary-butyl ether): Illinois EPA Notice of Health Advisory for Methyl Tertiary-Butyl Ether. Environmental Register No. 484, July 1994.

Vanadium: Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Provisional Peer-Reviewed Toxicity Values for Vanadium and Its Soluble Inorganic Compounds Other Than Vanadium Pentoxide (CASRN 7440-62-2 and Others). September 2009. Included as Attachment 1D 2, of the December 7, 2021, Initial Filing.

Appendix E, Table B Carcinogenic Health Effects

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
Inorganics					
7429-90-5	Aluminum	----	----	----	----
7440-36-0	Antimony	----	----	----	----
7440-38-2	Arsenic	1.50E+00	IRIS	Skin cancer	Skin
7440-39-3	Barium	----	----	----	----
7440-41-7	Beryllium	----	----	----	----
7440-42-8	Boron	----	----	----	----
7440-43-9	Cadmium	----	----	----	----
16887-00-6	Chloride	----	----	----	----
7440-47-3	Chromium (total)	----	----	----	----
7440-48-4	Cobalt	----	----	----	----
7440-50-8	Copper	----	----	----	----
143-33-9	Cyanide	----	----	----	----
7681-49-4	Fluoride	----	----	----	----
7439-89-6	Iron	----	----	----	----
7439-92-1	Lead	----	----	----	----
7439-93-2	Lithium	----	----	----	----
7439-96-5	Manganese	----	----	----	----
7487-94-7	Mercury (mercuric chloride)	----	----	----	----
7439-98-7	Molybdenum	----	----	----	----
7440-02-0	Nickel	----	----	----	----
14797-55-8	Nitrate as N	----	----	----	----
14797-73-0	Perchlorate	----	----	----	----
7440-14-4	Radium (combined 226+228)	----	----	----	----
7782-49-2	Selenium	----	----	----	----
7440-22-4	Silver	----	----	----	----

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
14808-79-8	Sulfate	----	----	----	----
	TDS (total dissolved solids)	----	----	----	----
7440-28-0	Thallium	----	----	----	----
7440-62-2	Vanadium	----	----	----	----
7440-66-6	Zinc	----	----	----	----
Organics					
83-32-9	Acenaphthene	----	----	----	----
67-64-1	Acetone	----	----	----	----
15972-60-8	Alachlor	5.60E-02	CALEPA	nasal trubinate tumors	Nasal
116-06-3	Aldicarb	----	----	----	----
120-12-7	Anthracene	----	----	----	----
1912-24-9	Atrazine	----	----	----	----
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)	6.30E+00	IRIS	Hepatic nodules and hepatocellular carcinomas	Liver
71-43-2	Benzene	5.50E-02	IRIS	Leukemia	Blood
56-55-3	Benzo(a)anthracene	1.00E-01	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
205-99-2	Benzo(b)fluoranthene	1.00E-01	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
207-08-9	Benzo(k)fluoranthene	1.00E-02	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
50-32-8	Benzo(a)pyrene	1.00E+00	IRIS	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
65-85-0	Benzoic acid	----	----	----	----
78-93-3	2-Butanone (methyl ethyl ketone)	----	----	----	----
1563-66-2	Carbofuran	----	----	----	----
75-15-0	Carbon disulfide	----	----	----	----

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
56-23-5	Carbon tetrachloride	7.00E-02	IRIS	Hepatocellular adenoma or carcinoma	Liver
12789-03-6	Chlordane	3.50E-01	IRIS	Carcinoma	Liver
108-90-7	Chlorobenzene	----	----	----	----
67-66-3	Chloroform	3.10E-02	CALEPA	renal tubular cell tumors	Kidney
218-01-9	Chrysene	1.00E-03	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)	----	----	----	----
75-99-0	Dalapon	----	----	----	----
53-70-3	Dibenzo(a,h)anthracene	1.00E+00	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)	8.00E-01	PPRTV	Renal tubular cell adenoma or carcinoma	Kidney
1918-00-9	Dicamba	----	----	----	----
95-50-1	*o*-Dichlorobenzene (1,2-dichlorobenzene)	----	----	----	----
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)	5.40E-03	CALEPA	Hepatocellular carcinomas and adenomas	Liver
75-71-8	Dichlorodifluoromethane	----	----	----	----
75-34-3	1,1-Dichloroethane	----	----	----	----
107-06-2	1,2-Dichloroethane	9.10E-02	IRIS	Hemangiosarcomas	Blood
75-35-4	1,1-Dichloroethylene	----	----	----	----
156-59-2	*cis*-1,2-Dichloroethylene	----	----	----	----
156-60-5	*trans*-1,2-Dichloroethylene	----	----	----	----
75-09-2	Dichloromethane (methylene chloride)	2.00E-03	IRIS	Hepatocellular carcinomas or adenomas	Liver

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
78-87-5	1,2-Dichloropropane	3.70E-02	PPRTV	Hepatocellular adenoma or carcinoma	Liver
117-81-7	Di(2-ethylhexyl)phthalate	1.40E-02	IRIS	Hepatocellular carcinoma and adenoma	Liver
84-66-2	Diethyl phthalate	----	----	----	----
84-74-2	Di-*n*-butyl phthalate	----	----	----	----
99-65-0	1,3-Dinitrobenzene	----	----	----	----
121-14-2	2,4-Dinitrotoluene	3.10E-01	CALEPA	Liver and mammary tumors	Liver, Mammary Glands
606-20-2	2,6-Dinitrotoluene	1.50E+00	PPRTV	Hepatocellular carcinomas	Liver, Mammary Glands
88-85-7	Dinoseb	----	----	----	----
123-91-1	1,4-Dioxane (*p*-dioxane)	1.00E-01	IRIS	Hepatocellular adenoma and carcinoma	Liver
145-73-3	Endothall	----	----	----	----
72-20-8	Endrin	----	----	----	----
100-41-4	Ethylbenzene	1.10E-02	CALEPA	Renal tumors	Kidney
106-93-4	Ethylene dibromide (1,2-dibromoethane)	2.00E+00	IRIS	Forestomach tumors, hemangiosarcomas, thyroid follicular cell adenomas or carcinomas	Forestomach and Thyroid
206-44-0	Fluoranthene	----	----	----	----
86-73-7	Fluorene	----	----	----	----
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)	1.10E+00	CALEPA	Liver tumors	Liver

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	----	----	----	----
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	----	----	----	----
76-44-8	Heptachlor	4.50E+00	IRIS	Hepatocellular carcinomas	Liver
1024-57-3	Heptachlor epoxide	9.10E+00	IRIS	Hepatocellular carcinomas	Liver
77-47-4	Hexachlorocyclopentadiene	----	----	----	----
193-39-5	Indeno(1,2,3-c,d)pyrene	1.00E-01	IRIS/RPF	forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal
98-82-8	Isopropylbenzene (cumene)	----	----	----	----
93-65-2	MCPP (mecoprop)	----	----	----	----
1634-04-4	MTBE (methyl tertiary-butyl ether)	----	----	----	----
72-43-5	Methoxychlor	----	----	----	----
90-12-0	1-Methylnaphthalene	----	----	----	----
91-57-6	2-Methylnaphthalene	----	----	----	----
95-48-7	2-Methylphenol (*o*-cresol)	----	----	----	----
91-20-3	Naphthalene	----	----	----	----
98-95-3	Nitrobenzene	----	----	----	----
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)	2.00E+00	IRIS	Liver hepatocellular adenomas, carcinomas, cholangiomas, or cholangiocarcinomas	Liver
375-73-5	PFBS (perfluorobutanesulfonic acid)	----	----	----	----

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
355-46-4	PFHxS (perfluorohexanesulfonic acid)	----	----	----	----
375-95-1	PFNA (perfluorononanoic acid)	----	----	----	----
335-67-1	PFOA (perfluorooctanoic acid)	1.43E+02	CALEPA	hepatocellular and pancreatic tumors	Liver, Pancreas
1763-23-1	PFOS (perfluorooctanesulfonic acid)	----	----	----	----
87-86-5	Pentachlorophenol	4.00E-01	IRIS	hepatocellular adenomas or carcinomas and adrenal benign or malignant pheochromocytomas	Liver
108-95-2	Phenol	----	----	----	----
1918-02-1	Picloram	----	----	----	----
129-00-0	Pyrene	----	----	----	----
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	----	----	----	----
122-34-9	Simazine	----	----	----	----
100-42-5	Styrene	----	----	----	----
118-96-7	TNT (2,4,6-trinitrotoluene)	----	----	----	----
93-72-1	2,4,5-TP (silvex)	----	----	----	----
127-18-4	Tetrachloroethylene	2.10E-03	IRIS	Hepatocellular adenomas or carcinomas	Liver
108-88-3	Toluene	----	----	----	----
8001-35-2	Toxaphene	1.10E+00	IRIS	Hepatocellular carcinomas and neoplastic nodules	Liver
120-82-1	1,2,4-Trichlorobenzene	----	----	----	----
71-55-6	1,1,1-Trichloroethane	----	----	----	----

CASRN	Constituent Name	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	Toxicity Source	SF_o Tumor Type	SF_o Target Organ
79-00-5	1,1,2-Trichloroethane	----	----	----	----
79-01-6	Trichloroethylene	4.60E-02	IRIS	Renal cell carcinoma, non-Hodgkin's lymphoma, and liver tumors (derived from IUR)	Kidney, Liver
75-69-4	Trichlorofluoromethane	----	----	----	----
99-35-4	1,3,5-Trinitrobenzene	----	----	----	----
75-01-4	Vinyl chloride	7.20E-01	IRIS	Total of liver angiosarcoma, hepatocellular carcinoma, and neoplastic nodules	Liver
1330-20-7	Xylenes	----	----	----	----

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