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 RESPONSES TO QUESTIONS** a copy of which is served upon you.

Respectfully submitted,

Dated: April 29, 2024

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY,

BY: /s/ Sara Terranova

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THIS FILING IS SUBMITTED ELECTRONICALLY

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ILLINOIS ENVIRONMENTAL PROTECTION AGENCY'S RESPONSES TO QUESTIONS

NOW COMES the Illinois Environmental Protection Agency (Illinois EPA or Agency), by and through one of its attorneys, and submits the following responses to questions:

Board Question 1. *Does the Agency have a response to participants' concerns of potential contamination of groundwater samples resulting from well sampling instruments and equipment and may be composed of Teflon or PFAS-containing plastics? Is there a need for additional requirements for sampling instruments and equipment? If so, please propose rule language.*

Agency Response 1.
The Agency believes these concerns have already been addressed. The Agency has already proposed for inclusion in the revisions to Part 620 the following documents as incorporations by reference in 620.125:

"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) ASTM D7979-20.

U.S. EPA, Office of Ground Water and Drinking Water, Standards and
Risk Management Division.
"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,"
November 2019.
https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf.

U.S. EPA, Office of Research and Development, Center for
Environmental solutions & Emergency Response
Shoemaker, J. and Dan Tettenhorst, 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.
Environmental Protection Agency, Office of Research and

Development, Center for Environmental Assessment, Washington,
DC. Version 2.0, March 2020.

Each of these methods has a section covering proper sample collection, preservation, and storage. Each method also requires Field Reagent Blanks be collected and analyzed to assess potential contamination in the field during sample collection. Therefore, following the procedures that have already been proposed for incorporation into Part 620 will address cross-contamination concerns.

The Agency provides the Board with the following draft language for consideration, to clarify which sampling and analytical procedures must be used when collecting PFAS-chemical samples.

620.510(b)(3)(C)

When sampling for Hexafluoropropylene oxide dimer acid (HFPO-DA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), the incorporations by reference in 620.125 that are applicable for sample collection, preservation, storage and analysis are:
"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) ASTM D7979-20.

U.S. EPA, Office of Ground Water and Drinking Water, Standards and
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"Method 533: Determination of Per- and Polyfluoroalkyl Substances
in Drinking Water by Isotope Dilution Anion Exchange Solid Phase
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Water by Solid Phase Extraction and Liquid
Chromatography/Tandem Mass spectrometry (LC/MS/MS). U.S.
Environmental Protection Agency, Office of Research and
Development, Center for Environmental Assessment, Washington,
DC. Version 2.0, March 2020.

Board Question 2.
In light of U.S. EPA's proposed drinking water MCL for PFOS of 4 ppt, the Board invites comment from IEPA on whether the proposed PFOS GWQS should be revised to 4 ppt.

Agency Response 2.
Illinois EPA agrees the proposed PFOS standard should be revised from 7.7 ppt to U.S. EPA's drinking water MCL of 4 ppt. Since Illinois EPA proposed its noncarcinogen standard in 2021, U.S. EPA and World Health Organization's International Agency for Research on Cancer (IARC) designated PFOS a carcinogen and U.S. EPA determined there is no safe level of the chemical in drinking water, setting the MCLG at zero. On April 10, 2024, U.S. EPA finalized a PFOS MCL of 4 ppt. As stated at First Notice (p. 2), once U.S. EPA finalizes its PFAS MCL proposal, the Board will propose amendments to Part 611 consistent with the federal rules. Part 620 Class I groundwater quality standards are based on MCLs if they are available. The appropriate PFOS standard is the U.S. EPA MCL of 4 ppt (0.000004 mg/L).

Board Question 3.
Please comment on the Board's proposal of setting the PFOA standard at 4 ppt, rather than 2 ppt.

Agency Response 3.
Illinois EPA agrees the proposed PFOA standard should be revised from 2 ppt to 4 ppt based on U.S. EPA's drinking water MCL, finalized April 10, 2024. As stated at First Notice (p. 2), once U.S. EPA finalizes its PFAS MCL proposal, the Board will propose amendments to Part 611 consistent with the federal rules. Part 620 Class I groundwater quality standards are based on MCLs if they are available. The appropriate PFOA standard is the U.S. EPA MCL of 4 ppt (0.000004 mg/L).

Board Question 4.
Please comment on the use of U.S. EPA's HBWC of 10 ppt as the basis for the Board's proposed PFNA GWQS of 12 ppt.

Agency Response 4.
When calculating health-based groundwater quality standards with Part 620, Subpart F and Appendix A methods, Illinois EPA extends the standard to two significant digits. Significant digits are simply the nonzero digits of a number. U.S. EPA extends its HBWCs to one significant digit. To be consistent with the HBWC for the U.S. EPA MCL hazard index calculation, Illinois EPA accepts presenting the standard with one significant digit (10 ppt or 0.00001 mg/L).

Board Question 5.
Please address the concerns raised by participants regarding the thyroid effects of PFHxS and whether the proposed standard should be based on U.S. EPA's HBWC.

Agency Response 5.
ATSDR reviewed four studies to determine an appropriate intermediate MRL for PFHxS, not a single study, as referenced by 3M in P.C. No. 56. Please refer to ATSDR's "Toxicological Profile for Perfluoroalkyls" (2021), for the studies evaluated to determine its PFHxS MRL. ATSDR is within U.S. EPA's toxicity hierarchy. Further, Illinois EPA notes U.S. EPA selected the ATSDR MRL, adjusted with an additional UF of 10 for a subchronic to chronic extrapolation, as its toxicological value to calculate a HBWC for the MCL hazard index calculation, finalized April 10, 2024. In its selection of thyroid alterations from the Buttenhoff, et al. 2009a study, ATSDR

noted a weakness in the study was the lack of thyroid hormone level measurements, which would have assisted in evaluating the alterations to the thyroid gland.

The American Chemistry Council noted increases in liver weight and hepatocellular hypertrophy may be related to activation of PPARα. ATSDR agrees histopathologic alterations in the thyroid may be the result of liver effects; however, not enough data was known to make that determination or to tie the thyroid alterations to PPARα. More recently, U.S. EPA's Integrated Risk Information System (IRIS), U.S. EPA's Tier 1 toxicity source, released its Draft Toxicological Profile for external peer-review and public comment in July 2023. This document is included as Attachment A. The draft profile selected an RfD of 2E-07 mg/kg-day, based on decreased total T4 hormone in Wistar rats. The Executive Summary of the draft profile states, "Overall, the available evidence indicates that PFHxS exposure is likely to cause thyroid and developmental immune effects in humans, given sufficient exposure conditions. For thyroid effects, the primary supporting evidence for this hazard conclusion included evidence of decreased thyroid hormone levels, abnormal histopathology results, and changes in organ weight in experimental animals." (xiv of Executive Summary). Therefore, additional studies confirm thyroid effects from PFHxS.

In addition, 3M discussed ATSDR's selection of a conservative elimination half-life ($t^{1/2}$) of 3,100 days (8.5 years), stating the selected half-life, based on a study of a group of retired fluorochemical production workers observed for a five-year period, is overly conservative for an exposed community. Illinois EPA agrees with ATSDR's selection of a human population of exposed workers following retirement is an appropriate population for determining an elimination half-life for PFHxS.

Illinois EPA agrees an additional UF of 10 should be applied to ATSDR's MRL to adjust the value for a subchronic to chronic duration. The revised MRL for chronic exposure is 2E-06 mg/kg-day. As stated at First Notice (page 2), once U.S. EPA finalizes its PFAS MCL proposal, the Board will propose amendments to Part 611 consistent with the federal rules. Part 620 Class I groundwater quality standards are based on MCLs if they are available. As U.S. EPA finalized its MCL hazard index calculation, Illinois EPA agrees the proposed standard should be based on U.S. EPA's HBWC for the U.S. EPA MCL hazard index calculation (10 ppt or 0.00001 mg/L).

Board Question 6.
Please address participants' concerns as to why the proposed HFPO-DA standard of 12 ppt is higher than U.S. EPA's HBWC of 10 ppt.

Agency Response 6.
When calculating health-based groundwater quality standards with Part 620, Subpart F and Appendix A methods, Illinois EPA extends the standard to two significant digits. U.S. EPA extends its HBWCs to one significant digit. To be consistent with the HBWC for the U.S. EPA MCL hazard index calculation, Illinois EPA accepts presenting the standard with one significant digit (10 ppt or 0.00001 mg/L).

Board Question 7.
Please provide additional justification to support the adoption of the proposed Class II standard of 0.05 mg/L for molybdenum as related to the beneficial use for irrigation of

crops and produce.

Agency Response 7.
The Class II standard of 0.05 mg/L for molybdenum is based on toxicity of animals from forage grown in soils with short term use of irrigation water. This value was selected from the National Academy of Sciences "Water Quality Criteria" (1972), which states, "Kubota et al. (1963) found that molybdenum concentrations of 0.01 mg/L or greater in soil solutions were associated with animal toxicity levels of this element in alsike clover." The United States Department of Agriculture notes at https://www.nrcs.usda.gov/sites/default/files/2022-12/AlsikeClover.pdf that alsike clover is "often grown in combination with other grasses for hay or pasture" and "is very palatable to all grazing animals." According to the Illinois Department of Natural Resources at https://dnr.illinois.gov/education/exoticshome/exoticherbaceous.html, alsike clover in Illinois "may be found statewide in roadsides, fields, and areas of disturbed soil."

Further, as discussed in Attachment 8 of Illinois EPA's May 6, 2022, pre-filed responses, the majority of soils in the state have pH levels between 6.0 and 7.5. Page 4,858 of the Initial Filing of PCB 2022-018 states, "the ability of the soil to inactivate molybdenum decreases with increase in pH, such that the amount of this element that could be added without producing excesses was higher in acidic soils." Therefore, in soils in the neutral to alkaline pH range, as in the majority of soils in Illinois, the capacity of the soil to remove or inactivate molybdenum is decreased. According to footnote (d) on page 4,858 of the Initial Filing of PCB 2022-018, the 0.05 mg/L value chosen for the Class II standard applies "for only acid fine textured soils or acid soils with relatively high iron oxide contents." Since the pH of most soils in Illinois is predominantly neutral, the capacity of the soil to remove or inactivate molybdenum is decreased. Upon further review of a comment regarding whether the proposed Class II standard was representative of Illinois soils, the Illinois EPA agrees that the standard for molybdenum should be 0.01 mg/L based on livestock toxicity. This value is less than the proposed health-based Class I groundwater quality standard of 0.019 mg/L. Therefore, both the Class I and Class II molybdenum standards should be 0.01 mg/L. The footnote accompanying the proposed Class I standard should be changed from ("c") to ("h") to reflect the Class I standard is based on beneficial use for livestock.

Board Question 8.
Please comment on whether the 2,500 feet setback zone maximum should be included as Class I groundwater under Section 620.210(a)(5).

Agency Response 8.
The Agency has reviewed the testimony provided to the Board at the March 19, 2022, public hearing regarding the definition of Class I groundwater under 620.210(a)(5) and the interplay of maximum setback zones and wellhead protection areas (WHPAs) as defined in 620.110.

The Agency believes, based on the requirements under Section 14.3(f) of the Illinois Environmental Protection Act (Act) (415 ILCS 5/14.3(f) for adoption of a 2,500-foot maximum setback zone and the definitions of Class I groundwater under 620.210(a)(2) and 620.210(a)(4), it is reasonable to include an adopted 2,500-foot maximum setback zone as Class I groundwater. Because the definition of a WHPA specifically describes them as outside of applicable setback zones, the Agency recommends the following changes to 620.210(a) to clarify the Class I groundwater designation in maximum setback zones.

a) Groundwater located 10 feet or more below the land surface and within:

> 1) The minimum setback zone of a well which serves as a potable water supply and to the bottom of the well;
>
> 2) Unconsolidated sand, gravel, or sand and gravel which is 5 feet or more in thickness and that contains 12% ~~percent~~ or less of fines (i.e., fines which pass through a No. 200 sieve tested according to ASTM Standard Practice D2487-06, incorporated by reference at Section 620.125);
>
> 3) Sandstone which is 10 feet or more in thickness, or fractured carbonate which is 15 feet or more in thickness; ~~or~~
>
> 4) Any geologic material which is capable of a:
>
> > A) Sustained groundwater yield, from up to a 12-inch borehole, of 150 gallons per day or more from a thickness of 15 feet or less; or
> >
> > B) Hydraulic conductivity of 1 x 10-4 cm/sec or greater using one of the following test methods or its equivalent:
> >
> > > i) Slug test; or ~~Permeameter;~~
> > > ii) Pump test ~~Slug test; or~~
> > > ~~iii) Pump test.~~
>
> 5) The Phase I and Phase II wellhead protection area of a community water supply well or well field, as defined in Section 620.110 and delineated according to the methods incorporated by reference in Section 620.125. ~~For the purposes of this Subpart, when a maximum setback zone has been adopted under Section 14.3 of the Act, the WHPA includes the delineated area within the maximum setback zone.~~
>
> 6) The maximum setback zone of a community water supply well adopted under Section 14.3 of the Act.

Board Question 9.
It is the Board's understanding that IEPA will address impacts of the proposed PFAS GWQS to landfills and other programs in separate, future rulemakings. Can the Agency provide any details regarding its timeline on this issue?

Agency Response 9. Once amendments to Part 620 are adopted, the Agency will identify and develop amendments needed in other rules address the impacts of the proposed PFAS GWQS to landfills and other Agency programs. It is an iterative process that requires multiple steps. The Agency has formulated internal working groups related to Bureau of Land program rules that will analyze these rules to evaluate the extent of amendments required. At this time, the Agency cannot provide a firm timeline as to specific proposed rulemakings but anticipates amending Parts 740 and 742 with certainty. Should any of the hazardous waste management facilities (Parts 702 through 750 generally) or solid waste disposal facilities (Parts 807, 811 through 817 generally) regulations require amendment in response to the revised Part 620 standards, then those rulemakings proposals will be prepared accordingly. Regarding hazardous waste regulations that are identical-in-substance, the Agency will review those as part of determining potential implementation impacts from the adoption of the Part 620 amendments, but the Agency does not foresee proposing any changes to those rules given the Board's identical-in-substance mandate under the Act. The specific timing of any of these proposed rule packages will depend upon Agency staffing resource availability, stakeholder outreach discussions, and the priority order established by Agency management.

Board Question 10.
The Board proposes striking the comparison of the total concentration of Atrazine plus Atrazine metabolites to the Atrazine standard of 0.003 mg/L in Section 620.410(c)(2) as the Table in subsection (c)(2) lists the applicable standards. Please comment on this change.

Agency Response 10.
The table in Section 620.410(c)(2) should not be stricken. The U.S. EPA has adopted a MCL for Atrazine only, not its metabolites. However, based on the documents submitted by the Agency in support of this proposal, the three listed Atrazine metabolites have been found in Illinois groundwater, and have health effects similar to Atrazine at concentrations equal to or below that of Atrazine. Those health effects are developmental delays and adverse effects on the reproductive system, liver, kidney, and heart. Therefore, a Class I groundwater standard equal to Atrazine's Class I groundwater standard (based on the U.S. EPA MCL) is reasonable for Desethyl-atrazine (DEA), Desisopropyl-atrazine (DIA), or Diaminochlorotriazine (DACT), or any combination of the three. It also follows therefore, given the health effects of the Atrazine and the Atrazine metabolites, that any combination of Atrazine and Atrazine metabolites should have a Class I groundwater quality standard, as one or more of these four constituents may be present in a sample.

WHEREFORE, the Illinois EPA asks the Board to accept these Responses to the Board Questions.

Respectfully submitted,
ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,

Dated: April 29, 2024

BY: /s/ Sara Terranova

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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 RESPONSES TO QUESTIONS** 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 April 29, 2024.

/s/ Sara Terranova

April 29, 2024

Attachment A

EPA/635/R-23/148a
External Review Draft
www.epa.gov/iris

IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 335-46-4) and Related Salts

July 2023

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

ADHD	attention deficit hyperactivity disorder
AIC	Akaike's information criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service registry number
CHO	Chinese hamster ovary (cell line cells)
CPHEA	Center for Public Health and Environmental Assessment
CL	confidence limit
CNS	central nervous system
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DDEF	data-derived extrapolation factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GLP	good laboratory practices
GSH	glutathione
GST	glutathione-S-transferase
HBCD	hexabromocyclododecane
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
ORD	Office of Research and Development
osRfD	organ-specific reference dose
PBPK	physiologically based pharmacokinetic
PFHxS	perfluorohexanesulfonic acid
PND	postnatal day
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SEM	Systematic Evidence Map
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOS	Web of Science

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Agency Review

This assessment was provided for review to scientists in EPA's program and regional offices. Comments were submitted by: Office of Air and Radiation (OAR), Office of Air Quality and Standards (OAQPS), Office of Land and Emergency Management (OLEM), Office of Children's Health Protection (OCHP), Office of Water, Region 1, Region 3, Region 4, and Region 8.

Interagency Review

This assessment was provided for review to other federal agencies and the Executive Office of the President (EOP). Comments were submitted by: The National Institute for Occupational Safety and Health (NIOSH), Department of Defense (DoD), National Institute of Environmental Health Sciences (NIEHS), Council on Environmental Quality (CEQ), Department of Health and Human Services (HHS), National Institute of Health (NIH), and the Centers for Disease Control and Prevention (CDC)/Agency for Toxic Substance and Disease Registry (ATSDR).

EXECUTIVE SUMMARY

Perfluorohexanesulfonic acid (PFHxS, CASRN 355-46-4)[1], and its related salts (such as potassium perfluorohexanesulfonate [PFHxS-K, CASRN 3871-99-6], ammonium perfluorohexanesulfonate [PFHxS-NH_4, CASRN 68259-08-5], and sodium perfluorohexanesulfonate [PFHxS-Na, CASRN 82382-12-5]), are members of the group per- and polyfluoroalkyl substances (PFAS). This assessment applies to PFHxS as well as nonmetal and alkali metal salts of PFHxS that would be expected to fully dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body) and not release other moieties that would cause toxicity independent of PFHxS. The synthesis of evidence and toxicity value derivation presented in this assessment focuses on the free acid of PFHxS and its potassium, sodium, and ammonium salts given the currently available toxicity data.

Concerns about PFHxS and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment. PFAS are not naturally occurring; they are man-made compounds that have been used widely over the past several decades in industrial applications and consumer products as many PFAS are resistant to heat and are used to confer resistance of products (e.g., textiles) to stains by repelling oil, grease, and water. PFAS are also used in a wide range of other applications, including as electrical insulation and to confer frictionless coatings onto surfaces. PFAS in the environment are found at industrial sites, military fire training areas, wastewater treatment plants, and found in commercial products (see Appendix A, Section 2.1.2).

The Integrated Risk Information System (IRIS) Program is developing a series of five PFAS assessments (i.e., perfluorohexane sulfonate [PFHxS], perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts) (see December 2018 IRIS Program Outlook) at the request of EPA national programs and regions. Specifically, the development of human health toxicity assessments for exposure to these individual PFAS represents only one component of the broader PFAS strategic roadmap at the EPA (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024). The systematic review protocol (see Appendix A) for these five PFAS assessments outlines the related scoping and problem-formulation efforts, including a summary of other federal and state assessments of PFHxS. The protocol also describes the systematic review

1 The CASRN given here is for linear PFHxS; the source of PFHxS used in toxicity studies was reported to be 98% pure and reagent grade, generally giving this CASRN. None of the studies referenced in this assessment explicitly state that only the linear form was used. Therefore, there is the possibility that a minor proportion of the PFHxS used in the studies were branched isomers and thus observed health effects may apply to the total linear and branched isomers in a given exposure source.

and dose-response methods used to conduct this review (see also Section 1.2). In addition to these ongoing IRIS PFAS toxicity assessments, EPA's Office of Research and Development is carrying out several other activities related to PFAS, including the creation of PFAS systematic evidence maps (SEMs) (Carlson et al., 2022; Radke et al., 2022)and consolidating and updating PFAS data on chemical and physical properties, human health toxicity, and pharmacokinetics, as well as ecotoxicity.

Human epidemiological studies have examined possible associations between PFHxS exposure and health outcomes including immune responses, birth weight, hematopoietic effects, thyroid hormone effects, liver enzyme effects, serum lipids effects, cardiovascular disease, hematological effects, reproductive effects, neurodevelopmental effects, and cancer. The ability to draw conclusions from the epidemiological evidence for the assessed health outcomes is limited (apart from immune effects) by the overall quality and lack of consistency in the available studies.

Animal studies of PFHxS exposure exclusively examined the oral exposure route; therefore, no inhalation assessment was conducted nor was an inhalation reference concentration (RfC) derived (see Section 5.2.3). The available animal studies of oral PFHxS exposure examined a variety of noncancer endpoints, including those relevant to the thyroid, immune system, developmental effects, hematopoietic system, hepatic effects, cardiometabolic effects, reproductive (male and female) system, nervous system, and renal effects. Some limitations in the animal database include the types of studies identified (e.g., few subchronic studies and no chronic exposure studies were available), and few studies per health outcome.

Overall, the available **evidence indicates** that PFHxS exposure is likely to cause thyroid and developmental immune effects in humans, given sufficient exposure conditions. For thyroid effects, the primary supporting evidence for this hazard conclusion included evidence of decreased thyroid hormone levels, abnormal histopathology results, and changes in organ weight in experimental animals. For immune effects, the primary supporting evidence included decreased antibody responses to vaccination against tetanus or diphtheria in children. Selected quantitative data from these identified hazards were used to derive toxicity values (see Table ES-1; see Sections 3.2.1 and 3.2.2 for evidence synthesis and integration analyses).

Evidence primarily from epidemiological studies **suggests** but is insufficient to infer that PFHxS exposure might affect fetal development, specifically resulting in decreased birth weight (see Section 3.2.3). However, due to limitations and uncertainties in the currently available studies, a hazard could not be clearly identified, and these data were not considered for use in deriving toxicity values. While no reference dose (RfD) was derived for developmental effects, a point of departure (POD) was derived and presented for comparison purposes (see Section 5.2.1).

In addition, evidence from human and animal studies **suggests** but is insufficient to infer that PFHxS exposure may cause hepatic, neurodevelopmental, and cardiometabolic effects in humans.

Lastly, although evidence from humans and or animals was also identified for hematopoietic, reproductive, renal, and carcinogenic effects, the currently available **evidence is inadequate** to assess whether PFHxS exposure may be capable of causing these health effects in humans, and these outcomes were not considered for use in deriving toxicity values.

Table ES-1. Health effects with evidence available to synthesize and draw summary judgments and derived toxicity values

Organ/ System	Evidence Integration judgment	Toxicity value	Value (mg/kg-d)	Confidence	UFA	UFH	UFS	UFL	UF D	UFC	Basis
Immune (i.e., developmental immune)	**Evidence indicates (likely)**	Lifetime osRfD	**2×10^{-10} (RfD)**	*Medium*	1	10	1	1	3	30	Decreased serum anti-tetanus antibody concentration in children at age 7 yrs (Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012)
		Subchronic osRfD	2×10^{-10}	*Medium*	1	10	1	1	3	30	Decreased serum anti-tetanus antibody concentration in children at age 7 yrs (Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012)
Thyroid	**Evidence indicates (likely)**	Lifetime osRfD	1×10^{-7}	*Medium*	3	10	1	1	3	100	Decreased serum total T4 levels in F1 Wistar rats (Ramhøj et al., 2018)
		Subchronic osRfD	1×10^{-7}	*Medium*	3	10	1	1	3	100	Decreased serum total T4 levels in Wistar rats (Ramhøj et al., 2018)

RfD = reference dose (in mg/kg-d) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposure; osRfD = organ-/system-specific reference dose (in mg/kg-d); UFA = animal to human uncertainty factor; UFC = composite uncertainty factor; UFD = evidence base deficiencies uncertainty factor; UFH = human variation uncertainty factor; UFL = LOAEL to NOAEL uncertainty factor; UFS = subchronic to chronic uncertainty factor.

ES.1 LIFETIME AND SUBCHRONIC ORAL REFERENCE DOSE (RfD) FOR NONCANCER EFFECTS

From the identified hazards with sufficient qualitative and quantitative information to support the derivation of candidate lifetime values (i.e., immune and thyroid), decreased serum anti-tetanus antibody concentrations in children (male and female) (Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012) was selected as the basis for the oral RfD of 4×10^{-10} mg/kg-day. A $BMDL_{½SD}$ of 2.82×10^{-4} mg/L in serum was identified for this endpoint and was used as the $POD_{Internal}$. The human equivalent dose POD (POD_{HED}) of 1.16×10^{-8} mg/kg-day was derived by multiplying the $POD_{Internal}$ by the human clearance of 4.1×10^{-5} L/kg-day to estimate human equivalent doses from an internal dose. The overall RfD for PFHxS was calculated by dividing the POD_{HED} by a composite uncertainty factor of 30 to account for interindividual differences in human susceptibility (UF_H = 10) and deficiencies in the toxicity evidence base (UF_D = 3). The immune organ-/system-specific osRfD is based on the lowest overall POD_{HED} and UF_C; therefore, the selected RfD based on decreased serum anti-tetanus antibody concentration in children (a susceptible lifestage for this effect) is considered protective of the observed health effects associated with lifetime PFHxS exposure. The selection considered both available osRfDs as well as the overall confidence and composite uncertainty for those osRfDs. The thyroid osRfD was based on application of a composite uncertainty threefold greater than that applied in deriving the immune osRfD (UF_c = 100 for thyroid versus UF_c = 30 for developmental immune effects). Further, when comparing the sensitivity of thyroid and immune osRfDs, the thyroid value is 500-fold higher than the developmental immune endpoint. Selection of the RfD on the basis of developmental immune effects is presumed to be protective of possible thyroid and other potential adverse health effects (including potential effects on birth weight) in humans. Finally, since the developmental immune osRfD is based on effects observed in males and females, the overall RfD would be protective for both sexes. The same study (Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012) endpoint (decreased serum anti-tetanus antibody concentration in children) and value were selected as the basis for the subchronic RfD of 4×10^{-10} mg/kg-day.

ES.2 CONFIDENCE IN THE ORAL REFERENCE DOSE (RFD) AND SUBCHRONIC RFD

The overall confidence in the RfD and subchronic RfD is *medium* and is driven by *medium* confidence in the overall evidence base for immune effects, *medium* confidence in the Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012) study (HAWC link), and *medium* confidence in quantitation of the POD (see Section 5.2. and Table 5-8).

ES.3 NONCANCER EFFECTS FOLLOWING INHALATION EXPOSURE

No studies that examine toxicity in humans or experimental animals following inhalation exposure are available and no acceptable physiologically based pharmacokinetic (PBPK) models are available to support route-to-route extrapolation; therefore, no RfC was derived.

ES.4 EVIDENCE FOR CARCINOGENICITY

Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), EPA concluded there is ***inadequate information to assess carcinogenic potential*** for PFHxS by either the oral or inhalation routes of exposure. This conclusion is based on the lack of adequate data to inform the potential carcinogenicity of PFHxS in the database. This precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS

A series of five PFAS assessments (Perfluorohexanesulfonic acid [PFHxS], perfluorohexanoic acid [PFHxA], perfluorobutanoic acid [PFBA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts; see December 2018 IRIS Outlook) is being developed by the Integrated Risk Information System (IRIS) Program at the request of the U.S. Environmental Protection Agency (EPA) national programs and regions. Appendix A is the systematic review protocol for these five PFAS assessments. The protocol outlines the scoping and problem-formulation efforts relating to these assessments, including a summary of other federal and state reference values for PFHxS. The protocol also lays out the systematic review and dose-response methods used to conduct this review (see also Section 1.2). This systematic review protocol was released for public comment in November 2019 and was subsequently updated based on those public comments. Appendix A includes a link to the updated protocol, including a summary of the updates in the protocol history section (see Section 12). In addition to these ongoing IRIS PFAS toxicity assessments, EPA's Office of Research and Development is carrying out several other activities related to PFAS, including creation of PFAS systematic evidence maps (SEMs) and consolidating and updating PFAS data on chemical and physical properties, human health toxicity, and pharmacokinetics, as well as ecotoxicity.

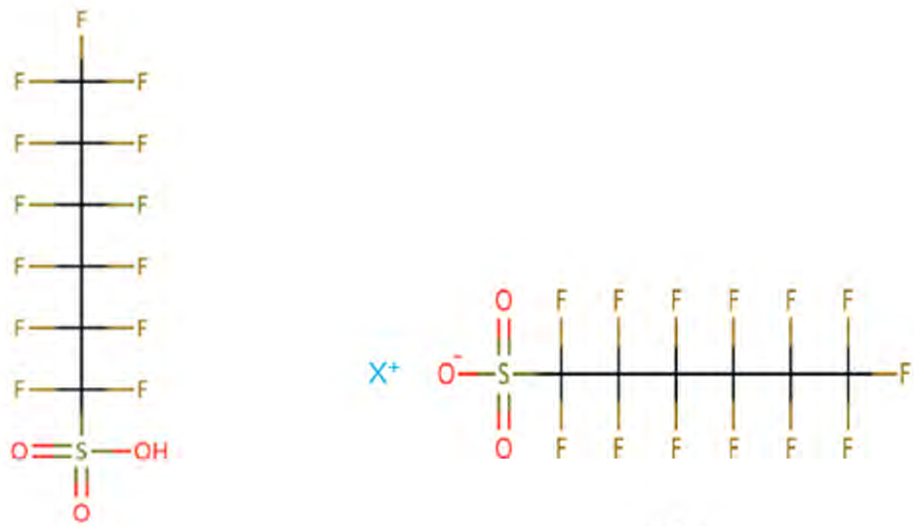
1.1. BACKGROUND INFORMATION ON PERFLUOROHEXANESULFONIC ACID (PFHxS)

Section 1.1 provides a brief overview of aspects of the physicochemical properties, human exposure, and environmental fate characteristics of perfluorohexanesulfonic acid (PFHxS; CASRN 335-46-4), and its related salts that might provide useful context for this assessment. This overview is not intended to provide a comprehensive description of the available information on these topics. The reader is encouraged to refer to the source materials cited below, more recent publications on these topics, and authoritative reviews or assessments focused on these topics.

1.1.1. Physical and Chemical Properties

PFHxS and its related salts such as potasium, sodium, and ammonium PFHxS salts covered in this assessment are members of the group per- and polyfluoroalkyl substances (PFAS). Buck et al. (2011) defines PFAS as fluorinated substances that "contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety

C_nF_{2n+1}–).” More specifically, PFHxS is classified as a perfluoroalkane sulfonic acid [PFSA; (OECD, 2015)]. PFSAs containing six or more perfluorinated carbons are considered long-chain PFASs (ATSDR, 2018b; OECD, 2015; Buck et al., 2011). Thus, PFHxS is a long-chain PFAS. The chemical structures of PFHxS[2] and its related salts are presented in Figure 1-1. The physical-chemical properties of PFHxS and related salts are provided in Table 1-1.



PFHxS
355-46-4

PFHxS
related salts

Figure 1-1. Chemical structure of PFHxS and related salts (see https://comptox.epa.gov/dashboard/). X represents the cations for potassium (CASRN 3871-99-6), sodium (CASRN 82382-12-5), and ammonium (CASRN 68259-08-5).

[2] While this figure shows the linear chemical structures, the assessment may also apply to other non-linear isomers of PFHxS and related salts as described in the Executive Summary.

Table 1-1. Physical-chemical properties of PFHxS and related salts[a]

Property (unit)	Value			
	PFHxS 355-46-4[b]	PFHxS Potassium salt 3871-99-6[c]	PFHxS Ammonium salt 68259-08-5[c]	PFHxS Sodium salt 82382-12-5[c]
Molecular weight (g/mol)	400	438	417c*	422*
Melting point (°C)	190	273	111*	217*
Boiling point (°C)	246	303*	228 *	238*
Density (g/cm^3)	1.84*	1.84*	1.84*	1.84*
Vapor pressure (mm Hg)	8.10×10^{-9}	8.19×10^{-9}*	8.19×10^{-9}*	8.19×10^{-9}*
Henry's law constant (atm-m^3/mol)	1.94×10^{-10}*	1.94×10^{-10}*	1.94×10^{-10}*	1.94×10^{-10}*
Water solubility (mol/L)	6.08×10^{-4}[d]	3.52×10^{-2}*	6.10×10^{-4}*	7.03×10^{-2}*
pKa	0.14*	ND	ND	ND
LogP	2.20[d]	2.71*	3.48 *	2.91*
Soil adsorption coefficient (L/kg)	2,300*	2,300*	2,300*	2,300*
Bioconcentration factor (BCF)	175*	271*	271*	5.94*

[a]This information is provided as part of a general overview providing background context only and should not be used for decision purposes. Up-to-date primary references should be consulted.
[b]CompTox Chemicals Dashboard (U.S. EPA, 2018a) for all values except pKa. The value of pKa was obtained from ECHA: https://echa.europa.eu/documents/10162/1f48372e-97dd-db9f-4335-8cec7ae55eee. Questions and corrections to the CompTox Chemicals Dashboard can be submitted at: https://comptox.epa.gov/dashboard/.
[c] (U.S. EPA, 2018a). Questions and corrections to the CompTox Chemicals Dashboard can be submitted at: https://comptox.epa.gov/dashboard/.
[d]As of April 2023 these values are indicated as 'experimental' in the CompTox Chemicals Dashboard (U.S. EPA, 2018a); however, they appear to be predicted values based on the citations provided, and therefore may be more uncertain. Note that these values are not used for dosimetric extrapolation in this assessment, which was based on available empirical pharmacokinetic data (see Section 3.1.7).
*Average predicted value. These values are more uncertain and, in general, less reliable than experimental values.
ND= No data

1.1.2. Sources, Production, and Use

PFAS are not naturally occurring in the environment (ATSDR, 2018a). They are man-made compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. PFHxS has been used as a surfactant to make fluoropolymers, and in water- and stain-protective coatings for carpets, paper, packaging, and textiles (Norwegian Environment Agency, 2018; NTP, 2018c). It may also be present in certain industrial and consumer products, such as electronics, industrial fluids, "food-contact papers, water-proofing agents, cleaning and polishing products either for intentional uses (as surfactants or surface protection agents) or as unintentional impurities from industrial production processes" (Norwegian Environment Agency, 2018). It has also been used in aqueous film-forming foam (AFFF) for fire suppression (Laitinen et al., 2014).

EPA has been working with companies in the fluorochemical industry since the early 2000s to phase out the production and use of long-chain PFAS such as PFHxS (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfass). However, in addition to the environmental persistence of PFHxS (see below), products containing PFHxS are still in use and may be imported into the United States; thus, there may continue to be a source of environmental contamination due to disposal or breakdown in the environment (Kim and Kannan, 2007).

No chemical reporting data on production volume are available in EPA's ChemView (U.S. EPA, 2019a) for PFHxS or its salts. As part of the National Defense Authorization Act for Fiscal Year 2020 (see Section 7321), 172 per- and polyfluoroalkyl substances including PFHxS were added to the EPA's Toxic Release Inventory (TRI) list (https://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals). The reporting requirements apply to a de minimus limit of 1% and a manufacture, process, or otherwise use threshold of 100 lbs. Currently, there is incomplete quantitative information available in EPA's Toxic Release Inventory or other informational repositories regarding PFHxS releases to the environment from facilities that manufacture, process, use imported/previously manufactured products that contain, or dispose of imported/previously manufactured products containing PFHxS.

1.1.3. Environmental Fate and Transport

PFAS, including PFHxS, are very stable and persistent in the environment (ATSDR, 2018a; Harbison et al., 2015), and many are found worldwide in the environment, wildlife, and humans (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfass). Long-chain PFAS have been found at sites, including private and federal facilities, and have been associated with various sources, including AFFF for fire suppression, and PFAS manufacturers and industries that use PFAS (e.g., textiles) (ATSDR, 2018a). Various long chain PFAS have estimated half-lives of 2 to 9 years in humans (ATSDR, 2018a). However, using an average volume of distribution of 255 mL/kg estimated from nonhuman primate data (see Table 3-1) and weighted geometric mean clearance of 0.031 mL/kg-day in humans (see Table 3-4), the half-life of PFHxS in humans is estimated by the EPA to be 15.6 years.

PFAS that are released to air exist in the vapor phase in the atmosphere and resist photolysis, but particle-bound concentrations have also been measured (Kim and Kannan, 2007).

In soil, the mobility of PFHxS depends on the soil adsorption coefficients (see Table 1-1). Volatilization of PFHxS from moist soil is not expected to be an important transport process (NLM, 2017, 2016, 2013). Furthermore, PFHxS is expected to adsorb to suspended solids and sediments in water (NLM, 2017, 2016, 2013).

1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure

The general population may be exposed to PFAS via inhalation of indoor or outdoor air, ingestion of drinking water and food, and dermal contact with PFAS-containing products (ATSDR, 2018a; NLM, 2017, 2013). Exposure may also occur via hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018a). However, the oral route of exposure has been considered the most important route of exposure among the general population. This conclusion is based on several studies that have investigated the various routes of PFAS exposure (Sunderland et al., 2019).

The presence of PFHxS in human blood provides evidence of exposure among the general population. PFHxS has been monitored in the human population as part of the National Health and Nutrition Examination Survey (NHANES). PFHxS was measured in serum samples collected in 2013–2014 from more than 2,000 survey participants (CDC, 2022). The results of these analyses are presented in Table 1-2.

Table 1-2. Serum PFHxS concentrations based on NHANES 2013–2014 data (μg/L)

Population group[a]	Value
Total Population (N = 2,168)	
geometric mean	1.35
50th percentile	1.40
95th percentile	5.60
3 to 5 yrs (N = 181)	
geometric mean	0.715
50th percentile	0.740
95th percentile	1.62
6 to 11 yrs (N = 458)	
Geometric mean	0.913
50th percentile	0.850
95th percentile	4.14
12 to 19 yrs (N = 402)	
Geometric mean	1.27
50th percentile	1.10
95th percentile	6.30
20 yrs and older (N = 1,766)	
Geometric mean	1.36
50th percentile	1.40
95th percentile	5.50

[a]This table provides only general context on serum PFHxS levels from a single study and within a narrow time-period (environmental PFHxS levels are changing over time). Note that PFHxS is expected to bioaccumulate over a lifetime (see Sections 1.1.3 and 3.1). Up-to-date information from authoritative bodies should be used in any decisional context.

Source: CDC (2022). Fourth National Report on Human Exposure to Environmental Chemicals.

Air and Dust

PFHxS has not been evaluated under the Air Toxics Screening Assessment (https://www.epa.gov/AirToxScreen). However, PFHxS was measured at concentrations ranging from less than the limit of detection to 1.56 pg/m^3 in the vapor and particle phases of air samples collected from an urban area of Albany, New York, in 2006 (Kim and Kannan, 2007).

PFAS, including PFHxS, have also been measured in indoor air and dust and may be associated with the indoor use of consumer products such as PFAS-treated carpets or other textiles (ATSDR, 2018a). For example, Kato et al. (2009) analyzed dust samples collected from 39 homes in the United States, United Kingdom, Germany, and Australia for PFAS, including PFHxS, which was detected in 79.5% of the samples. Furthermore, indoor air samples (N = 4) from a town in Norway had PFHxS mean concentrations of <4.1 pg/m^3 for PFHxS (Barber et al., 2007).

Water

EPA conducted monitoring for several PFAS in drinking water as part of the third Unregulated Contaminant Monitoring Rule (UCMR) (U.S. EPA, 2016c). Under the UCMR3, all public water systems (PWSs) serving more than 10,000 people and a representative sample of 800 PWSs serving 10,000 or fewer people were monitored for 30 unregulated contaminants between January 2013 and December 2015. PFHxS was among the 30 contaminants monitored and was detected above the minimum reporting level (MRL) of 0.03 µg/L in 55 of the 4,920 PWSs tested and in 207 of the 36,971 samples collected. Kim and Kannan (2007) analyzed lake water, rainwater, snow, and surface water from Albany, New York, and reported concentrations of PFHxS ranging from less than the LOD to 0.0135 µg/L. PFAS were detected at higher concentrations in groundwater samples from an industrial site (3M Cottage Grove) in Minnesota. PFHxS was detected in all seven wells that were sampled at concentrations ranging from 6.47 to 40 µg/L (WS, 2007) as cited in ATSDR (2018b).

Aqueous Film-Forming Foam (AFFF) Training and Military Sites

The levels of PFHxS in soil and sediment surrounding perfluorochemical industrial facilities has been measured at concentrations ranging from less than the LOD to 3,470 ng/g (ATSDR, 2018b). PFHxS was also detected at an Australian training ground where AFFFs had been used (Baduel et al., 2015). PFHxS was detected at 10 U.S. military sites in 76.9% of the surface soil samples and 72.7% of sediment samples (ATSDR, 2018b). Table 1-3 shows the concentration of PFHxS in soil and sediment at these military sites.

Table 1-3. PFHxS levels at 10 military installations

Media	Value
Surface Soil	
Frequency of detection (%)	76.92
Median (µg/kg)	5.70
Maximum (µg/kg)	1,300

Media	Value
Subsurface Soil	
Frequency of detection (%)	59.62
Median (μg/kg)	4.40
Maximum (μg/kg)	520
Sediment	
Frequency of detection (%)	72.73
Median (μg/kg)	9.10
Maximum (μg/kg)	2,700
Surface Water	
Frequency of detection (%)	88.00
Median (μg/kg)	0.710
Maximum (μg/kg)	815
Groundwater	
Frequency of detection (%)	94.93
Median (μg/kg)	0.870
Maximum (μg/kg)	290

Source: Anderson et al. (2016); ATSDR (2018a).

Other Exposures

Schecter et al. (2012) collected 10 samples of 31 food items from five grocery stores in Texas and analyzed them for persistent organic pollutants, including PFHxS, which was detected in cod fish at a concentration of 0.07 ng/g wet weight. Stahl et al. (2014) characterized PFAS in freshwater fish from 164 U.S. urban river sites and 157 Great Lakes sites. PFHxS was detected in 45% of the samples at maximum concentrations of 3.5 ng/g and method detection limit of 0.12 ng/g (Stahl et al., 2014). PFHxS was not detected in U.S. grocery store finfish and shellfish samples (Ruffle et al., 2020). Apart from fish, overall dietary data for the United States are limited. Data from other countries (e.g., South Korea, Brazil, Saudi Arabia) suggest that long-chain PFAS such as PFHxS can sometimes be detected in samples of food products including shellfish, dairy products, meats, vegetables, food packaging materials, and water (both tap and bottled) (Chen et al., 2018b; Surma et al., 2017; Heo et al., 2014; Moreta and Tena, 2014; Pérez et al., 2014). The relevance of these detects (and the associated PFHxS levels) to U.S. products is unknown.

Populations with Potentially Greater Exposures

Populations that may experience exposures greater than those of the general population may include individuals in occupations that require frequent contact with PFHxS-containing products, such as individuals who install and treat carpets or firefighters (ATSDR, 2018a). Rotander et al. (2015a) analyzed serum samples from 149 Australian firefighters at an AFFF training facility. Mean and median PFHxS concentrations were 10 to 15 times higher than those of the general population of Australia and Canada. Laitinen et al. (2014) evaluated eight firefighters exposure to PFHxS after three training sessions in Finland in which AFFF had been used. The authors found that the firefighters "serum PFHxS concentrations seemed to increase during the three training sessions although it was not the main PFAS used in AFFF." Populations living near fluorochemical facilities where environmental contamination has occurred may also be more highly exposed (ATSDR, 2018b).

Populations that rely primarily on seafood for most of their diet, possibly including some native American tribes (Byrne et al., 2017), may also be disproportionately exposed to PFHxS. Christensen et al. (2017) and Haug et al. (2010) used data on serum PFAS levels and 30-day self-reported fish and shellfish ingestion rates from NHANES 2007–2014 to explore potential relationships between PFAS exposures and fish consumption. PFHxS was detected in the serum of at least 30% of the NHANES participants, and after adjusting for demographic characteristics shellfish consumption was associated with elevated levels of PFHxS (Christensen et al., 2017).

1.2. SUMMARY OF ASSESSMENT METHODS

The methods used to conduct this systematic review and dose-response analysis are summarized in the remainder of this section. A more detailed description of the methods for each step of the assessment development process is provided in the systematic review protocol released in 2019 (see Appendix A); the literature inventory for PFHxS in the protocol was not updated after its release (see Section 2.1). The protocol includes additional problem-formulation details, including the specific aims and key science issues identified for this assessment.

1.2.1. Literature Search and Screening

The detailed search approach, including the query strings and populations, exposures, comparators, and outcomes (PECO) criteria (see Table 1-4), are provided in Appendix B. The results of the literature search and screening efforts are documented in Section 2.1. Briefly, a literature search was first conducted in 2017 and regular yearly updates are performed. The most recent literate search update that was fully incorporated into the assessment is from April 2022. The literature from the past year (through March 2023) is in the process of being screened while the document is undergoing public comment. The results of this literature update and any additional unscreened studies identified during public comment will be screened against the PECO criteria and presented in a table that will be included as an Appendix to the assessment. The table will provide the identified studies that met PECO criteria or certain supplemental evidence categories (i.e., in vivo mechanistic or MOA studies, including non-PECO routes of exposure and populations; in vitro and in silico models; and ADME and pharmacokinetic studies) and EPA's judgment on whether the studies would have a material impact on the assessment conclusions (i.e., identified hazards or toxicity values) presented in the public comment draft. The external peer reviewers are asked to consider EPA's disposition of these newly identified studies and make recommendations, as appropriate (see Charge Question 1).

The literature search queried the following databases (no date or language restrictions were applied):

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- Toxline (National Library of Medicine)
- TSCATS (Toxic Substances Control Act Test Submissions)

In addition, relevant literature not found through database searching was identified by:

- Review of citations in studies meeting the PFHxS PECO criteria or published reviews of PFHxS; finalized or publicly available U.S. federal and international assessments (e.g., the 2021 Agency for Toxic Substances and Disease Registry [ATSDR] PFAS toxicity profile).
- Searches of published PFAS Systematic Evidence Maps (SEMs) (Carlson et al., 2022; Pelch et al., 2022) starting in 2021.
- Review of studies submitted to federal regulatory agencies and brought to the attention of EPA. For example, studies submitted to EPA by the manufacturers in support of requirements under the Toxic Substances Control Act (TSCA).
- Identification of studies during literature screening for other EPA PFAS assessments. For example, epidemiology studies relevant to PFHxS were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the Integrated Risk Information System (IRIS) Program.
- Other gray literature (e.g., primary studies not indexed in typical databases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to the attention of EPA.

All literature is tracked in the U.S. EPA Health and Environmental Research Online (HERO) database (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2630). The PECO criteria (see Table 1-4) identify the evidence that addresses the specific aims of the assessment and to focus the literature screening, including study inclusion/exclusion.

Table 1-4. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	Human: Any population and lifestage (occupational or general population, including children and other sensitive populations). The following study designs will be included: controlled exposure, cohort, case control, and cross-sectional. (Note: Case reports and case series will be tracked as potential supplemental material.) Animal: Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages).

PECO element	Evidence
	Other: In vitro, in silico, or nonmammalian models of genotoxicity. (Note: Other in vitro, in silico, or nonmammalian models will be tracked as potential supplemental material.)
Exposures	Human: Studies providing quantitative estimates of PFHxS exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational-setting measures (e.g., water levels or air concentrations, residential location and/or duration, job title, or work title). (Note: Studies that provide qualitative, but not quantitative, estimates of exposure will be tracked as supplemental material.) Animal: Oral or inhalation studies including quantified exposure to PFHxS based on administered dose, dietary level, or concentration. (Note: Nonoral and noninhalation studies will be tracked as potential supplemental material.) PFHxS mixture studies are included if they employ an experimental arm that involves exposure to a single PFHxS. (Note: Other PFHxS mixture studies are tracked as potential supplemental material.) Studies must address exposure to following: PFHxS (CASRN 355-46-4), PFHxS potassium salt (CASRN 3871-99-6) or PFHxS ammonium salt (CASRN 68259-08-5).
Comparators	Human: A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. Animal: Includes comparisons to historical controls or a concurrent control group that is unexposed, exposed to vehicle-only or air-only exposures. (Note: Experiments including exposure to PFHxS across different durations or exposure levels without including one of these control groups will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)
Outcomes	All cancer and noncancer health outcomes. (Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing toward toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)

In addition to those studies meeting the PECO criteria and studies excluded as not relevant to the assessment, studies containing supplemental material potentially relevant to the specific aims of the assessment were inventoried during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues (see Appendix A, Section 2.4) and other potential scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as “potentially relevant supplemental material” included the following:

- In vivo mechanistic or mode of action studies, including nonPECO routes of exposure (e.g., intraperitoneal injection) and populations (e.g., nonmammalian models)
- In vitro and in silico models

- Absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic studies (excluding models)[3]
- Exposure assessment or characterization (no health outcome) studies
- Human case reports or case series studies

The literature was screened by two independent reviewers with a process for conflict resolution, first at the title and abstract level and subsequently the full-text level, using structured forms in DistillerSR (Evidence Partners; https://distillercer.com/products/distillersr-systematic-review-software/). Literature inventories for PECO-relevant studies and studies tagged as "potentially relevant supplemental material" during screening were created to facilitate subsequent review of individual studies or sets of studies by topic-specific experts.

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiologic and animal toxicological studies used in the PFHxS assessment are provided in the systematic review protocol (Appendix A, see Section 6). The general approach for evaluating PECO-relevant health effect studies is the same for epidemiology and animal toxicological studies, although the specifics of applying the approach differ; thus, they are described in detail in Appendix A (see Sections 6.2 and 6.3, respectively). Approaches for study evaluation for mechanistic studies is described in detail in Appendix A (see Section 6.5).

The key concerns for the review of epidemiology and animal toxicological studies are potential bias (systematic errors or deviations from the truth related to internal validity that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect and can lead to a false negative). For example, any types of random measurement error that may lead to attenuation of study results (i.e., bias toward the null). In evaluating individual studies, two or more reviewers independently arrived at judgments regarding the reliability of the study results (reflected as study confidence determinations; see below) with regard to each outcome or outcome grouping of interest; thus, different judgments were possible for different outcomes within the same study. The results of these reviews were tracked within EPA's version of the Health Assessment Workplace Collaboration (HAWC). To develop these judgments, each reviewer assigned a category of *good*, *adequate*, *deficient* (or *not reported*, which generally carried the same functional interpretation as *deficient*), or *critically deficient* (listed from best to worst methodological conduct; see Appendix A, Section 6 for definitions) related to each evaluation domain representing the different characteristics of the study methods that were evaluated based on the criteria outlined in HAWC.

[3]Given the known importance of ADME data, this supplemental tagging was used as the starting point for a separate screening and review of pharmacokinetics data (see Appendix A, Section 9.2 for details).

Once all evaluation domains were evaluated, the reviewers collectively considered the identified strengths and limitations to reach a final study confidence classification:

- *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.
- *Medium* confidence: Possible deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree or to have a notable impact on the results.
- *Low* confidence: Deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. *Low* confidence results were given less weight than *high* or *medium* confidence results during evidence synthesis and integration (see Sections 1.2.4 and 1.2.5).
- *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

Using the HAWC platform (and conflict resolution by an additional reviewer, as needed), the reviewers reached a consensus judgment regarding each evaluation domain and overall (confidence) determination. The specific limitations identified during study evaluation were carried forward to inform the synthesis (see Section 1.2.4) within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform judgments in isolation).

Additional Epidemiology Considerations

While the detailed methods for epidemiology study evaluation are described in the systematic review protocol (see Appendix A, Section 6.2.1), a few considerations have been developed further; these are described here.

As noted above, study sensitivity is an important consideration given that it could lead to false negative (i.e., null) results (Type II error) if a study is underpowered or not designed with adequate sensitivity to detect an association that may exist. A key element for study sensitivity, along with others described in the systematic review protocol, is whether exposure contrasts/gradients are sufficient across populations to detect differences in risk. For example, if measurement error results in inaccurate exposure estimates, this can lead to exposure misclassification and also influence the ability to detect an association as well as an exposure-response relationship that may be evident of a biologic gradient.

Confounding across PFAS is a potential source of uncertainty when interpreting the results of epidemiology studies of individual PFAS (e.g., quantifying the effect of an individual PFAS can potentially be confounded by other PFAS). For confounding to occur, co-pollutants would have to be associated with PFAS of interest, associated with the endpoint, and not act as an intermediate in the causal pathway. One way to begin to assess whether co-exposure is occurring is through examination of correlations. While some PFAS pairs have correlation coefficients consistently above

0.6 (e.g., PFNA and PFDA), the correlations for most PFAS, including PFHxS, vary from 0.1 to 0.6 depending on the study (see Appendix A, Section 6). For this reason, it was not considered appropriate to assume that co-exposure to other PFAS was necessarily an important confounder in all studies. The potential for confounding across PFAS is incorporated in individual study evaluations and assessed across studies in evidence synthesis. In most studies, it is difficult to determine the likelihood of confounding without considering additional information not typically included in individual study evaluation (e.g., associations of other PFAS with the outcome of interest and correlation profiles of PFAS within and across studies). In addition, even when this information is considered or the study authors perform analyses to adjust for other PFAS, it is often not possible to fully disentangle the associations due to high correlations. This challenge stems from the potential for amplification bias in which bias can occur following adjustment of highly correlated PFAS (Weisskopf et al., 2018). Thus, in most studies, there may be some residual uncertainty about the risk of confounding by other PFAS. A "Good" rating for the confounding domain is reserved for situations in which there is minimal concern for substantial confounding across PFAS as well as for other sources of confounding. Examples that would obtain this rating include results for a PFAS that predominates in a population (such as a contamination event) or studies that demonstrate robust results following multi-PFAS adjustment (i.e., similar results to single-PFAS models), which would also indicate minimal concern for amplification bias. Because of the challenge in evaluating individual studies for confounding across PFAS, this issue is also assessed across studies during the evidence synthesis phase, as described in the systematic review protocol (see link in Appendix A, Section 6.2), primarily when there is support for an association with adverse health effects in the epidemiology evidence (i.e., *moderate,* or *robust* evidence in humans, as described below). Analyses used include comparing results across studies in populations with different PFAS exposure mixture profiles, considering results of multipollutant models when available, and examining strength of associations for other correlated PFAS. In situations for which there is considerable uncertainty regarding the impact of residual confounding across PFAS, a factor is captured that decreases the overall strength of evidence (see link in Appendix A, Section 10).

1.2.3. Data Extraction

The detailed data extraction approach is provided in Appendix A, Section 8. Briefly, data extraction and content management were carried out using HAWC for all health effects for animal studies and some health effects for epidemiological studies. Data extraction elements collected from epidemiological, controlled human exposure, animal toxicological, and in vitro studies are described in HAWC (https://hawcprd.epa.gov/about/). For epidemiological studies not extracted in HAWC, extraction was performed into Word tables and the extraction elements depended on information needed for presentation. Not all studies that meet the PECO criteria went through data extraction: studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction, and outcomes determined to be less relevant during PECO refinement did not go through data extraction. The same was true for *low* confidence studies when *medium* and *high* confidence studies (e.g., on an outcome) were available. All findings are considered for extraction, regardless of the statistical significance of their findings. The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). For quality control, data extraction was performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction were resolved by discussion or consultation within the evaluation team.

1.2.4. Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes (see Appendix A, Sections 9 and 10 for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from Hill (1965). Mechanistic evidence is also synthesized as necessary to help inform key decisions regarding the human and animal evidence; processes for synthesizing mechanistic information are covered in detail in Appendix A, Section 9.2.

The syntheses of the human and animal health effects evidence focus on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies. The evidence synthesis is based primarily on studies of *high* and *medium* confidence. *Low* confidence studies could be used if few or no studies with higher confidence are available to help evaluate consistency, or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect. If *low* confidence studies are used, a careful examination of the study evaluation and sensitivity with potential effects on the evidence synthesis conclusions will be included in the narrative. When possible, results across studies are compared using graphs and charts or other data visualization strategies. The synthesis of mechanistic information informs the integration of health effects evidence for both hazard identification (e.g., biological plausibility or coherence of the available human or animal evidence; inferences regarding human relevance, or the identification of susceptible populations and lifestages across the human and animal evidence) and dose-response evaluation (e.g., selection of benchmark response levels, selection of uncertainty factors). Evaluations of mechanistic information typically differ from evaluations of phenotypic evidence (e.g., from routine toxicological studies) primarily because mechanistic data evaluations consider the support for and involvement of specific events or sets of events within the context of a broader research question (e.g., support for a hypothesized mode of action; consistency with known biological processes), rather than evaluations of individual apical endpoints considered in relative isolation.

Following the synthesis of human and animal health effects data and mechanistic data, integrated judgments are drawn across all lines of evidence for each assessed health effect. During evidence integration, a structured and documented two-step process is used, as follows:

Building from the separate syntheses of the human and animal evidence, the strength of the evidence from the available human and animal health effect studies are summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Sir Bradford Hill (Hill, 1965). This process is similar to that used by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Morgan et al., 2016; Guyatt et al., 2011; Schünemann et al., 2011), which arrives at an overall integration conclusion based on consideration of the body of evidence. These summaries incorporate the relevant mechanistic evidence (or mode of action [MOA] understanding) that informs the biological plausibility and coherence within the available human or animal health effect studies. The terms associated with the different strength of evidence judgments within evidence streams are *robust*, *moderate*, *slight*, *indeterminate*, and *compelling evidence of no effect*.

The animal, human, and mechanistic evidence judgments are then combined to draw an overall judgment that incorporates inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed. The final output is a summary judgment of the evidence base for each potential human health effect across evidence streams. The terms associated with these summary judgments are *evidence demonstrates*, *evidence indicates (likely)*, *evidence suggests*, *evidence inadequate*, and *strong evidence of no effect*. The decision points within the structured evidence integration process are summarized in an evidence profile table for each considered health effect.

As discussed in the protocol (see Appendix A), the methods for evaluating the potential carcinogenicity of PFAS follow processes laid out in the EPA cancer guidelines (U.S. EPA, 2005); however, for PFHxS, data relevant to cancer were sparse and did not allow for such an evaluation (see Appendix A, Section 3.3).

1.2.5. Dose-Response Analysis

The details for the dose-response employed in this assessment can be found in Appendix A, Section 11. Briefly, a dose response assessment was performed for noncancer health hazards, following exposure to PFHxS via the oral route, as supported by existing data. For oral noncancer hazards, oral reference doses (RfDs) are derived when possible. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002). The derivation of reference value like the RfD depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer outcomes, a dose response assessment was conducted for evidence integration conclusions of **evidence demonstrates** or **evidence indicates (likely)**. In general, toxicity values are not developed for noncancer hazards with **evidence suggests** conclusions (see Appendix A, Section 10.2 for exceptions). Consistent with EPA practice, the PFHxS assessment applied a twostep approach for dose response assessment that distinguishes analysis of the dose response data in the range of observation from any inferences about responses at lower environmentally relevant exposure levels (U.S. EPA, 2012, 2005):

- Within the observed dose range, the preferred approach was to use dose-response modeling to incorporate as much of the dataset as possible into the analysis. This modeling to derive a point of departure (POD) ideally includes an exposure level near the lower end of the range of observation, without significant extrapolation to lower exposure levels.
- As derivation of cancer risk estimates and reference values nearly always involves extrapolation to exposures lower than the POD; the approaches to be applied in these assessments are described in more detail in Appendix A, Section 11.2.

When sufficient and appropriate human and laboratory animal data are available for the same outcome, human data are generally preferred for the dose-response assessment because use of human data eliminates the need to perform interspecies extrapolations. For reference values, this assessment will derive a candidate value from each suitable dataset. Evaluation of these candidate values will yield a single organ/system-specific value for each organ/system under consideration from which a single overall reference value will be selected to cover all health outcomes across all organs/systems. While this overall reference value represents the focus of these dose-response assessments, the organ/system-specific values can be useful for subsequent cumulative risk assessments that consider the combined effect of multiple PFAS (or other agents) acting at a common organ/system. For noncancer toxicity values, uncertainties in these estimates are characterized and discussed.

For dose-response purposes, EPA has developed a standard set of models (http://www.epa.gov/bmds) that can be applied to typical datasets, including those that are nonlinear. In situations for which there are alternative models with significant biological support (e.g., pharmacodynamic models), those models are included as alternatives in the assessment(s) along with a discussion of the models strengths and uncertainties. EPA has developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results [see the EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012)]. For each modeled response, a POD from the observed data was estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses. The POD is used as the starting point for subsequent extrapolations and analyses. For noncancer effects, the POD is used in calculating the RfD.

2. LITERATURE SEARCH AND STUDY EVALUATION RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 4,432 records, of these records 162 were identified from additional sources, such as posted National Toxicology Program (NTP) study tables and during review of reference lists from other authoritative sources (ATSDR, 2018b) (see Figure 2-1). No studies were submitted to EPA. After deduplication, 1,935 unique records were identified, 862 were excluded during title and abstract screening, and 806 were reviewed at the full text level. Of the 806 screened at the full text level, 446 were considered to meet the populations, exposures, comparators, and outcomes (PECO) eligibility criteria (see Table 1-4). The studies meeting PECO at the full text level included 415 epidemiologic studies and 20 animal studies. High throughput screening data on perfluorohexane sulfonate (PFHxS) are currently available from the EPA's Chemicals Dashboard (U.S. EPA, 2019b) and relevant information is presented and analyzed in Appendix D (see Section 3).

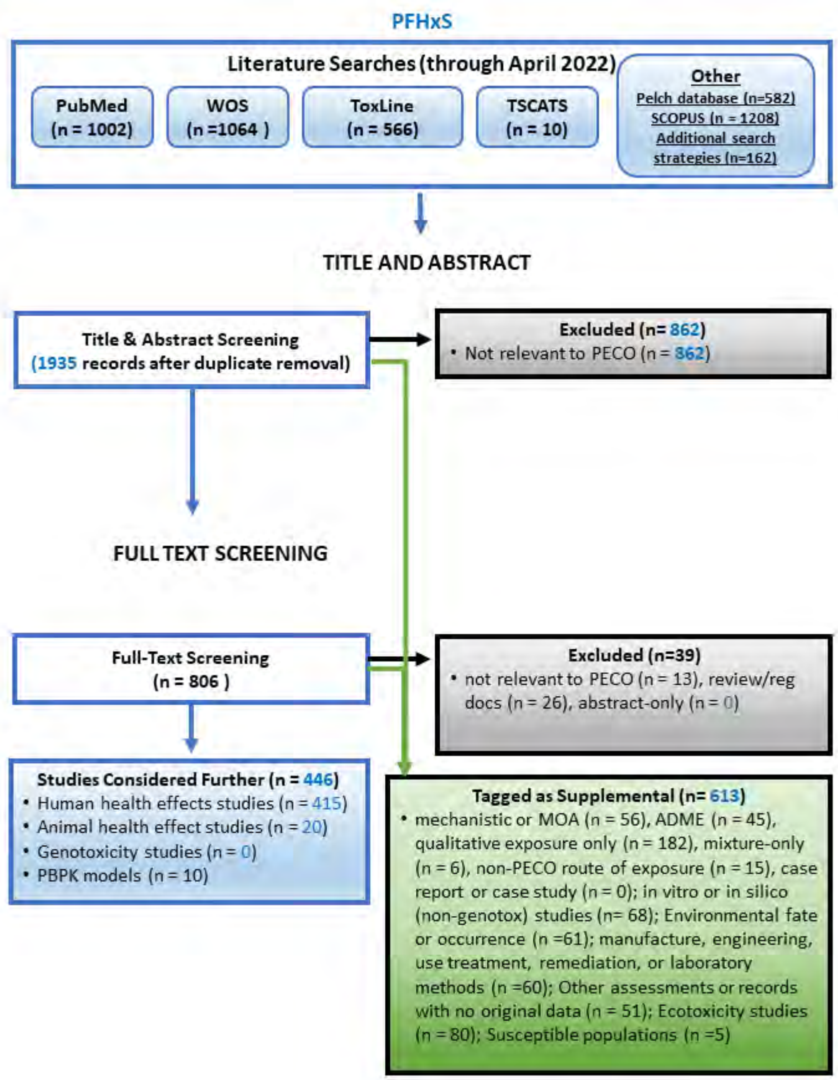


Figure 2-1. Literature search for perfluorohexanesulfonic acid and related salts.

2.2. STUDY EVALUATION RESULTS

One hundred seventeen epidemiologic studies were identified that met the PECO criteria and report on the potential association between PFHxS and human health effects. The database of animal toxicity studies for PFHxS consists of two short-term oral exposure studies using rats (NTP, 2018a; 3M, 2000a), one subchronic study using mice (Bijland et al., 2011), and three multigenerational studies using rats or mice (Ramhøj et al., 2020; Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003).

Graphical representations of outcome-specific study evaluations are presented and discussed within the hazard sections (see Sections 3.2.1−3.3.1). In cases for which a study was rated *medium* or *low* confidence for one or more of the evaluated outcomes, the specific limitations are explained in the synthesis section(s). Detailed rationales for each domain and overall confidence rating are available in Health Assessment Workspace Collaborative (HAWC).

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND INTEGRATION

3.1. PHARMACOKINETICS

The following sections review the scientific evidence for the absorption, distribution, metabolism, and excretion (ADME) of perfluorohexane sulfuric acid (PFHxS). In general, the evidence described below demonstrates that PFHxS has ADME characteristics of comparable with other perfluoroalkyl acids (PFAA) that are readily absorbed in the gastrointestinal tract following oral exposure irrespective of sex or species.

Multiple PFHxS isomers have been identified. Benskin et al. (2009) found evidence of three PFHxS isomers as minor fractions in a PFOS standard generated using electrochemical fluorination. They identified the most prevalent of these as the linear isomer (n-PFHxS), and the two others as branched isomers. The branched isomers were present as a small fraction relative to the linear isomer[4] but were a majority of the PFHxS found in urine 3 days after dosing, as branched isomers are eliminated more quickly than n-PFHxS. By day 38 the branched isomers, but not n-PFHxS, were essentially absent in blood (Benskin et al., 2009). Some pharmacokinetic studies specifically identified the isomer used (e.g., Sundström et al. (2012) used the linear isomer), but others did not. Results from other studies based on measured PFHxS concentrations in blood were therefore assumed to represent n-PFHxS unless otherwise specified. The current evidence is too sparse to draw separate judgments for branched and linear isomers, although this review of PFHxS ADME is interpreted as primarily focused on evidence for n-PFHxS. While branched PFHxS isomers are likely to have many similar pharmacokinetic (and pharmacodynamic) properties as n-PFHxS, their contribution to the summary information below (and the toxicity data in Section 3.2) cannot currently be specified.

Both animal and human data suggest that PFHxS has a high affinity for protein binding. Bischel et al. (2011) measured 99% bound in a solution of bovine serum albumin and Kim et al. (2018b) estimated less than 0.08% free in rat plasma and 0.03% free in human plasma. Significant sex differences in urinary excretion have been reported, suggesting hormonal regulation of transporters involved in renal reuptake (Yang et al., 2009). The PFHxS serum concentrations reported at the end of the 28-day NTP bioassay (NTP, 2019) were in fact strongly suggestive both of sex differences and of saturable resorption in the elimination of PFHxS by rats (see Figure 3-1). While the dose range was greater for female rats (0–50 mg/kg-day) than male rats (0–10 mg/kg-

[4] Based on peak height in a representative chromatogram shown in Figure 1 of Benskin et al. (2009), quantified by digitization of the published plot, the two branched isomers had concentrations of about 8% and 15% of the linear isomer in the dosing solution.

day), it is still clear that plasma levels in the males at 10 mg/kg-day (198 mg/L) were three times higher than the plasma concentration in females given 12 mg/kg-day (64 mg/L) at the end of the 28-day study. This sex difference was clearly reflected by the differences in clearance and half-life for male and female rats seen in multiple studies, discussed subsequently. The NTP (2019) data also clearly indicated strong pharmacokinetic nonlinearity (see Figure 3-1). If absorption and clearance were independent of concentration the plasma concentrations in Figure 3-1 would be approximately linear with dose. The PK data discussed below also indicated nonlinearity in either or both the absorption and clearance. In particular, Huang et al. (2019a) estimated clearance levels 1.5 to 2 times higher after a 32 mg/kg dose than after 4 and 16 mg/kg and a decrease in bioavailability of about 50% between 4 and 32 mg/kg in both male and female rats. However, because those PK experiments only used a single dose, they may not have achieved plasma concentrations high enough to demonstrate the extent of the difference in clearance that might be needed to explain the NTP data.

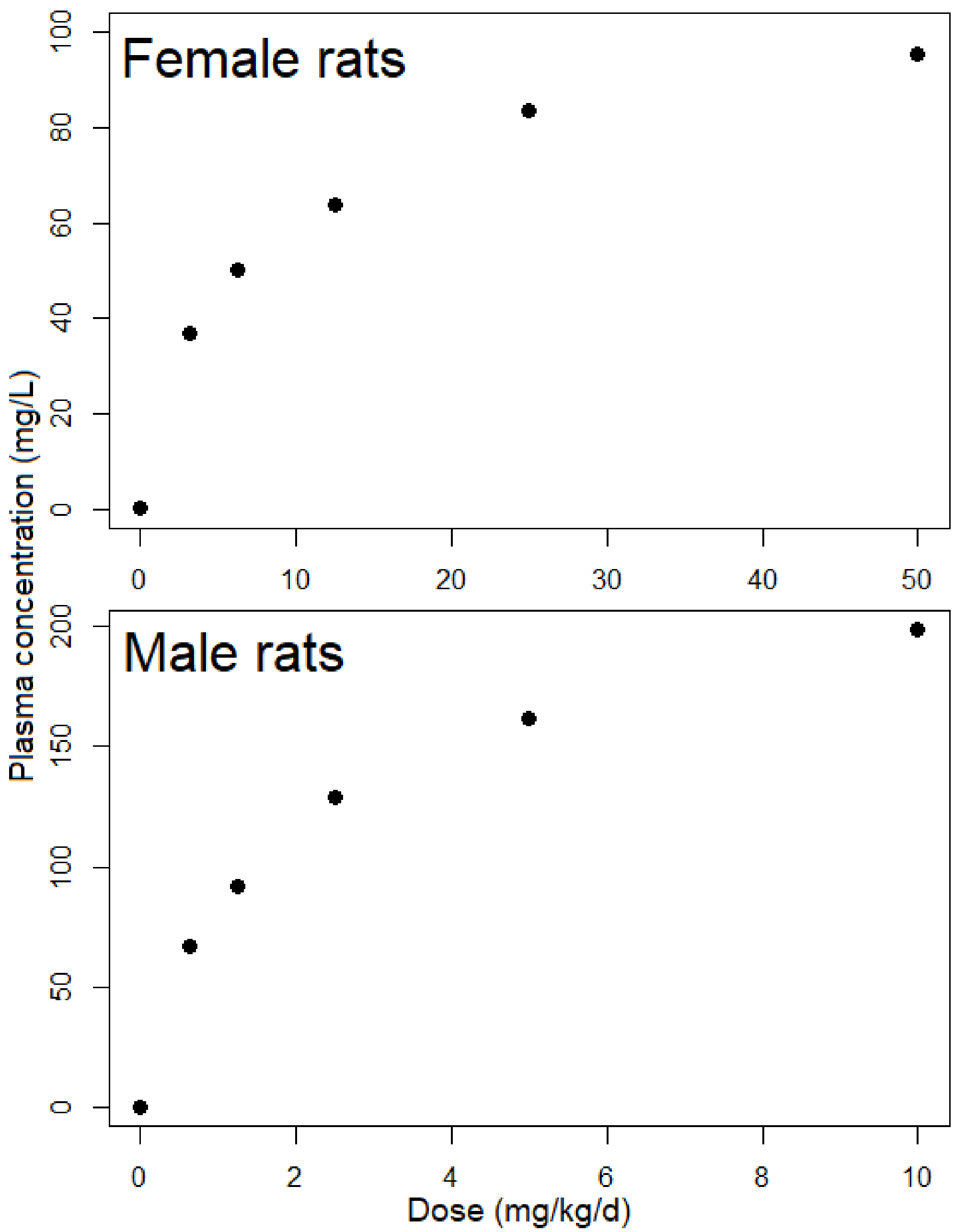


Figure 3-1. Observed end-of-study of PFHxS in female and male rats in the NTP bioassay (NTP, 2019) as a function of dose. The plasma concentrations were measured one day after the final dose, i.e., day 29. While the two data sets look similar as shown with their respective dose scales, note that significant saturation occurs in male rats by a dose of 5 mg/kg-day, where the plasma concentration is 80% of that observed at the highest administered dose, while a dose of about 20 mg/kg-day is needed to achieve the same degree of saturation in females, while the highest concentration in males is twice that in females. The similarity in shape may occur because binding of PFHxS to the same transporter determines the nonlinearity in both sexes.

Serum binding also appears to limit distribution of PFHxS into other tissues, with the tissue:blood or plasma ratio reported as less than 0.2 for liver and much lower for all other tissues (Kim et al., 2016b; Benskin et al., 2009). After the liver, the next highest tissue levels were observed in kidney, lung, heart, and spleen. Similar to other PFAAs, PFHxS has been presumed to be metabolically inert, but Sundström et al. (2012) only recovered 45%–55% of material between serum, liver, urine, and feces 96 hours after dosing to Sprague Dawley (SD). The majority (~90%) of PFHxS was excreted in the urine rather than the feces (Kim et al., 2018b).

A pharmacokinetic (PK) approach was used to extrapolate toxicity points of departure from animal PFHxS doses and human blood PFHxS levels to a human equivalent (external oral) dose. A review of the ADME information for rats and humans directly informed the PK approach. Although no endpoints in mice or monkeys were advanced for dose-response modeling, evaluation of ADME in those species provided a broader context for interpreting the results in rats and humans. For example, to what extent might significant differences between PK in male and female rats be predictive of possible sex differences in humans? Differences or similarities between rats and monkeys can likewise be indicative of the comparison between rats and humans.

Two key parameters determined were clearance (CL; L/kg-day) and volume of distribution (Vd; L/kg). For convenience, the following analysis of published data used units of mL/kg-day. Options for PBPK, and PK modeling were evaluated (see Section 3.1.5). That evaluation informed the specific choice for dose extrapolation, described in Approach for Animal Human Extrapolation of PFHxS Dosimetry in Section 3.1.7), while the literature used to support the selection of the PK parameters and rationale for the approach used are discussed in the relevant Pharmacokinetics sections below.

3.1.1. Absorption

For the most part, PFHxS data showed near complete absorption after oral dosing. Kim et al. (2016b) estimated total AUC in blood ($AUC_{0-\infty}$) that was greater after oral compared with IV doses (4 mg/kg PFHxS) in both male and female rats. This result is counter to general pharmacokinetic understanding, which assumes that the oral AUC will be lower than the IV AUC due to incomplete absorption in the gastrointestinal tract. These results may have been an artifact of experimental variability and the PK analysis used but they indicated complete absorption. Kim et al. (2018b) then estimated ~90% absorption in female SD rats (92% and 88% absorption at 1 and 4 mg/kg doses, respectively) and 96% in male SD rats (10 mg/kg dose) based on observations to 14 days postexposure. While Sundström et al. (2012) showed results indicating only 50% oral uptake in SD rats, this was based on only two animals for the oral PK and observations only to 24-hour post dose, so are more uncertain. Huang et al. (2019a) estimated a decline in the fraction of PFHxS absorbed with increasing dose in rats: 98%, 82%, and 52% absorbed in males and apparent values of 142%, 112%, and 71% in females at respective doses of 4, 16, and 32 mg/kg. As noted above, reduced absorption at higher doses would explain in part the observed dose-dependence seen in Figure 3-1.

While the results discussed above indicate a decrease in bioavailability at higher doses, pharmacokinetic extrapolation from animals to humans is focused on low doses for which most of the available data indicated complete absorption, if not greater bioavailability after oral exposure than IV dosing. A more comprehensive computational analysis of the PK data was conducted (see Section 3.1.6), including consideration of less than 100% bioavailability; however, that analysis was unable to resolve the uncertainty in bioavailability. Therefore, 100% bioavailability was assumed for the purpose of low-dose extrapolation from rats to humans.

The rate of absorption appeared to be more rapid in female rats than in males. Kim et al. (2016b) reported a T_{max} of 1.4–1.5 hours (0.06 days) in female rats, but 3 days in male rats and Kim et al. (2018b) likewise reported 1.4 hours in females and 3.1 days in males. However, this difference in timing may also be confounded by the much slower clearance in male versus female rats (see below). Huang et al. (2019a) obtained a T_{max} of 2–3 hours in female rats and 5–7 hours in male rats, with a decreasing trend as dose increased. Transporter-mediated processes and protein binding may have caused dose-dependence of T_{max} for PFHxS, but the differences in T_{max} between dose groups was not reported as statistically significant by Huang et al. (2019a) and the range of values for each sex was not large enough to be of consequence for dose extrapolation.

While these results indicated somewhat slower absorption in male than female rats, it is only by a factor of 2 or 3 (Huang et al., 2019a). Sundström et al. (2012) observed a T_{max} of only 0.5 hours in female SD rats and could not estimate a value for male rats due to the short 24-hour window of observation. The cause for the discrepancy from other studies discussed just above was unclear. Plotted data indicated very rapid initial absorption in both males and females (Kim et al., 2018b; Kim et al., 2016b) and by definition peak concentration occurs when the rate of clearance equals the rate of absorption (which decreases as the remaining dose in the gastrointestinal tract declines). So, it may simply be that it took longer for the absorption rate to fall below the slow clearance rate of PFHxS in male rats than female rats.

In male CD-1 mice Sundström et al. (2012) the observed T_{max} was 8 hours at a dose of 1 mg/kg and 4 hours at a dose of 20 mg/kg, while T_{max} was 2 days in females at 1 mg/kg, but only 4 hours in female mice at 20 mg/kg. Thus, the predominant results indicated that the majority of absorption occurs in less than 8 hours in mice, consistent with uptake being in the range of 90% or higher. It was unclear why T_{max} was lower at the higher doses in both males and females. No specific methodological flaws were identified, but the exact value of T_{max} from an experiment depends on the timing of blood samples (experimental design) and can be affected by experimental variability. Serum concentrations were measured starting at 2 hours and it is possible that the value of “2” for female mice dosed with 1 mg/kg PFHxS was actually 2 hours, rather than 2 days. While bioavailability was not measured in primates, it is reasonable to assume that uptake in monkeys and humans is likewise fairly efficient.

A study on the toxicological response upon dermal exposure to a technical mixture containing PFHxS showed the presence of PFHxS in serum during the 28-day dosing period and after a 14-day recovery period (3M, 2004). Male and female rats were exposed to the product as a liquid on cotton gauze or as a solid dried onto cotton gauze. PFHxS from both the liquid and dried product entered systemic circulation through the skin as determined by measurements of serum PFHxS levels. Male rats showed higher PFHxS serum levels compared with female rats, which was likely an effect of differential excretion, rather than differential absorption. Male rats showed a clear accumulation of PFHxS in serum over the duration of the 28-day dosing period and levels appeared to decrease during the recovery period in the group exposed to the dried formulation. Male rats exposed to the liquid formulation had peak levels observed after the recovery period. In female rats, peak concentrations were seen after 14 days of exposure and lower levels were seen after 28 days of exposure. Levels were lower still after the recovery period. These data suggested a concern for dermal exposure to PFHxS in both liquid and dried formulations, but further research is needed to quantify rates of absorption, the resulting relationship between external and internal dose, and the extrapolation of this information to human exposure.

No data on absorption of PFHxS through the respiratory tract has been found.

There is no direct quantification of oral absorption of PFHxS in humans. However, an epidemiological study by Stubleski et al. (2016) identified a qualitative association between PFHxS concentrations in human serum and concentrations in drinking water. Specifically, a 54% increase in serum levels was observed during the observation period after a large contamination event, but serum levels only declined 20% after an intervention that decreased drinking water levels by 60%. The lack of exact correlation may have been due to the timing of sampling versus the contamination event, as well as to the long half-life of PFHxS in humans.

Given the generally high absorption reported in rats (e.g., 90% for female rats and 96% for male rats) by Kim et al. (2018b), humans will be assumed to absorb 100% of ingested PFHxS, which is slightly more health protective compared with assuming 90%–96%.

3.1.2. Distribution

While PFHxS was found at some level in all tissues evaluated, the largest amounts have been in the liver, followed by the kidneys and lung, with much lower levels in other tissues. For example, Benskin et al. (2009) reported tissue:blood ratios in male rats on day 3 of dosing at 0.03 mg/kg as being 17% for liver, 10% for lungs, 5% for heart and kidney, with other tissues being 4% or lower. Kim et al. (2016b) measured ratios after 72 days in male and 14 days in female rats from 4 mg/kg doses and obtained ratios of 17% and 11% for male and female liver, respectively; 13% and 8% for kidney; 5% and 4% for heart; 4% and 3% for lung (each for males and females, respectively); and 2% for spleen in both sexes. This distribution appears to be fairly rapid compared with the overall time-course in blood: Huang et al. (2019a) showed essentially constant tissue:plasma ratios in female rat liver and kidney from day 0 to day 8 and in the male rat kidney from 0 to 50 days after a 16 mg/kg dose. Interestingly, the ratio in the male rat liver quickly rose to 50%–60% but then gradually increased to over 80% on day 50 (Huang et al., 2019a). This time-dependence may have been due to slower clearance from the male rat liver than the blood and other tissues which may confound interpretation of PK data. If the percent distribution to the liver (relative to plasma) increased over time, then the observed decline in plasma concentrations was not proportional to whole-body elimination.

The order of tissue concentrations was observed to be the same in mice as in rats, but with the mouse liver having 25%–40% of serum levels and the kidney ~10% (Sundström et al., 2012). However, measurements of PFAS levels in human cadavers indicated a different ordering of concentration, with highest levels in kidney (median 18 ng/g), followed by lung (median 5.7 ng/g), then brain, liver, and bone (2.3, 1.8, and 1.2 ng/g, respectively) (Pérez et al., 2013). These human results should be interpreted with some caution since they do not provide ratios from matched samples and the specific method of collecting tissues likely differed to some extent (details on the human tissue collection are not available). But the difference between kidney and liver may be large enough to suggest a difference between human and rodent PFHxS distribution for these tissues.

Karrman et al. (2010) also examined postmortem liver concentrations in 12 human samples and compared those to serum concentrations previously observed in the region. This comparison is severely limited as the serum and liver samples were sourced from different individuals.

Yeung et al. (2013) evaluated PFHxS concentrations in liver versus serum of humans with hepatocellular carcinoma (HCC) or cirrhosis due to chronic hepatitis C virus (HCV). In these patients, the liver concentration was 15% of the serum in HCC patients (n = 11) and 9% of the serum in HCV patients (n = 32). These results need to be interpreted with caution because of the disease status, but they indicated somewhat lower distribution into the human liver than observed in rodents. The authors did not have paired liver and serum from healthy individuals for comparison. In addition to the evidence of distribution to the brain in cadavers, PFHxS has been observed in the cerebrospinal fluid of neonates, with a median cerebrospinal fluid: blood serum ratio of 0.0290 from 2 paired samples (Liu et al., 2022b). Based on evidence from other PFAS in humans and rats that the authors reviewed, this ratio is expected to be higher in neonates compared to adults due to ongoing development of the blood-cerebrospinal fluid barrier. Intracellular concentrations of PFHxS in the brain are expected to be much higher than the concentration in the cerebrospinal fluid due to interactions between PFHxS and cytoplasmic proteins.

A recent study evaluated levels of several PFAS, including PFHxS, in human serum as a function of various measures of body composition as well as localized measurements of adipose content throughout the body generated by dual-energy X-ray absorptiometry (DXA) and whole-body magnetic resonance imaging (WB-MRI) (Lind et al., 2022). There was not an association with traditional measures of body composition, such as body-mass index (BMI). PFHxS was however inversely related to total lean mass, leg lean mass, subcutaneous adipose tissue in the arms, trunk and thigh, and skeletal muscle volume in the arms and legs in men but not in women. Given the minimal distribution of PFHxS to adipose and muscle tissues described above, one might expect essentially no effect of the volume of these tissues on serum levels. However, one would predict a negative correlation between Vd and body fat, the results in men may be consistent with that prediction if glomerular filtration increases with body mass or surface area. It is also possible that the correlation was due to variation in exposure related to body fat or muscle volume that occurs particularly in males. Matched estimates of exposure from dietary surveys or samples, or matched measures of urinary clearance (PFAS concentrations in urine) are ultimately needed to determine whether or not the correlations actually reflect PK variation.

Kang et al. (2020) measured the levels of PFAS in the follicular fluid of women undergoing oocyte retrieval for in vitro fertilization in relation to their serum levels and observed a median ratio of 0.84, which is much higher than seen for other various tissues described above. This result suggested that PFHxS can pass readily through the follicular walls (theca and granulosa cells), and that binding to proteins in the follicular fluid is similar to that in serum.

Zhao et al. (2015) and Zhao et al. (2017) investigated the role of renal transporters known to be involved in enterohepatic recirculation of bile acids. Zhao et al. (2015) showed that PFHxS is a substrate for the human and rat Na^+/taurocholate co-transporting polypeptide (NTCP) expressed in vitro and Zhao et al. (2017) showed that multiple human and rat organic anion transporting polypeptides (OATPs) likewise transported PFHxS. These active transport processes may contribute to the relatively high distribution of PFHxS observed in the liver and its long half-life in rats and humans by limiting biliary excretion. Excretion is also limited by protein binding in the liver, for example observed in interactions with human liver fatty acid-binding protein (hL-FABP) (Yang et al., 2020a; Sheng et al., 2016), and in serum, discussed subsequently in the ***Distribution in Blood/Proteins*** section. The impact of serum protein binding on renal clearance is also discussed in the **Excretion** section (Section 3.1.4) under the ***Clearance Versus Glomerular Filtration Rate and Free Fraction in Serum*** subsection.

Volume of Distribution

Vd is a pharmacokinetic parameter that quantifies the extent to which a chemical distributes between the blood and the body as a whole and is effectively an average of tissue-specific distribution ratios. Vd is key in evaluating internal dose because it quantifies the blood concentration for a given total amount in the body. See Section 3.1.6, Empirical Pharmacokinetic Analysis, for details of EPA's computational analysis. In rats, mean Vd ranged from 123 to 327 mL/kg among studies, doses, and routes of administration, without a clear sex difference (Huang et al., 2019a; Kim et al., 2018b; Kim et al., 2016b; Sundström et al., 2012). Only Sundström et al. (2012) evaluated the Vd in mice at two oral doses, and while the values were approximately 25% lower in females than in males at a given dose, the value for female mice given 20 mg/kg was between the values for male mice given 1 versus 20 mg/kg. The overall range of Vd in mice (96–195 mL/kg) strongly overlapped the observed range in rats. The Vd in monkeys was also evaluated by Sundström et al. (2012), though only at a single IV dose (10 mg/kg) and was likewise in the range reported for rats: 213 mL/kg in female monkeys and 287 mL/kg in male monkeys.

The fact that reported values of Vd were generally below 300 mL/kg and that most tissue-specific levels were low compared with blood (see previous section) indicated that PFHxS primarily distributes with extracellular fluid, with the exception of the liver.

Reported values of Vd are listed in Table 3-1, grouped by species and sex. No data to determine Vd in humans were found.

The biochemical and physiological factors that determine tissue distribution have been generally presumed to be evolutionarily conserved among mammalian species, an assumption which was supported by the overall similarity of values across species seen in Table 3-1. However, species differences in Vd can occur, especially given that the tissue fraction in the body varies among species, and as shown by Kim et al. (2018b) the distribution to different tissues varies several-fold. Since nonhuman primates were expected to be closer to humans in body composition than rats or mice, the Vd values in human males and females was assumed equal to the values estimated by Sundström et al. (2012) for male and female monkeys, respectively. There is uncertainty in this assumption, that would be reduced by measurements of the PFHxS Vd in humans.

A Bayesian PK analysis was conducted that combines data from across studies and doses listed in Table 3-1 for male and female rats and mice (summary in Section 3.1.6, details provided in Appendix E). This analysis provided both an overall mean and a credible interval for the Vd for each of these species and sexes. The analysis for rats was restricted to oral dosimetry data because the reported PK parameters indicated some discrepancy between the results for IV and oral dosimetry that were unlikely to be resolved by the empirical modeling approach used here, and the bioassay results that will be extrapolated using the PK parameters are from oral exposures. Because only IV route data were available for monkeys, those data were used for that species.

Table 3-1. Estimated Volume of distribution (Vd) values in rats, mice, and monkeys

Study	Vd (mL/kg)	Notes
Male rats		
Sundström et al. (2012)	275 ± 5[a]	10 mg/kg IV, n = 4, 10 w time-course
Kim et al. (2016b)	269 ± 52[b]	4 mg/kg IV, n = 5, 72 d
	278 ± 4[b] 264.4 (255.6–272.6)	4 mg/kg oral, n = 5, 72 d
Kim et al. (2018b)	315 ± 23[b]	10 mg/kg IV, n = 5, 14 d
	327 ± 10[b] 293.4 (262.9–323.9)	10 mg/kg oral, n = 5, 14 d
Huang et al. (2019a)	224 ± 32[c]	4 mg/kg IV, n = 3/time point, 50 d
	123 ± 11[d] 137.8 (116.2–159.6)	4 mg/kg oral, n = 3/time point, 50 d
	137 ± 9[d] 144.2 (121.1–166.5)	16 mg/kg oral, n = 3/time point, 50 d
	192 ± 17[d] 210.7 (176.9–243.2)	32 mg/kg oral, n = 3/time point, 50 d
Population mean	216.5 (149.2–281.4)	
Female rats		
Sundström et al. (2012)	278 ± 66[a]	10 mg/kg IV, n = 3, 24 h
	126 ± 14[a]	10 mg/kg IV, n = 4. 10 w
Kim et al. (2016b)	289 ± 24[b]	4 mg/kg IV, n = 5, 14 d
	256 ± 18[b] *286.9 (264.5–309.6)*	4 mg/kg oral, n = 5, 14 d
Kim et al. (2018b)	176 ± 11[b]	0.5 mg/kg IV, n = 5, 14 d
	191 ± 7.5[b]	1 mg/kg IV, n = 5, 14 d
	130 ± 5.5[b]	4 mg/kg IV, n = 5, 14 d
	154 ± 20[b]	10 mg/kg IV, n = 5, 14 d
	187 ± 3.5[b] *196.0 (117.2–213.6)*	1 mg/kg oral, n = 5, 14 d
	159 ± 7.8[b] *236.3 (215.5–257.6)*	4 mg/kg oral, n = 5, 14 d
Huang et al. (2019a)	144 ± 18[c]	4 mg/kg IV, n = 3/time point, 22 d
	155 ± 9[d] *162.8 (142.9–183.2)*	4 mg/kg oral, n = 3/time point, 22 d
	186 ± 14[d] *187.9 (166.5–208.5)*	16 mg/kg oral, n = 3/time point, 22 d

Study	Vd (mL/kg)	Notes
	264 ± 20[d] *261.9 (231.9–290.2)*	32 mg/kg/ oral, n = 3/time point, 22 d
Population mean	*224.2 (182.7–266.4)*	
Male mice		
Sundström et al. (2012)	129[b]	1 mg/kg oral, n = 4/time point, 23 w
	195[b]	20 mg/kg oral, n = 4/time point, 23 w
Population mean	*154.6 (122.6–185.5)*	
Female mice		
Sundström et al. (2012)	96[b]	1 mg/kg oral, n = 4/time point, 23 w
	147[b]	20 mg/kg oral, n = 4/time point, 23 w
Population mean	*123.0 (104.5–140.6)*	
Male monkeys		
Sundström et al. (2012)	287 ± 52[b] *282.4 (251.9–314.9)*	10 mg/kg IV, n = 3, 171 d
Female monkeys		
Sundström et al. (2012)	213 ± 28[b] *228.5 (204.4–252.5)*	10 mg/kg IV, n = 3, 171 d

Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix E (oral exposure data).
[a]VdSS from two-compartment PK model.
[b]Vd from noncompartmental PK analysis.
[c]Sum of central and peripheral compartment volumes obtained with a 2-compartment PK model.
[d]Vd from one-compartment PK model.

While Vd in rodents for a number of PFAS have generally been found to be less than 1,000 mL/kg (1 L/kg), reported values do vary considerably. For example, Huang et al. (2019a) reported respective male and female rat values for total Vd of:

- 170–340 and 170–420 mL/kg for PFBS;
- 300–680 and 220–420 mL/kg for PFOS given doses of 2 mg/kg; but
- 79 and 56 mL/kg for PFOS given a dose of 20 mg/kg

(Dzierlenga et al., 2019) reported respective male and female rat values for total Vd of:

- 300–620 and 223–560 mL/kg for PFHxA;
- 150–200 and 79–340 mL/kg for PFOA; and
- 410–630 and 270–410 mL/kg for PFDA.

In part, these ranges, and differences in reported Vd values between laboratories reflected both experimental variability and differences in the pharmacokinetic analyses used, which may have been more or less sensitive to variability in the data. Experimental design, such as the timepoints selected for measurement and duration of a PK study also impact Vd estimates. But some of the variability demonstrated here between different PFAS almost certainly represents true differences in their chemical properties. A comprehensive review of such factors is beyond the scope of this assessment, but these data indicated that the reported Vd values for PFHxS were well within the overall range observed for several other PFAS.

The only study to evaluate Vd in humans directly from human data for PFHxS (vs. using a value obtained for other PFAS or in other species) was that of Chiu et al. (2022), who applied a one-compartment PK model in a Bayesian analysis of human serum concentrations matched with drinking water (DW) concentrations of several PFAS, including PFHxS, from multiple community studies. The analysis only included adults who were determined unlikely to have occupational exposure (i.e., for whom DW was likely to be the primary exposure) with corresponding DW concentrations measured prior to measurement of their serum concentration. The overall approach and parameter estimation method were considered sound. The value of Vd obtained for PFHxS (95% CI) was 0.25 (0.15, 0.42) L/kg, which is almost identical to the average of the Vd values estimated for male and female monkeys (Table 3-1).

Distribution in Blood/Proteins

The low estimated volume of distribution of PFHxS reflects the relatively high amount of the chemical found in plasma. A major factor in this distribution was attributed to the interaction between PFHxS and proteins in plasma, including albumin and transthyretin (Alesio et al., 2022; Forsthuber et al., 2020; Bischel et al., 2011; Weiss et al., 2009). An investigation of protein binding showed that in human plasma PFHxS was 99.98% bound to protein with no sex-specific difference (Kim et al., 2018b). The same study reported 99.92% binding to protein in male rat plasma and 99.93% binding to protein in female rat plasma (Kim et al., 2018b). Binding to plasma proteins may also drive the partitioning of PFHxS within blood components for which greater levels of PFHxS were measured in serum and plasma compared with whole blood. Poothong et al. (2017) found median ratios of 1.06 between serum and plasma, 1.88 between serum and whole blood, and 1.75 between plasma and whole blood in adult men and women. Hanssen et al. (2013) found a median ratio of 1.58 between plasma and whole blood in women just after the delivery of a child. Jin et al. (2016) determined a mass fraction in plasma of 0.87 in adult men and women. Liu et al. (2023) obtained a similar mean fraction in plasma of 0.84 specifically for *n*-PFHxS, but higher fractions of 0.9 and 0.93 for two branched isomers.

Fetal Blood and Placenta

Studies of the associations between maternal serum levels and umbilical cord blood levels of PFHxS demonstrated transfer through the placenta (Kang et al., 2021; Li et al., 2020a; Chen et al., 2017; Hanssen et al., 2013; Lee et al., 2013; Zhang et al., 2013a; Fromme et al., 2010; Monroy et al., 2008). Lee et al. (2013), Chen et al. (2017), Kang et al. (2021), Li et al. (2020a) and Zhang et al. (2013a) showed greater concentrations of PFHxS in maternal serum relative to cord serum, a phenomenon that also has been observed for other PFAS such as PFOA and PFOS (e.g., Li et al. (2020a)). Lee et al. (2013) analyzed pairwise data to determine a cord serum: maternal serum ratio of 0.57 ± 0.29 (mean ± SD). Chen et al. (2017) similarly found a geometric mean cord serum:maternal serum ratio of 0.54. Kang et al. (2021) calculated an arithmetic mean cord serum:maternal serum ratio of 0.365. Hanssen et al. (2013) observed a median cord:maternal ratio of 0.53 in plasma and a median cord:maternal ratio of 0.43 in whole blood from pairwise data. Zhang et al. (2013a) also examined the ratio in whole blood and found a cord:maternal blood ratio of 0.294. Li et al. (2020a) compared cord: maternal serum ratios from preterm versus full-term deliveries and reported a median ratio of 0.40 for preterm versus 0.72 for full-term, with the difference being statistically significant. The authors suggest that this increase in distribution may be due to placental aging, resulting in a reduced capacity to limit transfer of xenobiotics, though they also consider simple accumulation with time as a mechanism (Li et al., 2020a). Li et al. (2020a) also evaluated the role of nine placental transporters, testing for correlation between their expression and the cord:maternal serum ratio. However, the only significant correlation was with folate receptor alpha (FRα) in preterm deliveries (i.e., not full term), with a positive correlation coefficient, indicating that FRα facilitates transfer to the fetus.

In contrast, Monroy et al. (2008) observed cord serum concentrations that were significantly higher than maternal serum concentrations based on a paired *t*-test and linear regression analysis. However, these data were highly censored, with the prevalence of samples above the level of detection in umbilical cord serum (20%) lower than in maternal serum (45.5%).

The observed relationship between maternal serum and umbilical cord serum could be an artifact due to the higher prevalence of umbilical cord samples below the level of detection.

To quantitatively compare the distribution between tissues and maternal blood matrices among different studies, adjustment were made to correct for the distribution among blood components. As described above, Poothong et al. (2017) measured a median ratio of 1.88 for serum: whole blood, 1.75 for plasma: whole blood, and 1.06 for serum: plasma concentrations of PFHxS. These values were used to adjust subsequent tissue:blood matrix ratios to tissue:serum, when reported for whole blood or plasma.

Serum and plasma are components of whole blood, with the main other component (by volume) being red blood cells. Assuming that PFHxS partitions completely into the plasma and not the red blood cells, a theoretical maximum ratio between the plasma and whole blood was calculated, that is, as if whole blood is a dilution of plasma with red blood cells. The small additional volume contribution from other components of whole blood not present in plasma or serum were assumed to not substantially affect this theoretical ratio. The most common metric for the composition of whole blood is the hematocrit (Hct), which is the ratio of the volumes of red blood cells and whole blood. In terms of Hct, the theoretical maximum ratio of plasma:whole blood was calculated as 1/(1-Hct). The normal range of hematocrit for men is 42–52 % and for women is 37–48 % (Jordan et al., 1992). Inputting a typical human male Hct of 45% gave a plasma:whole blood ratio of 1.82. In females, Hct is typically lower, which resulted in a lower estimated maximum plasma:whole blood ratio. Using the reported plasma:whole blood ratio of 1.7 and a Hct of 45% the fraction of PFHxS in plasma (Fp) was calculated to be 1.7 × (1-Hct) = 93.5%, which is very high but consistent with the high level of plasma protein binding described above. The median ratio of 1.88 serum:whole blood reported by Poothong et al. (2017) is greater than the theoretical maximum and implies a Hct of ≥46.8%, which is in the normal range for men, but slightly higher than the normal range for women. The population of Poothong et al. (2017) was approximately 75% women, which may indicate a deviation from the ideal behavior assumed for the calculation, variation in Hct, or an experimental error in the measurement of concentrations or in the separation. Partitioning of PFHxS and other PFAAs between human plasma and blood cells was also investigated by Jin et al. (2016), who obtained a mean Fp = 91% and report a mean serum:whole blood ratio of 1.6. The average of serum: blood ratio of 1.6 from Jin et al. (2016) and 1.88 from Poothong et al. (2017) is 1.7. Given Hct = 0.45, this value implies 95.7% of PFHxS is in serum, which is still reasonable. Therefore, a serum:blood ratio of 1.7 was used to convert tissue partitioning data relative to whole-blood concentrations to serum-based concentrations below.

The empirical data of Hanssen et al. (2013), although limited by a modest number of subjects with data over the limit of detection, indicated generally higher serum:whole blood ratios in cord serum and blood than maternal serum and blood, with ratios for multiple samples (subjects) reported as 2.2 or higher. This difference can be explained in part by a higher hematocrit in later gestation and newborns than in adults (mean hematocrit ~51% for gestation week 42 and full-term newborns) (Jopling et al., 2009). One study included in Table 3-2 below (Zhang et al., 2013a) reported concentrations of PFHxS for whole maternal and cord blood, rather than serum levels. Therefore, the resulting ratios for matched samples (obtained from the supplemental data of Zhang et al. (2013a)) were adjusted by the ratio 0.55;0.49, that is, $(1\text{-}Hct_{adult})/(1\text{-}Hct_{fetus})$ to account for the expectation that serum:whole blood concentrations will be higher in the fetal cord blood than in the adult.

With the adjustment noted above, median (mean) values of cord serum:maternal serum ratios in humans at childbirth were 0.53 on average (see Table 3-2). That the value is roughly 50% indicated that the placenta may limit transfer of PFHxS from the mother to the fetus, but if distribution to fetal tissues is increased in proportion to water content of tissue, as discussed below, then an overall higher concentration in the fetus versus maternal tissue is predicted. There was not an apparent trend in the ratio related to the maternal sample timing relative to childbirth (i.e., whether taken before, at, or after childbirth) or the fraction of cord or maternal serum measurement below the limit of detection, although as described above Li et al. (2020a) reported a significant increase in the ratio from preterm to full-term deliveries. Examination of the standard deviation of the mean of medians and mean of means shows that the two values are, on average, similar, suggesting that the distribution of cord serum:maternal serum ratio is symmetric. However, it is notable that the reported median value is lower than the mean value in almost every study.

Table 3-2. Measured cord serum: maternal serum ratios

	Cord serum: maternal serum ratio		% > LOD		
Study	**Median**	**Mean**	**Cord**	**Maternal**	**Maternal sample timing**
Chen et al. (2017)	0.55	0.6	97%	97%	Within 3 d prior to delivery
Hanssen et al. (2013)	0.54	0.63	100%	100%	3–5 d after delivery
Kang et al. (2021)	0.315	0.365	97%	100%	At delivery, exact timing not clear
Kim et al. (2011b)	0.65	0.64	100%	100%	20–41st wk of pregnancy, mostly in 3rd trimester
Lee et al. (2013)	0.5	0.57	100%	100%	At delivery, exact timing not clear
Liu et al. (2011)	0.73	0.95	96%	98%	Within 1 wk after delivery
Yang et al. (2016b)	0.35	0.43	100%	100%	1–2 d before delivery
Yang et al. (2016c)[a]	0.52	0.63	96%	100%	Within 1 wk after delivery
Zhang et al. (2013a)	0.332	0.387	100%	100%	Within 1 hr prior to delivery
Li et al. (2020a) preterm	0.40	NR	81%	81%	Within 1 wk before delivery
Li et al. (2020a) full-term	0.72	NR	94%	94%	Within 1 wk before delivery

	Cord serum: maternal serum ratio		% > LOD		
Study	**Median**	**Mean**	**Cord**	**Maternal**	**Maternal sample timing**
Overall mean[b]	0.50±0.14	0.58±0.17			

NR = not reported.
[a]Cord: maternal serum ratios for this study are the ratio of the reported median (mean) values for cord and maternal serum.
[b]Mean and standard deviation of the set of medians or means.

After correction for the serum:whole blood ratio as described above, comparisons between maternal serum and placenta were reasonably consistent: Chen et al. (2017) observed median (mean) placenta:maternal serum = 0.421 (0.429) and applying the serum:whole blood factor of 1.7 to the results of Zhang et al. (2013a) the EPA obtained median (mean) = 0.266 (0.289). Chen et al. (2017) suggested that the difference between their results and those of Zhang et al. (2013a) was due to variation in isomeric composition between the two study populations or the greater range in concentration in the placentas in the study of Zhang et al. (2013a), but with the correction applied here it appears to be modest. The volume of distribution estimated for PFHxS in female monkeys was Vd = 0.213 L/kg (Sundström et al., 2012), which represents the average of distribution into all tissues. While the placenta distribution measurements in humans of Chen et al. (2017) and Zhang et al. (2013a) were 1.5 to 2 times higher than this value for female monkeys, Kim et al. (2018b) showed greater variability in PFHxS concentrations between specific tissues of rats. Hence, the reported placenta: serum levels of Chen et al. (2017) and Zhang et al. (2013a) were not outside the range one would expect for a specific tissue given an overall Vd of 0.213 L/kg, i.e., if distribution to adipose and muscle was substantially less than internal organs, as was observed for rats by Kim et al. (2018b).

As umbilical cord blood followed the same trend as in adult blood, the results from Chen et al. (2017) and Zhang et al. (2013a) were consistent with a concentration trend of cord serum > placenta > cord whole blood.

One study that distinguished between isomers of PFHxS found the greatest prevalence of the linear relative to the branched isomer in cord serum (97% linear), followed by maternal serum (86% linear) and placenta (77% linear) (Chen et al., 2017).

Distribution in Fetal Tissues and Children

One study provides a relatively unique dataset of PFHxS concentrations in human fetal tissues obtained from voluntary abortion (gestation week < 12) or after intrauterine fetal death in the second and third trimester, and in maternal serum collected at these times (Mamsen et al., 2019). However, PFHxS was detected in only 6% of fetal tissues, making it difficult to interpret these data quantitatively.

Pharmacokinetic modeling of PFOA dosimetry in humans by Goeden et al. (2019) suggested a reason why observed tissue levels of PFAS in the fetus and young children may have been greater than in adults: the greater amount of extracellular water in the tissues of fetuses and children (Friis-Hansen, 1961) led to a greater distribution of PFAS into these tissues. As noted above, the Vd values estimated for adult rats, mice, and monkeys are consistent with the assumption of distribution in body water. The amount of extracellular water in newborns was estimated to be 2.4 times higher than adults (Friis-Hansen, 1961) (see Figure 3-2).

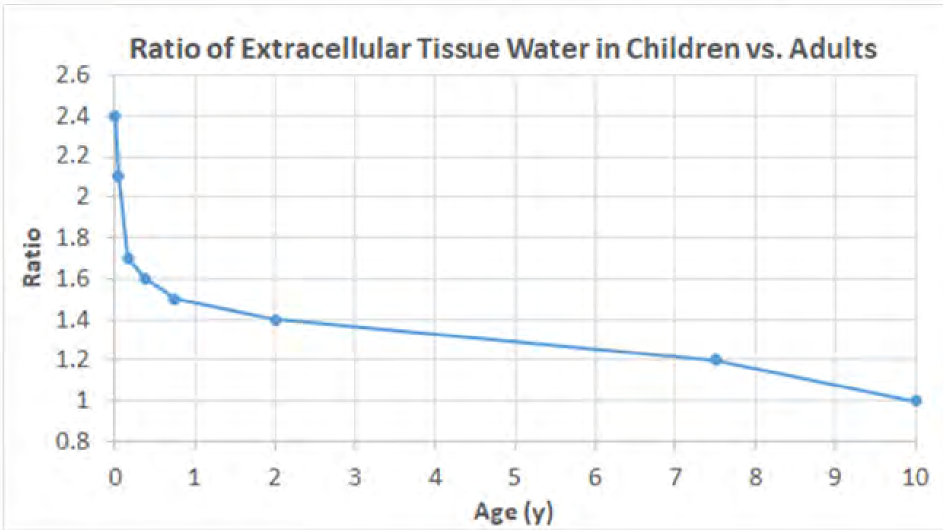


Figure 3-2. Ratio of extracellular water (% of body weight) in children versus adults. Values (points) were calculated from results in **Friis-Hansen (1961)** and plotted at the mid-point for the corresponding age ranges evaluated.

Mamsen et al. (2019) (described briefly above) only detected PFHxS in 6% of fetal tissue samples and did not report ratios of fetal tissue to maternal serum for PFHxS. So, while their data may indicate that average fetal levels are much lower than maternal levels, they cannot be used to quantify the fetal-maternal relationship. Since PFHxS is amphiphilic, with Vd < 1 in adults, it is not expected to distribute with or in proportion to body fat and therefore fetal body fat content is not considered an appropriate predictor of fetal PFHxS distribution. Given the overall lack of data on fetal distribution of PFHxS, EPA considers any estimate of such distribution to be uncertain. In the face of this uncertainty, EPA chose the simplest assumption for prediction of fetal body burdens: that distribution between fetal serum and fetal tissues is the same as the distribution between serum and tissues in the newborn. The alternative, which would be to assume that there is a discontinuity (sudden increase or decrease) in the body burden of the offspring at the moment of birth, would require a more specific assumption about the magnitude and direction of that discontinuity. Likewise, assuming any other change in Vd over the time of fetal development and birth would also have no supporting data and therefore involve equal or greater uncertainty. There are no clear developmental PK data for PFHxS that could be used to guide a choice among these alternatives. Hence, EPA simply assumed that the ratio of body water in the newborn versus adults (2.4) also applies to the fetus.

Since the Vd in a human woman (mother) is assumed to be the same as in monkeys, given the assumption that Vd in a fetus is 2.4 times higher than an adult, the estimated Vd in a female fetus relative to fetal serum is 2.4 × 0.213 L/kg = 0.511 L/kg and in a male fetus 2.4 × 0.287 L/kg = 0.689 L/kg. But as described above, the average ratio of PFHxS in cord serum, which is assumed to be fetal serum, compared with maternal serum was $r_{f:m}$ = 0.52. Together, these values and assumptions led to the prediction that relative to *maternal* serum, the Vd for the fetus as a whole is 0.52 × 0.511 L/kg = 0.266 L/kg for females and likewise 0.358 L/kg for males, indicating average fetal tissue concentrations is 25% higher than average maternal tissues for girls and 68% higher for boys. Hence, the body burden in the newborn can be estimated using the following equation:

$$\text{amount of PFHxS in newborn} = r_{f:m} \times C_{mother} \times Vd_{newborn} \times BW_{newborn}, \qquad (3\text{-}1)$$

where $r_{f:m}$ = 0.52 and $Vd_{newborn}$ is 0.511 L/kg for girls and 0.689 L/kg for boys.

The average weight of a newborn is only 5% of maternal body weight (3.4 versus 68 kg), so while distribution into the male fetus was estimated to be 68% higher than maternal tissues, the effect on Vd of the mother and fetus together (i.e., total amount in the mother and fetus compared with maternal serum concentration) was thereby estimated to be less than 3.4% (5% × 68%). Therefore, the Vd for mother and fetus together during pregnancy was simply assumed equal to the value for the adult woman (0.213 L/kg), although the amount in the newborn child was calculated as described above. Because the maternal weight just after childbirth is reduced by more than the weight of the newborn, reflecting the loss of amniotic fluid, placenta, etc., this choice effectively assumed slightly less PFHxS mass is lost with those fluids than would be calculated if total maternal and fetal Vd were increased. The interpolation function shown in Figure 3-2 can be multiplied by the adult Vd (L/kg) to obtain the corresponding value for children under 10 years of age, as was done by Goeden et al. (2019). However, an opposing factor is the approximately 20% larger blood volume as a fraction of BW in young children compared with older children and adults (Darrow et al., 1928), given that a high fraction of PFHxS is bound to blood proteins. More specifically, the mass of PFHxS bound to blood proteins would increase in proportion to the total mass of those proteins, which one might expect to increase in proportion to blood volume. Hence, a 20% larger blood volume could be expected to reduce the PFHxS available for distribution to tissues by 20%. So, instead of an increase of 2.4-fold in Vd in newborns one might predict an increase of 1.9-fold (i.e., 80% × 2.4).

Trend in Pregnancy

Four studies investigated how PFHxS levels tend to change during pregnancy and nursing. Monroy et al. (2008) found that mean maternal serum PFHxS concentration did not change between sampling at 24–28 weeks and sampling at delivery. Likewise, Oh et al. (2022) observed only a slight average decrease in maternal PFHxS over the course of pregnancy, not statistically significant. (Varsi et al., 2022) observed PFHxS serum concentrations in pregnant women at 18, 28, and 36 weeks. Total PFHxS concentrations were relatively constant during this time, but there were differences observed between PFHxS isomers. Linear PFHxS decreased during pregnancy and was lower than concentrations observed in women who had never been pregnant at all timepoints. Branched PFHxS however was highest at the 36 week timepoint, compared to concentrations at 18 and 28 weeks and compared to the non-pregnant women. Glynn et al. (2012) presented data for other PFAS on the relative serum concentrations during pregnancy and nursing but did not present that information for PFHxS, although PFHxS was included in other analyses in that study.

Breast Milk

PFHxS has been observed in human breastmilk, indicating that nursing acts as a route of excretion for the mother and a route of exposure for her infant (Kim et al., 2011b; Karrman et al., 2010; Kärrman et al., 2007). Blomberg et al. (2023) evaluated longitudinal changes in breast milk concentrations of PFHxS between delivery and up to 8 months postpartum; while milk concentrations declined among the women with the highest levels at 0-2 months postpartum (i.e., over 500 pg/mL), they were more constant among those with early concentrations of 300 pg/mL or lower. This decrease can be viewed as supporting this hypothesis, but some caution is needed in interpreting these data as the drinking water source for the most highly exposed part of the cohort was switched to a less contaminated source as soon as the contamination was identified, i.e., decreased exposure through drinking water could also drive decreased breast milk concentrations, independent of excretion through breast milk. However, Oh et al. (2022) observed a significant decline in maternal serum levels (average decline of 5.6%) during the first six months postpartum in a population with typical PFHxS exposure (with no intervention to reduce exposure). This provides some additional potential evidence of increased excretion of PFHxS after giving birth, without an artificial change in PFHxS exposure.

In paired milk and maternal serum samples, the concentrations were highly correlated (Pearson $r^2 = 0.8$) (Kärrman et al., 2007). The concentration of PFHxS in breastmilk was reported to be lower than the concentration in paired maternal serum, with ratios between milk and maternal serum of 0.02 (Kärrman et al., 2007) and 0.008 (Kim et al., 2011b). Karrman et al. (2010) reported PFHxS concentrations in breast milk samples but did not have paired maternal blood levels, which limits the ability to specify the distribution into breast milk compared with other body compartments. Another study found that PFHxS was below the limit of detection in all breast milk samples collected (Liu et al., 2011). Mondal et al. (2014) investigated the association between PFHxS concentration in maternal and infant serum and the length of breastfeeding and found that, although there were associations consistent with breastfeeding acting as a route of excretion for the mother and a route of exposure for the infant, none of the associations rose to the level of significance. Significant associations were found for other PFAS studied and negative associations for maternal serum and length of breastfeeding and positive associations for infant serum and length of breastfeeding were consistent across PFAS. Varsi et al. (2022) observed paired maternal and infant serum concentrations, with one infant timepoint at 6 months of age, and six maternal timepoints, three during pregnancy and four postpartum. At 6 months after delivery, the relative concentrations of PFHxS in the infant and mother differed by isomer, with the infants having a higher median linear PFHxS concentration and a lower median branched PFHxS compared to the mothers. Similarly, the branched:linear isomeric ratio was lower in the infant compared to the mother. This could indicate a preferential transfer of the linear isomer to the infant, either during gestation or lactation. Potential evidence for gestational transfer is the increase in maternal branched:linear isomeric ratio that the authors observed between the 28th and 36th week of pregnancy. Evidence for lactational transfer is the association the authors observed between infant linear PFHxS concentration and months of exclusive breastfeeding, a relationship that was not present for the branched isomer.

3.1.3. Metabolism

Due to the high stability of the perfluoroalkyl bonds, PFHxS is thought to not be metabolized in mammals, as was seen for similar PFAS (Lau et al., 2007). Studies have examined similar PFAS, including perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) and identified only the parent compound in excreta (Vanden Heuvel et al., 1991a, b). The sulfonate analog of PFOA, perflurosulfonic acid (PFOS), is also not metabolized (Lau et al., 2007).

3.1.4. Excretion

Animals

Several studies examined the excretion of PFHxS from animals, particularly rats, after a controlled exposure (Huang et al., 2019a; Kim et al., 2018b; Kim et al., 2016b; Sundström et al., 2012; Benskin et al., 2009). Excretion has been observed in urine and feces, with renal excretion being the most prominent route. Other studies have only indirect observation of excretion through the decreasing amounts of PFHxS in the serum over time. As PFHxS is not metabolized, decreases in serum concentration after the distribution phase were attributed to excretion, assuming a constant serum:tissue ratio. As noted above, the distribution phase may not be complete after a relatively short time given the shifts in liver: serum ratio observed over 50 days (Huang et al., 2019a). To quantify the impact of such a shift on estimated excretion would require a PBPK model for PFHxS that accounts for the time-dependence in specific tissue volumes and distribution, which is not in the realm of available science. Since the extended time-dependent distribution appears to be confined to the liver, the analysis based on empirical evaluation of excretion was still assumed to provide a sufficient approximation for dosimetric extrapolation.

In animal studies, urinary excretion was greater than fecal excretion. There was a strong sex-dependence in rats and mice in renal excretion with female rats excreting more of the total dose in urine. Specifically, Kim et al. (2018b) reported 15.9% of the initial IV dose was excreted in urine and 1.3% of the dose was excreted in feces in male rats and 39.1% of the dose was excreted in urine and 3.1% of the dose was excreted in feces in female rats after 14 days. Similarly, after an oral dose, in male rats 18.5% of the dose was excreted in urine and 2.8% in feces, while in female rats 36.8% of the dose was excreted in urine and 3.3% in feces. In another study Kim et al. (2016b), reported that female rats excreted 28.02% of an IV dose in urine after 14 days while male rats excreted 8.26% of the dose in urine after 72 days. Sundström et al. (2012) reported that twenty-four hours after an IV dose, female rats excreted 13.28% of the dose, while male rats excreted 0.70% of the dose.

In mice, the total dose excreted in 24 hours was dose dependent, with 0.882% of a 1 mg/kg dose and 1.654% of a 20 mg/kg dose excreted in males and 0.317% of a 1 mg/kg dose and 2.552% of a 20 mg/kg dose excreted in females (Sundström et al., 2012). The lower excretion in female versus male mice for the 1 mg/kg dose was the only situation with a greater male rodent excretion (Sundström et al., 2012). Urinary excretion was slower in monkeys, with 0.102% of an IV dose excreted in urine in 24 hours in male monkeys and 0.055% of the dose in female monkeys (Sundström et al., 2012). Unlike rodents, there was not a clear difference between monkey sexes in the amount of urinary excretion.

In addition to observations in excreta, multiple studies also estimated the rate of decrease in serum or plasma levels of PFHxS in the form of a half-life or clearance (CL) in rats (Huang et al., 2019a; Kim et al., 2018b; Kim et al., 2016b; Sundström et al., 2012; Benskin et al., 2009). While all of these studies appear to have been conducted with appropriate quality, there is significant variation in the results. For example, Kim et al. (2018b) estimated a CL of 228 mL/kg-day in female rats after an intravenous (IV) dose of 4 mg/kg, while Huang et al. (2019a) estimated a CL of 46 mL/kg-day in female rats after an oral dose of 4 mg/kg. Despite the significant variability in the results between studies, routes of exposure, and to an extent, doses of PFHxS, a quite consistent result is that the CL in male rats is about an order of magnitude lower than female rats, and so the subsequent analysis evaluates parameters for male and female rats separately.

An issue found in the PK data is that for some studies that used both IV and oral doses, the blood AUC was higher after the oral dose than after the same dose given IV, which contradicts classical PK analysis. For example, given doses of 4 mg/kg Kim et al. (2016b) reported an AUC almost twice as great after oral dosing than after IV dosing in female rats, and Huang et al. (2019a) reported an AUC 40% higher after oral dosing than after IV. By classical PK analysis one expects that only a fraction of an oral dose will be absorbed but that the subsequent distribution and elimination are otherwise identical to what is observed after IV dosing. In that case, the AUC after oral dosing would be less than or equal to the AUC after IV dosing, to the extent that there is limited oral bioavailability. A key assumption in this classical analysis is that distribution and elimination are independent of the exposure route, and EPA interpreted these discordant empirical results as suggestive that this assumption is incorrect. EPA's analysis of PK data supported this possibility, with a trend of greater clearance following IV exposure compared to gavage in female rats (see 3.1.6 Empirical Pharmacokinetic Analysis). The mechanistic explanation for this difference is not obvious. Excretion could be greater after IV dosing if, immediately after dosing, a smaller proportion of PFHxS is bound to tissue phospholipids and serum proteins compared with the oral dosing scenario. This could occur if equilibration between bound and free PFHxS takes some time. Absorption from the GI tract is slower and PFHxS first passes through the liver (where a significant fraction is retained) before systemic distribution, which would allow for equilibration between free and bound states as PFHxS enters the blood. Thus, a higher fraction of PFHxS could have been bound when first reaching general circulation after oral dosing than after IV dosing, such that the urinary excretion after oral dosing was slower. A similar mechanistic explanation for differences in protein binding is that passage through the acidic environment of the stomach results in a greater proportion of the PFHxS anion, which could facilitate binding and thus limit excretion compared to IV exposure.

Because the toxicological bioassays that will be interpreted with the PK model used oral administration, it was considered clearly preferable that the PK parameters used should reflect that route of exposure. Given the oral-IV discrepancies noted above, only results from oral PK experiments were evaluated for rats and mice. Key PK parameters from these oral PK experiments are listed in Table 3-3.

A factor to be noted in Table 3-3 and discussed previously was that the data of Huang et al. (2019a) indicate higher CL in male and female rats given a dose of 32 mg/kg compared with 4 and 16 mg/kg. While the difference was not indicated as statistically significant, it was consistent with a mechanism of saturable renal resorption (Weaver et al., 2010; Yang et al., 2009) and with the end-of-study serum concentration data shown in Figure 3-1 (NTP, 2019). Comparing results for the lower two doses, the CL estimated by Huang et al. (2019a) for 16 mg/kg in female rats was 25% higher than that estimated at 4 mg/kg and the CL for 16 mg/kg in male rats was 19% higher than that estimated at 4 mg/kg. Although not statistically significant, this was interpreted as likewise consistent with some dose-dependence. On the other hand, the CL reported for female rats at 32 mg/kg by Huang et al. (2019a) was below that reported by Kim et al. (2016b) at 4 mg/kg and the CL for male rats at 32 mg/kg by Huang et al. (2019a) was below that estimated from the results of Benskin et al. (2009) presumably due to inter-study variability. Hence, subsequent PK analyses included data for all dose levels from Huang et al. (2019a).

Overall mean CL values and confidence intervals for male and female rats, mice, and monkeys were obtained by Bayesian PK analysis of all the oral PK data for each sex of rodents and the IV PK data for each sex of monkeys (summary in in Section 3.1.6, analysis details provided in Appendix E).

Table 3-3. Summary of estimated clearance values in animals

Citation	Dose (mg/kg)	CL[a] (mL/kg-d)	n
Male rats			
Benskin et al. (2009)	0.03	9.85[b]	7
Kim et al. (2016b)	4	7.15 *5.71 (5.46–5.69)*	5
Kim et al. (2018b)	10	6.65 *6.58 (3.34–9.68)*	5
Huang et al. (2019a)	4	4.82 *5.37 (4.61–6.14)*	3[c]
	16	5.74 *5.91 (5.09–6.75)*	3[c]
	32	9.02 *9.74 (8.47–11.03)*	3[c]
Population mean		*7.15 (3.73–10.26)*	
Female rats			
Kim et al. (2016b)	4	124.8 *117.8 (110.7–125.3)*	5
Kim et al. (2018b)	1	81.1 *83.02 (77.22–89.38)*	5
	4	65.3 *106.3 (98.58–113.8)*	5
Huang et al. (2019a)	4	46.1 *50.14 (45.03–55.01)*	3[c]
	16	59.0 *61.36 (55.58–67.17)*	3[c]
	32	92.2 *94.54 (85.43–103.3)*	3[c]
Population mean		*84.10 (64.72–103.8)*	
Male Mice			
Sundström et al. (2012)	1		4
	20		4
Population mean		*3.86 (3.27–4.41)*	
Female mice			
Sundström et al. (2012)	1		4
	20		4
Population mean		*3.18 (2.83–3.52)*	
Male monkeys			
Sundström et al. (2012)	10	1.33 ± 0.12 *1.39 (0.94–1.83)*	3
Female monkeys			

Citation	Dose (mg/kg)	CL[a] (mL/kg-d)	n
Sundström et al. (2012)	10	1.93 ± 0.41 *2.12 (1.81–2.44)*	3

Only oral exposure results are shown for rats because there were discrepancies between oral and IV data that could not be resolved and the oral route was used in the bioassays evaluated for toxicity. Only oral dosimetry data were available for mice and only IV dosimetry data were available for monkeys (results shown; (Sundström et al., 2012)).

[a]Values in italics are mean (90% credible interval) from Bayesian analysis (details in Appendix E).

[b]Calculated from reported half-life ($T_{0.5}$) for n-PFHxS as CL = ln(2)*Vd/$T_{0.5}$ using the geometric mean of Vd values for male rats listed in Table 3-1. Serum time-course data were not available from Benskin et al. (2009), so results from this study were not used in the Bayesian analysis.

[c]Number of rats per time point, but each rat had blood taken at no more than two time points, so the total number of rats used per dose level where much higher (Huang et al., 2019a).

While the results summarized in Table 3-3 were obtained by empirical analysis for total clearance, it is worth noting the fraction of PFHxS eliminated in feces reported by Kim et al. (2018b) was used as a means of estimating fecal clearance in humans. These data were used to estimate total clearance for studies where renal clearance was measured and were deemed most appropriate as primate and human-specific data were unavailable. The ratio of average PFHxS excretion in feces versus urine was 8.2% and 7.9% in male and female rats, respectively, after IV dosing and 15.1% and 9.0%, respectively, after oral dosing (Kim et al., 2018b). The higher fraction eliminated in feces after oral dosing was attributed in part to incomplete absorption by that route. Therefore, an average value of 8% from the IV data was used for extrapolation to humans.

The excretion of PFHxS has been observed in humans both directly through measurement of PFHxS in urine and indirectly through the observation of changes in serum or plasma concentrations over time. Changes in serum or plasma concentrations are informative of excretion because PFHxS is not metabolized, thus any observations of decreasing concentrations in blood after the distribution of the chemical were attributed to excretion. Most observations were within populations with higher exposure than the general population, either workers in fluorochemical production (Fu et al., 2016; Gao et al., 2015; Olsen et al., 2007), workers at a fishery where the waters were contaminated with PFAS (Zhou et al., 2014), or with increased exposure via contaminated drinking water (Li et al., 2018; Worley et al., 2017). For measures of clearance and half-life, geometric means were presented unless otherwise specified because geometric means are less influenced by extreme values that are common in these skewed distributions.

Humans

Half-life estimates

Four studies reported half-life values for PFHxS based on observations of decreasing serum levels in individual subjects at multiple time points after decreased exposure, either due to retirement after occupational exposure (Olsen et al., 2007), replacement of the foam used by firefighters (Nilsson et al., 2022) or to the introduction of drinking water filtration at an occupational site (Li et al., 2022b; Li et al., 2018). Li et al. (2022b) is a follow-up analysis of the population evaluated by Li et al. (2018). All four studies, the Nilsson et al. study and the Olsen et al. study fit the data for each person separately. Several plots in Olsen et al. (2007) showed declines in serum levels over time that were very close to log-linear (i.e., showed negligible positive curvature), which is suggestive of little effect of ongoing exposure for those subjects. However, Li et al. (2022b) obtained a shorter half-life using data collected between six months and one year after the end of exposure (mean $t_{1/2}$ = 3.85 years) compared to using data collected 1-2.5 years after the end of exposure (mean $t_{1/2}$ = 4.33 years) or 2.5-4.5 years after the end of exposure (mean $t_{1/2}$ = 4.62 years). Positive curvature in a serum time-course plot after a decrease in exposure (for example retirement), which is indicated by these results from Li et al. (2022b), is evidence of background exposure, as can be observed by examining Eq. 2 in (Bartell, 2012). The differences among half-lives values for the time periods of evaluation reported by Li et al. (2022b), less than 20%, are not statistically significant, however. Li et al. (2018) reported a mean half-life of 7.4 years in males (n = 20) and 4.7 years in females (n = 30) aged 15–50 years old while Li et al. (2022b) reported a median (5th, 95th percentile) half-life of 5.4 (2.34, 9.29) years (n = 114). Olsen et al. (2007) reported a half-life of 8.5 years in their cohort, which consisted of 2 females and 24 males at retirement.

The population of Li et al. (2022b) included children and the mean half-life for those participants 1–14 years of age was 3.01 years compared to 5.26 years for participants 15–50 years of age and 6.41 years in participants over 50 years. The much lower apparent half-life in the 1–14 year old group is almost certainly the result of PFHxS dilution into the growing bodies of the youth. The intermediate half-life for participants 15–50 years of age may be partly attributed to the difference between males (mean 5.39 years) and females (mean 4.48 years) which correlates with the expected higher clearance due to menstrual fluid loss for women in that age range. This difference of 17% in half-life is in contrast to minimal differences of less than 2% between males and females aged 1–14 and less than 3.7% between males and females over age 50.

Nilsson et al. (2022) analyzed PFHxS concentrations in firefighters after PFHxS was removed from the formulation of the foam used for fire suppression. (97.5% of the recruited population were male and the exact number of women in each sub-cohort was not reported, so the results will be assumed to represent males.) The subjects had a range of serum concentrations at the start of the study that overlapped with those found in the general population, which would come from other exposure sources that are presumed to be shared by the study subjects. Since the level of these other exposure sources is not precisely known and likely varies over time, the contribution from them represents an uncertainty that would particularly impact half-life estimates of subjects with initial concentrations in the general population range. Therefore, EPA chose to use the results reported for only those subjects who's initial PFHxS concentration was greater than the 95th percentile of the general population, which ranged from just above that 95th percentile to over 20 times higher. Nilsson et al. (2022) reported a mean (95% CI) half-life of 7.7 (7.1, 8.3) years for this group without background subtraction and a mean (95% CI) half-life of 6.7 (6.2, 7.2) years after subtracting age-specific average concentrations reported for the general Australian population. The half-life calculation assumes a simple exponential decay, which would only be accurate with no ongoing exposure or if background exposure is constant, allowing it to be addressed by simple subtraction, and is reasonable estimated based on results from other study populations, albeit from the same country. The modest difference in the mean half-lives obtained with and without background subtraction for the highly exposed group indicates that background exposure had some impact on the observed changes in serum levels for that group, but less than 15%. Hence, the value obtained for the highly exposed group with subtraction is considered to be appropriate for describing the elimination of the PFHxS from occupational exposure of this cohort with a minimal level of uncertainty due to the assumptions involved.

Worley et al. (2017) estimated a population half-life by fitting a PK model to population mean serum concentrations at two timepoints with an estimated ingestion rate for that population. Because Worley et al. (2017) did not evaluate individual elimination, only measured serum levels at two time points, and relied on an estimated exposure level, their study was considered to have greater uncertainty than the other studies, with results that are more difficult to interpret in terms of being a mean or geometric mean of individual values. In particular, it is possible that the drinking water concentration was not constant as was assumed by Worley et al. (2017) or that there were other significant sources of ongoing exposure. Because of these methodological concerns, the results of Worley et al. (2017) were not used in estimating an overall average clearance for humans, although it is noted that the corresponding clearance (0.031 mL/kg-day) is identical to the estimated geometric mean across other studies (see Table 3-4).

As described in Volume of Distribution (in Section 3.1.2), Chiu et al. (2022) applied a one-compartment PK model in a Bayesian analysis of human serum concentrations matched with drinking water (DW) concentrations of several PFAS, including PFHxS, from multiple community studies. Since the overall approach and parameter estimation method were considered sufficiently sound, the resulting clearance was combined with other published human parameters in estimating overall population clearance and volume of distribution (Table 3-4).

Clearance rates estimated from half-lives

The clearance rate for a single-compartment PK model is related to the half-life and volume of distribution by the following equation:

$$CL = \ln(2) \cdot Vd / T_{0.5}$$

The approach for Bayesian analysis of PK data described in Appendix E was used to re-analyze the monkey PK data from Sundström et al. (2012), resulting in mean volumes of distribution of 278 mL/kg for males and 228 mL/kg for females, for which the average is 253 mL/kg. Using either the sex-specific Vd for corresponding segregated human studies, or the average

Vd for results from mixed populations, values for total human clearance were estimated from the half-life values:

- Li et al. (2018): 0.071 mL/kg-day in males and 0.092 mL/kg-day in females (same participants as Li et al. (2022b)).
- Li et al. (2022b): 0.098 mL/kg-day in male participants aged 15–50 years, 0.064 mL/kg-day in females aged 15-50 years and 0.075 mL/kg-day in males and females aged > 50 years (participants below age 15 not included due to impact of growth)
- Nilsson et al. (2022): 0.079 mL/kg-day in adults (age 22–82, 97%–98% males).
- Olsen et al. (2007): the clearance for each subject was calculated as described above for the 24 men and 2 women in the study.
    - The geometric mean (arithmetic mean) of the resulting values is 0.072 (0.077) mL/kg-day in males.
    - Clearance in the two women ranked second and third lowest in the entire set.
- Worley et al. (2017): 0.031 mL/kg-day in men and women

These total clearance values also incorporate routes of clearance in addition to renal and menstrual clearance, which could consist of fecal clearance, shedding of skin, and clearance due to childbirth and lactation, to the extent that these occurred in the study populations.

Urinary clearance estimates

Four studies directly evaluated urinary clearance of PFHxS in humans from matched serum and urine concentrations (Yao et al., 2023; Fu et al., 2016; Gao et al., 2015; Zhang et al., 2013b). Of these studies, the ones with occupational cohorts Gao et al. (2015) and Fu et al. (2016) had much greater exposure than the general population (Yao et al., 2023; Zhang et al., 2013b). Yao et al. (2023) estimated clearance in infants, while all other studies were in adults. Their results are as follows:

Fu et al. (2016) measured serum and urine PFHxS concentrations in matched samples from occupationally exposed workers, and while they converted the results to half-lives for reporting, the paper states that Vd = 230 mL/kg was used for the estimate. Given a reported geometric mean (GM) half-life of 19.9 years in men, the corresponding clearance is 0.022 mL/kg-day. The GM urinary clearance for women in the study (reported in the text) was 0.024 mL/kg-day. That the overall population GM was reported to be 0.023 mL/kg-day increases confidence in the CL in men back-calculated here (0.022 mL/kg-day).

Gao et al. (2015) did not distinguish between sexes but did distinguish between isomers of PFHxS and found much greater clearance for the branched isomer, GM = 0.18 mL/kg-day, compared with the linear (n-) isomer, GM = 0.04 mL/kg-day, with an overall clearance GM of 0.05 mL/kg-day for total PFHxS, in a mixed population of men and women. The values for n- and total are between those estimated from the half-lives of Li et al. (2018) and Olsen et al. (2007) (0.06-0.07 mL/kg-day) and the urinary clearance values estimated by Fu et al. (2016) and Zhang et al. (2013b) (0.02-0.03 mL/kg-day).

Zhang et al. (2013b) obtained GM values of 0.018 for men and older women and 0.028 for younger women, which is in the range of total clearance estimated from Worley et al. (2017). That the GM values of Zhang et al. (2013b) are within an order of magnitude of the overall population GM provides confidence that the true value is within an order of magnitude of those reported.

These route-specific clearance estimates do not include fecal elimination. After IV dosing Kim et al. (2018b) measured fecal/urinary excretion rates of 8.2% and 7.9% in male and female rats, respectively. Therefore, total excretion for Fu et al. (2016), Gao et al. (2015), and Zhang et al. (2013b) was estimated as 1.08 times) the estimated urinary excretion rates (i.e., 100% of urinary excretion plus 8% of urinary excretion for fecal clearance) for the purpose of determining an overall total clearance in humans. The value estimated from a rat study was deemed appropriate as there is no human or primate data on the relative amount of fecal and urinary excretion. There is uncertainty in assuming that relative amount of fecal and urinary excretion in human is similar to rats, that could be reduced by additional relevant human or primate data.

Yao et al. (2023) estimated urinary clearance of PFHxS and other PFAS in infants, based on the ratio of the estimated urinary excretion rate to estimated cord serum concentration. Cord blood was collected at delivery and the concentration multiplied by two to account for the serum-to-whole-blood ratio. Urine was collected in disposable diapers collected over the first postnatal week and later extracted for measurements. The methods do not specify how a daily average urine concentration was then determined from the set of samples for each infant, but it is presumed that the extracted urine from all diapers collected during the week was mixed prior to analysis, resulting in a "mixing cup" average concentration for the week. The resulting concentration was then multiplied by a reported average urine elimination rate in infants of 48 mL/kg-day, rather than using the actual urine volume collected. Since the serum concentrations and resulting urinary elimination of breast-fed infants are expected to increase significantly after child-birth based on reported breast milk: maternal serum distribution and breast milk ingestion rates, while the cord blood concentration might only match the infant blood concentration at the moment of birth, the resulting estimate of infant clearance is likely to be an over-prediction of the true clearance rate. From a population of 20 infants the median (15^{th}, 75^{th} percentile) urinary clearance was 0.270 (0.108, 0.781) mL/kg-day, with a mean value 0.956 mL/kg-day, i.e., an order of magnitude higher than the rate estimated in adults. The sample distribution is clearly skewed, with a maximum estimated value of 11.7 mL/kg-day perhaps due to the urine sample timing issue discussed here. While glomerular filtration is still developing in neonates, the expression of renal OAT1 and OAT3 is also below adult levels (Bueters et al., 2020), and urinary excretion of PFNA will depend on both of these opposing factors in a manner that cannot be quantitatively predicted. Given these uncertainties, the results of this study will not be used quantitatively, though they indicate that neonates will have lower serum levels of PFNA per unit exposure than adults.

Sex differences in human PFHxS PK

Zhang et al. (2013b) shows a small quantitative difference in urinary clearance between men and older women and younger women (i.e., 0.01 mL/kg-day). It is possible that this difference derives from differences in renal expression of renal transporters between men and women (Murray, 2017), but it could also be due to random inter-subject variability, given the overall range of clearance observed across studies, and based on the overall range of clearance in each group, the difference is not statistically significant. Hence, there does not appear to be a systematic difference between men and women in the urinary clearance of PFHxS, except to the extent that menstrual blood loss accounts for the difference reported by Li et al. (2018) and in participants between 15 and 50 years of age reported by Li et al. (2022b). However, a menstrual loss term of 0.033 mL/kg-day was used for EPA's analysis for women of reproductive age, based on the analysis of Verner and Longnecker (2015) of corresponding blood and fluid loss reported by Hallberg et al. (1966). Applying the Vd values estimated from male and female monkeys (Sundström et al., 2012) to men and women respectively also led to some difference in the corresponding half-life estimates.

Zhang et al. (2013b) calculated a rate for menstrual clearance based on a study of PFOA and PFOS that estimated menstrual blood loss using measurements of the blood quantity excreted (Harada et al., 2005). This estimate of menstrual blood loss was not specific to PFOA or PFOS and is also applicable to PFHxS. However, Harada et al. (2005) cite Hallberg et al. (1966) as the source for a menstrual blood loss of 70 mL per cycle, but according to Hallberg, "The mean value of the menstrual blood loss was 43.4 ± 2.3 mL in the entire series" [of experimental groups] and "the upper normal limit of the menstrual blood loss is situated between 60–80 mL." Thus, 70 mL/cycle appears to be closer to an upper bound for healthy women. More recently Verner and Longnecker (2015) reviewed Hallberg et al. (1966), evaluated both blood loss and total fluid loss from menstruation and concluded that the fluid lost in addition to blood was likely to be serum, with the corresponding serum binding proteins and associated PFAS. Including this serum loss and assuming 12.5 menstrual cycles per year, Verner and Longnecker (2015) estimated an average yearly total serum loss of 868 mL (69.4 mL/cycle or 72.3 mL/month). Assuming an average human female body weight of 72 kg (mean value for women 21-30 years of age from Table 8-5 of (U.S. EPA, 2011a)), the corresponding average rate of clearance is 868 mL/(365 day)/(72 kg) = 0.033 mL/kg-day.

Lorber et al. (2015) examined the effects of ongoing blood loss through menstruation or through frequent blood withdrawal as a medical treatment. Male patients with frequent blood withdrawal had serum concentrations 40%–50% less than males from the general population for the chemicals observed in the study (PFOA, PFNA, PFDA, PFHxS, and PFOS). Female patients also had a lower serum concentration than females from the general public. The trend in relation to the number of recent blood draws or in the recency of the last blood draw was not examined for PFHxS. It was examined for PFOA and PFOS, and significant associations were observed in PFOS only. This study's analysis of the impact of menstrual blood loss was purely a modeling exercise, which was performed for PFOA and PFOS. The authors estimated a monthly blood loss of 35 mL (which is close to the median loss of 43.4 mL reported by Hallberg et al. (1966)), 50% of which was serum, resulting in a clearance of 17.5 mL/month, or 0.0081 mL/kg-day in a 72 kg woman. This value is also chemical-independent and could be applied to PFHxS instead of the menstrual clearance estimated by Verner and Longnecker (2015).

Jain and Ducatman (2022) compared serum levels of PFHxS and other PFAS in US females and males as a function of age. While serum PFHxS concentrations were similar at age 12–13 and after age 55, they declined in females compared to males between these ages until the concentrations in females were approximately one half of those in males between ages 30 and 45. Qualitatively similar results, though with a smaller magnitude, were seen for PFOA, PFOS and PFNA (Jain and Ducatman, 2022). Similarly, Li et al. (2022b) estimated a shorter half-life (corresponding to more rapid clearance) for females than males 15–50 years of age, but not for 1–14 years of age or over 50 years of age, although the difference between males and females aged 5–50 is only about 15%. These results are strongly suggestive that menstrual clearance is a significant factor in the clearance of these PFAS. Further, the results of Jain and Ducatman (2022) that for the US population (rather than a highly exposed Swedish cohort) menstrual clearance results in an approximate doubling of total clearance, supporting use of the menstrual clearance rate of 0.033 mL/kg-day estimated from the results of Verner and Longnecker (2015) above.

As mentioned in the distribution section (see Section 3.1.2), PFHxS has been observed in breast milk, so lactation can act as an excretion route for a nursing mother. One study that examined the association between maternal serum concentrations and the length of breastfeeding and found a weak, nonsignificant inverse association. There were stronger inverse associations for the other PFAS studied, PFOA, PFOS and PFNA, suggesting that there may be less transfer of PFHxS to breast milk than other PFAS, or that the variation between people in serum level is large compared with the impact of breastfeeding.

Dosimetry of linear versus branched isomers

Gao et al. (2015) is the only PK study to provide separate estimates of elimination for linear versus branched isomers in humans. With the clearance of the branched isomer being so much higher than the linear, the body burden is expected to be much higher for the linear than the branched isomer, given equal exposures. Using the clearance for the sum of PFHxS accounts for the relative prevalence of the different isomers in the serum of the participants. Therefore, the result for mixed or total PFHxS from Gao et al. (2015) will be used in combination with the results of the other PK studies. The result is interpreted as reasonably health-protective across all forms.

Summary of human PFHxS excretion

A summary of the clearance values reported or estimated from each of the adult human elimination studies is provided in Table 3-4.

Table 3-4. Summary of clearance values estimated for humans

Study (basis)	Clearance (mL/kg-d)	N	Notes
Chiu et al. (2022) (serum levels vs. drinking water exposure	0.068	41	Geometric mean; 37 individuals and 4 population mean results
Fu et al. (2016) (urinary clearance with fecal estimatea)	0.025	207	Geometric mean; 136 men, 71 women
Gao et al. (2015) (urinary clearance with fecal estimate[a])	0.054	36	Geometric mean for total linear and branched PFHxS; result based on 57 paired samples from 22 men, 14 women
Li et al. (2018) (empirical half-life)	0.071	20	Men aged 15–50; CL calculated from mean half-life using Vd = 278 mL/kg
Li et al. (2018) (empirical half-life)	0.059	30	Women aged 15–50; CL calculated from mean half-life using Vd = 228 mL/kg and subtracting 0.033 mL/kg-d for menstrual clearance (Verner and Longnecker, 2015)
Olsen et al. (2007) (empirical half-life)	0.072	26	Geometric mean of individual clearance values, calculated from reported half-lives as described above; 24 men, 2 women (all ≥59 yrs)
Li et al. (2022b) (empirical half-life)	0.098	22	Males, ages 15–50; CL calculated from mean half-life using Vd = 278 mL/kg
Li et al. (2022b) (empirical half-life)	0.064	30	Females, ages 15–50; CL calculated from mean half-life using Vd = 228 mL/kg and subtracting 0.033 mL/kg-d for menstrual clearance (Verner and Longnecker, 2015)
Li et al. (2022b) (empirical half-life)	0.075	33	Age > 50; CL calculated from mean half-life using Vd = 253 mL/kg
Nilsson et al. (2022) (empirical half-life)	0.079	99	Age 22–82, 97–98% males; CL calculated from mean half-life using Vd = 278 mL/kg
Worley et al. (2017) (half-life fitted for PK modelb)	0.031	45	Clearance calculated using Vd = 230 mL/kg (value used in the PK model); 22 men, 23 women
Zhang et al. (2013b) (urinary clearance with fecal estimate[a])	0.030	19	Geometric mean; women ≤50 yrs
Zhang et al. (2013b) (urinary clearance with fecal estimate[a])	0.019	64	Geometric mean; all men and women >50 yrs
Weighted geometric mean	**0.041[c,d]**	**447**	**Exp $\Sigma[\log(CL_i)\cdot N_i] / \Sigma[N_i]$**

[a]Reported urinary clearance was multiplied by 1.08 based on observed fecal/urinary elimination in rats after IV dosing (Kim et al., 2018b).
[b]Half-life determined from fitting PK model to geometric mean of serum concentrations measured in 2010 and 2016, accounting for estimated ongoing exposure.
[c]Calculated for all studies except Worley et al. (2017) due to methodological issues identified for that study and Li et al. (2018) since data for that population are included in the data of Li et al. (2022b) (see "Half-life estimates").
[d]Variance around this value can be described by a weighted geometric standard deviation of 1.6, which is a multiplicative factor, or a weighted geometric coefficient of variance of 22%.

In Table 3-4, the subset of clearance values estimated from empirical half-lives (Li et al., 2022b; Li et al., 2018; Olsen et al., 2007) are fairly similar to each other after adjustment for (subtraction of) menstrual blood loss, and similar to the results of Chiu et al. (2022), but are higher than most of the urinary clearance values and the results of Worley et al. (2017), which were based on exposure estimated from drinking water concentrations measured at one time point and may not reflect higher exposure concentrations in preceding years. While Kim et al. (2018b) observed fecal excretion of PFHxS in rats to be only 8% of urinary excretion after IV exposure, it is possible that fecal excretion and other routes such as shedding of dead skin contribute enough to the overall clearance to account for the two- to three-fold difference between those estimated from empirical half-lives (Li et al., 2022b; Li et al., 2018; Olsen et al., 2007) and the estimates of urinary clearance. In this case, the weighted geometric mean clearance shown in Table 3-4 will underpredict overall clearance to that extent. However, it also possible that the empirical half-lives reflect urinary clearance under conditions of saturated renal resorption, which is not representative of the general population at lower exposure levels, but Chiu et al. (2022) attempted to exclude very highly exposed individuals (i.e., with occupational exposure) and also obtained a relatively high clearance.

Data on how clearance may vary as a function of age (i.e., in rat pups or children compared with adults) and during pregnancy are mostly lacking. Li et al. (2022b) did estimate the half-life in individuals 1–14 years of age and found it to be about one half of that in older individuals (3 years vs. 6 years), but this is an apparent half-life that likely includes the impact of growth. As discussed above, Yao et al. (2023) estimated urinary clearance of PFHxS in infants to be almost an order of magnitude higher than the estimated clearance rates in adults, 0.27 vs. 0.036 mL/kg-day, but the approach used may have over-estimated the rate. Renal excretion varies in proportion to body surface area with age over most of the lifetime but is still developing in newborns along with expression of organic anion transporters (OATs) (Bueters et al., 2020) that are associated with renal resorption of PFAS, and the volume of distribution may also vary with age. In the preceding section, "Distribution in fetal tissues and children," the possible effect of changes in extracellular water and blood volume as a fraction of BW in children was discussed. Finally, the absence of a reliable pharmacokinetic model which can account for these factors and the likely differences in accumulation of PFHxS in humans exposed chronically versus in experimental animals during relatively short-term health effects studies creates uncertainty in simpler pharmacokinetic extrapolation based on clearance. Nevertheless, the results of Jain and Ducatman (2022) indicate strongly that menstrual fluid loss creates an approximately two-fold difference in clearance between women of reproductive age and men, which is quite consistent with the weighted geometric mean clearance of 0.041 mL/kg-day (in the absence of menstrual fluid clearance) and the average menstrual fluid clearance of 0.033 mL/kg-day from Verner and Longnecker (2015) and the limited data available for neonates and children indicate that their clearance is higher than adults.

While the range of values in Table 3-4 represent a range of uncertainty of five-fold, given the number of estimates it seems unlikely that the true clearance in humans would be lower than the minimum value of 0.019 mL/kg-day from Zhang et al. (2013b). The weighted geometric mean clearance of 0.041 mL/kg-day is 2.2 times higher than this minimum and based on the overall evidence was considered sound for use in estimating human equivalent doses (HEDs) for points of departure (PODs) estimated from animal toxicity studies or blood concentrations estimated from epidemiological evaluations, with an additional 0.033 mL/kg-day for menstrual fluid loss in women of reproductive age.

The clearance values shown in Table 3-4 were compared with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding, to evaluate the possible role of those mechanisms. Considering the time period of Davies and Morris (1993), this comparison used their value for average human BW, 70 kg, which results in an estimated GFR/BW of 2.57 L/kg-day in humans, 83,000 times greater than the empirically estimated geometric mean clearance for humans. Kim et al. (2018b) reported an average PFHxS free fractions (f_{free}) of 0.00025 in humans, which led to GFR× f_{free} = 0.64 mL/kg-day, which is still almost 16 times greater than the geometric mean empirical clearance. Thus, it appears likely that there is significant renal resorption of PFHxS in humans.

Comparing the human CL values to those predicted from allometric scaling of mouse and rodent CL values shows that allometric scaling appears to overpredict human clearance rats. $BW^{3/4}$ allometric scaling suggested that CL in an 80 kg human should be 4.2 times lower than in a 0.25 kg rat and 7.2 times lower than in a 30 g mouse. Applying a factor of 4.2 to the population mean CL values for male and female rats in Table 3-3, resulted in predictions of human male CL of 1.7 mL/kg-day and female CL of 20 mL/kg-day, one to three orders of magnitude higher than the values estimated from human data in Table 3-4. Likewise using the CL in mice and the allometric factor of 7.2 resulted in an estimated human male CL of 0.54 mL/kg-day and female CL of 0.44 mL/kg-day, roughly an order of magnitude higher than observed. Performing this analysis for a 6 kg male monkey or a 4 kg female monkey produces a similar overprediction, with extrapolated clearance values of 0.73 and 1.0 mL/kg-day after applying scaling factors of 1.9 and 2.1. In summary, this analysis indicated that use of $BW^{3/4}$ scaling would have led to an overprediction of HEDs (effectively an underprediction of risk) by one to three orders of magnitude, depending on the animal species and sex in which a POD was identified. Hence, the use of $BW^{3/4}$ scaling was avoided for PFHxS, but comparisons of $BW^{3/4}$ scaling to the selected approach (see Section 3.1.6) was provided for context.

Excretion Summary

The estimated average clearance values for adult humans are listed in Table 3-5. Since menstrual blood loss was subtracted as appropriate from the data in Table 3-4 when estimating the general, nonspecific clearance in humans, a corresponding rate should be added for women of childbearing age. In particular, the higher estimate of Verner and Longnecker (2015) (0.033 mL/kg-day) appears to be consistent with the empirical comparison of PFHxS serum concentrations in men and women (Jain and Ducatman, 2022). This additional term is considered appropriate for deriving HEDs for reproductive effects in women. Since newly available data show that maternal serum levels remain constant or decline during pregnancy and the early postpartum period, the additional clearance term for menstrual loss is also considered appropriate for estimating HEDs for effects occuring in-utero or otherwise correlated with maternal serum concentrations measured during pregnancy and post-partum.

However, since the current analysis should protect younger children, men and older women, it was considered appropriate not to include menstrual clearance when evaluating dosimetry in humans for health effects that can occur at any point in life, even though they may have been observed in laboratory animals of reproductive age. This choice follows the typical approach when assessing susceptible sub-populations.

Table 3-5. Summary clearance values for humans

Population	Clearance (mL/kg-d)	References
Human geometric mean (general population)	0.041[a,b]	(Chiu et al., 2022; Li et al., 2022b; Nilsson et al., 2022; Fu et al., 2016; Gao et al., 2015; Zhang et al., 2013b; Olsen et al., 2007)
With menstrual fluid loss (women of reproductive age)	0.074	Includes average menstrual fluid loss of 0.033 mL/kg-day from Verner and Longnecker (2015)

[a]Human clearance estimates also depend in part on volumes of distribution estimated for monkeys by Sundström et al. (2012); does not include estimated clearance due to menstrual fluid loss.
[b]Measurements of urinary clearance only were corrected for estimated fecal/urinary clearance ratio of 1.08 based on observations in rats by Kim et al. (2018b).

3.1.5. Evaluation of PBPK and PK Modeling

The PFAS protocol (Supplemental Information document, Appendix A) recommends the use of scientifically sound and validated physiologically based pharmacokinetic (PBPK) models as the preferred approach for dosimetry extrapolation from animals to humans, while allowing for the use of data-informed extrapolations (such as the ratio of serum clearance values) for PFAS that lack a scientifically sound and sufficiently validated PBPK model. If chemical-specific information is not available or too uncertain, the protocol then recommends that doses be scaled allometrically using body weight $(BW)^{3/4}$ methods. Selection from among this hierarchy of decisions considered both the inherent and chemical-specific uncertainty (e.g., data availability) for each approach option. This hierarchy of recommended approaches for cross-species dosimetry extrapolation is consistent with EPA's recommendations on using allometric scaling for the derivation of oral reference doses (U.S. EPA, 2011b). This hierarchy preferentially prioritizes adjustments that result in reduced uncertainty in the dosimetric extrapolation.

A PBPK model was identified for PFHxS in rats and humans (Kim et al., 2018b). The computational code for this model was obtained from the model authors and evaluated for consistency with the written description in the published paper, the PK data for PFHxS, known physiology, and the accepted practices of PBPK modeling. Unfortunately, several flaws were found in the model. One flaw, an error in the balance of blood flow through the liver, had only a moderate impact on model predictions. A much larger issue identified is that the model had only been calibrated to fit the oral PK data for rats and the set of model parameters selected by the model authors to match those data included an oral bioavailability (BA) lower than is otherwise supported by the empirical PK data. For example, the fraction absorbed by the male rat was effectively set to 39% in the model when the empirical PK analysis showed 88%–92% bioavailability. Further, when the model was used to simulate the intravenous PK data, data to which a PK model should be calibrated, the parameters were found to be completely inconsistent with these data. Figure 3-3 compares results obtained with a replication of the PBPK model, which exactly matches the published PBPK model results for oral dosimetry, to the data and empirical PK fit for a 10 mg/kg IV dose to male rats.

The overprediction (approximately three to four times higher than the data for male rats) of the IV data by the Kim et al. (2018b) model indicated that distribution into the body is significantly underpredicted by the model, which was offset in the simulations of oral dosimetry data by use of an unrealistically low oral bioavailability. Initial efforts to refit the model to the data did not produce acceptable fits to both the IV and oral dose PK data and involved changing model assumptions in a way that would require separate experimental validation before use. In particular, to match the observed rate of decline in the blood as well as the observed accumulation in urine and feces required an assumption of another route of excretion, for which there are no data. It was therefore determined that the published model structure and underlying assumptions did not allow a sufficiently sound calibration of the model to the PK data, given the currently available understanding of PFAS pharmacokinetics.

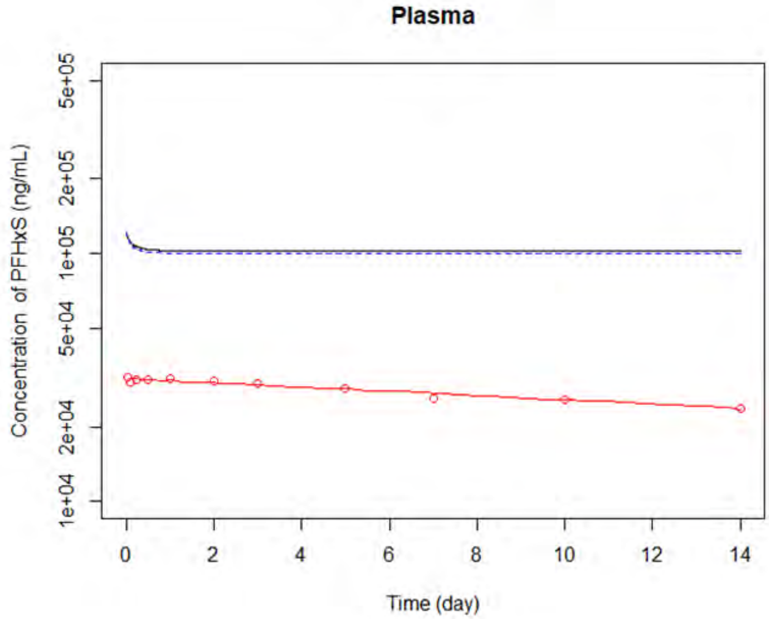


Figure 3-3. Comparison of PFHxS PBPK model predictions to IV dosimetry data (circles) of Kim et al. (2018b) for a 10 mg/kg dose. The red, solid line was the result of an empirical PK analysis shown by **Kim et al. (2018b)** (digitized). EPA's replication of the PBPK model (solid black line) exactly reproduced the PBPK model results of **Kim et al. (2018b)** for oral dosimetry (results not shown – simulation shown here was for IV dose) hence was considered an accurate reproduction of the model. The blue dashed line shows model results after correction of the blood flow rate exiting the liver. The discrepancy between the PBPK model prediction for a 10 mg/kg dose and the data demonstrated that the published model structure and parameters are very inconsistent with the empirical data, hence that there was a significant flaw in the model.

Fàbrega et al. (2015) developed a PBPK model describing the dosimetry of multiple PFAS in humans, including PFHxS. A concern with this model is that the tissue:blood partition coefficients were estimated by comparing tissue concentrations measured in cadavers with blood concentrations from different (living) subjects, albeit from the same geographic region. Also, the brief description provided for the estimation of the parameters for saturable renal resorption was considered not sufficient to allow for independent reproduction of that process and it was unclear how the two constants can be independently identified from such data. Finally, model results for PFHxS shown by the authors underpredict an epidemiological dataset (Rylander et al., 2009) by about an order of magnitude. Therefore, the model was not considered further for use in this review.

Verner et al. (2016) developed a coupled classical PK model, wherein single-compartment models represented the mother and fetus or child, which incorporated growth of the fetus and child, maternal body weight changes, and a time-varying rate of milk intake to account for the decline in g/kg-day ingested with the child's age. With parameter samples selected from distributions by Monte Carlo sampling, maternal exposure levels for individuals from two studies were selected to match the observed maternal serum concentration at delivery (i.e., given the sample set of parameter values) and the PFAS concentrations in the mother and child simulated for the first three years of the child's life. Measured plasma levels in children at 6 months of age were fairly well predicted, though the model tended to under-predict the plasma levels at age three, with many observations more than two-fold higher than predicted. A version of the PK model was implemented and its ability to predict rat PK data was evaluated as described in Appendix E.2. Unfortunately, based on the under-prediction of PFHxS concentrations in three-year-old children shown by Verner et al. (2016) and the poor performance of the model in predicting rat PK data using parameter values estimated for that species (Appendix E.2), model predictions were not considered sufficiently reliable for use in this assessment.

It is also noted that EPA's high throughput toxicokinetics (httk) computational model package (Pearce et al., 2017) predicts dosimetry for PFHxS. However, this model currently does not account for the activity of transporters, in particular those involved with renal resorption, so clearance (in the absence of metabolism) is estimated as the free fraction in blood times the glomerular filtration rate. The httk package estimates the half-life of PFHxS in humans to be 38 days or 0.11 years, corresponding to CL = 3.9 mL/kg-day (using Vd for female monkeys), over two orders of magnitude higher than that estimated from the empirical in vivo human data. Hence, the httk model was also not considered further for use in this review.

Bil et al. (2022) used a classical two-compartment PK model structure to estimate internal dose relative potency factors for liver toxicity observed in male rats for nine PFAS, including PFHxS. Since the PK model parameter estimation was performed separately for each PFAS, only the results for PFHxS need to be discussed here, but it is noted that the objective of the paper was to develop a method for prediction of toxicity from exposure to PFAS mixtures. For, PFHxS, Bil et al. (2022) used the PK data of Huang et al. (2019a), one of the studies included in EPA's analysis, and obtained results for a single compartment (monophasic clearance) with a volume of distribution of 137 mL/kg and a half-life of 16.5 days using the data for the 16 mg/kg dose. These values are similar to those reported by Huang et al. (2019a) for that dose (144 mL/kg and 16.9 days, respectively), but somewhat lower than the results of EPA's analysis of multiple data sets including Huang et al. (2019a) (mean values of 217 mL/kg and 21 days). Because EPA's clearance value is obtained from analyzing data from all three dose levels used by Huang et al. (2019a) and data from two other studies (Kim et al., 2018b; Kim et al., 2016b), it is considered superior for use in pharmacokinetic extrapolation from animal to human points of departure.

Sweeney (2022) developed a PBPK model for PFHxS in humans. Model simulations were conducted for individuals from 0–70 years of age and results analyzed (compared with data) for individuals from 12–70 years of age. The text indicates that an adjustment factor for ingestion in children 0–10 years of age was employed, but gestational and lactational exposure are not mentioned and pregnancy was not simulated. The model structure and assumptions and adjustments for physiological changes with age appear to be sound and the author has compared model results to a comprehensive set of human PK data.

Unfortunately, the model code for Sweeney (2022) contains a mass-balance error in which the unbound fraction in plasma (CAFREE) is calculated as the total amount in plasma (APLAS) divided by the plasma volume, which effectively means that distribution to tissues and urinary elimination are not restricted by the plasma protein binding. If instead one interprets APLAS as only being the amount free in plasma, then the corresponding total amount in plasma (APLAS/FREE) is not included in the mass balance check for the model code. EPA's review of the model code suggested that the variable APLAS is consistent with the total amount in the plasma, not the free amount. For example, the differential equation for APLAS sums all the PFHxS that distributes out of the liver after absorption from the stomach (based on the amount free in the liver), rather than being only assigned the fraction that is free in blood. However, if the total amount in blood is APLAS/FREE, making this correction would add an amount approximately 40 times APLAS to the overall mass balance equation, which would then likely demonstrate an overall mass balance error.

It is possible that the mass balance error in Sweeney (2022) is related to the inability of Kim et al. (2018b) to correctly replicate the IV dosimetry in rats, noted above, in that both point to a central assumption that appears to be incorrect. Kim et al. (2018b) correctly calculates the mass balance in the plasma based on the assumption that only the free fraction in the plasma can distribute to tissues, but then fails to predict that tissue distribution after IV dosing. The central model code used by Sweeney (2022) was originally developed by Loccisano et al. (2011), who may have inadvertently introduced the mass balance error in an attempt to correct for an inability of the model to predict tissue distribution and urinary elimination. The resolution of this issue may require relaxing the assumption that the free fraction and bound fraction in the serum are strictly at equilibrium at all times, as opposed to being treated as a dynamic equilibrium with distinct rates of association and dissociation. In the latter case, the rate of distribution to tissues and urinary elimination would be limited by the rate of dissociation, which may be more rapid than the equilibrium fraction free multiplied by the blood flow rate to the tissues (or glomeruli). A mathematical model that incorporates the kinetics of plasma binding and release to describe uptake of drugs by the brain has been previously described by Robinson and Rapoport (1986), but adaptation of this model to the tissue distribution of PFHxS would require measurement of the separate rates of association and dissociation, data which have not been reported. Hence, appropriate revision of the PBPK models was not possible for use in this assessment.

Irrespective of the potential impact of the mass balance error, from Table 1 of Sweeney (2022), the model predicts urine concentrations around 2.5 times higher than Fu et al. (2016) and 3.75 times higher than measured by Zhang et al. (2013b), indicating an overall predicted clearance of 0.06-0.07 mL/kg-day, consistent with the results of Li et al. (2018), whose data were used for calibration. However, the result means that application of the Sweeney (2022) would be less health-protective than use of the weighted geometric mean clearance, 0.041 mL/kg-day (Table 3-5) and would not address some of the other uncertainties noted here. For both this reason and the mass balance issue, the model was not further considered for use in the current analysis.

Most recently, Chiu et al. (2022) applied a one-compartment PK model in a Bayesian analysis of human serum concentrations matched with drinking water (DW) concentrations of several PFAS, including PFHxS, from multiple community studies. Since the one-compartment model structure is essentially identical to that already evaluated by the EPA and only addresses exposure of adults, for whom body weight is presumed fixed, it was not considered further for use as a PK model, but the overall approach and parameter estimation method were considered sufficiently sound that the resulting parameters were combined with other published human parameters in estimating overall population clearance and volume of distribution (Table 3-4).

Yao et al. (2023) used a one-compartment PK model to estimate the time-course of multiple PFAS, including PFHxS, in human children from birth to one year of age. However, the model used a constant level of intake by the child, based on the breast milk concentration measured just after birth and the volume of breast milk ingested per day for infants < 1 month of age, and did not account for the dilution due to growth of the child over that time. Breast milk intake is expected to peak between 3 and 6 months of age and the intake per kg BW of the infant to decline from the first month of age through the first year (https://www.epa.gov/expobox/exposure-factors-handbook-chapter-15), while concentrations of PFHxS in maternal serum declined on average in the first month after birth (Oh et al., 2022). Hence, the simulations of Yao et al. (2023) likely over-predict the actual PFHxS time-course in children after the first month of life.

3.1.6. Empirical Pharmacokinetic Analysis

To estimate sex-specific PK parameters with measures of uncertainty for male and female rats based on all of the published studies, including Kim et al. (2018b), a hierarchical Bayesian analysis was conducted using either a one- or a two-compartment empirical PK model. Details of the analysis are provided in Appendix E. Results for a one-compartment model are described here for mice and rats and results for a two-compartment model for monkeys.

Estimation of Pharmacokinetic Parameters

In classical PK theory, it is expected that once a chemical is absorbed or distributed to the blood, its excretion (clearance) is then independent of the route of administration. With IV administration, 100% of the dose is delivered directly to the blood, while only a fraction of an oral dose may be absorbed. Therefore, the area-under-the-curve (AUC) for blood or serum concentration after an oral dose should be less than or at most equal to the AUC after the same dose administered IV, and the fraction absorbed, or bioavailability, is estimated as AUC_{oral}/AUC_{IV}. However, when both the IV and oral PFHxS exposure data for rats (at identical doses) were analyzed from Kim et al. (2016b), Kim et al. (2018b) and Huang et al. (2019a) by EPA, the estimated serum concentration AUC was consistently lower for the IV-dose data than the oral dose data for a number of the datasets, with the result that the corresponding CL values were quite different, in some cases with non-overlapping data-set-level credible intervals (see Figure 3-4). This difference was especially evident in the female, where CL after IV dosing was higher in all cases examined. This outcome does not match general pharmacokinetic theory, which depends on a number of assumptions, including that distribution into body tissues is independent of dose route.

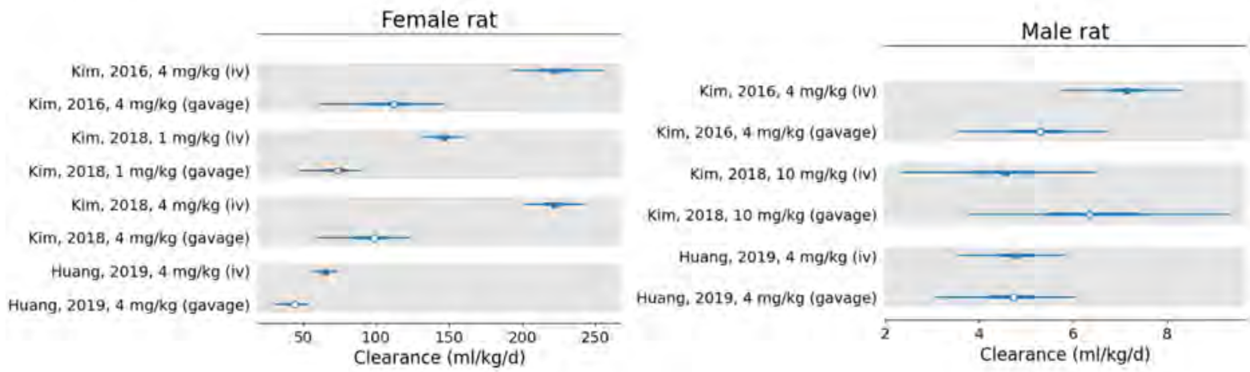


Figure 3-4. Comparison of Female (left) and Male (right) CL values for IV and gavage exposure of equivalent dose levels from Kim et al. (2016b), Kim et al. (2018b) and Huang et al. (2019a). The central point, a triangle for IV and a circle for gavage, denotes the mean CL, the thicker portion of the lines are the quartiles, and the thinner extent of the lines denote the 95th confidence interval. Note that these clearance values are slightly different than presented in Table 3-6, because those values were based on an analysis of only the gavage datasets, whereas the values in the figure above are based on analysis of the gavage and IV data together in a hierarchical Bayesian framework.

Since data of Kim et al. (2018b) show nearly identical urinary and fecal excretion after IV versus oral dosing, it is possible that distribution into body tissues was much greater after IV dosing, perhaps because more of the IV-infused PFHxS could distribute to various tissues before it became bound to serum proteins, while the slower absorption from oral dosing led to lower tissue distribution. Tissue dosimetry data after both IV and oral doses, which could be used to evaluate this hypothesis, were not available and resolution of the apparent discrepancy was considered beyond the scope of this analysis. Because the objective was to extrapolate dosimetry from oral exposures in animal toxicity studies to humans, given the unusual quantitative results from classical PK analysis for IV versus oral dosimetry, only the oral dosimetry data were included in the final analysis for rats and mice. Only IV dosimetry data were available for monkeys, so those data were analyzed recognizing that it may not exactly represent oral kinetics. Because the empirical data indicted the blood AUC after IV exposure was less than after oral exposure to the same dose for most of the experiments, it was assumed that oral bioavailability was 100% and that was assumed in subsequent analyses.

A single study reported PK data that could be used for parameter estimation for mice and monkeys (Sundström et al., 2012). While Sundström et al. (2012) did collect PK data after both IV and oral administration in mice, they did not estimate a bioavailability for male mice and the estimate of 50% availability in female mice was based on only two animals for oral dosimetry. Therefore, the more complete datasets for 1 and 20 mg/kg oral doses provided separately were analyzed similarly to the analysis for rats described above, assuming 100% bioavailability. The resulting PK model fits (see Appendix E, Figure E-5) were quite good, showing that the oral PK data for mice were consistent with this assumption; the model did not over-predict the serum concentration time-course.

Only IV data were available for monkeys (Sundström et al., 2012), so those data were analyzed for that species, recognizing the resulting uncertainty in bioavailability and that there may be differences in distribution and clearance between the two routes of administration. While the mouse and rat PK data were adequately fit with a one-compartment model (see Appendix E, Figures E-1 to E-5), the monkey PK clearly showed biphasic clearance from the serum, requiring a two-compartment model, that is, one including both central and a deep tissue compartment (see Appendix E, Figure E-6). No critical dose-response endpoints were identified in monkey, so no determination needed to be made considering the best approach for pharmacokinetic extrapolation from monkeys.

Values for the volume of distribution (Vd, mL/kg) and clearance (CL, mL/kg-day) were also estimated from the Bayesian analysis for each study and dose, as well as overall population mean values (Appendix E). An average half-life ($T_{1/2}$) was calculated from these results using the formula, $T_{1/2} = \ln(2) \times Vd/CL$. Interestingly, while the analysis showed a clear, large sex difference in clearance and the corresponding half-life between male and female rats, almost no difference appeared between male and female mice. The monkey results should be interpreted with some caution, as they were based on only three animals per sex, but they suggest an intermediate case between rats and mice, with clearance in male monkeys being 73% of female monkeys. The much slower clearance in male rats compared with female rats is assumed to result from higher expression of renal transporters that resorb PFHxS. The data for mice and monkeys suggest that expression of the transporters is much less sex-dependent in those species.

Table 3-6. Pharmacokinetic parameters for rats, mice, monkeys, and humans

Study	Dose (mg/kg)	n	Clearance (mL/kg-d)[a]	Volume of distribution (mL/kg)[a]	T1/2[b] (d)
Male rats					
Kim et al. (2016b)	4	5	5.71 (5.46–5.69)	264.4 (255.6–272.6)	32.1
Kim et al. (2018b)	4	5	6.58 (3.34–9.68)	293.4 (262.9–323.9)	30.9
Huang et al. (2019a)	4	3[c]	5.37 (4.61–6.14)	137.8 (116.2–159.6)	17.8
	16	3[c]	5.91 (5.09–6.75)	144.2 (121.1–166.5)	16.9

Study	Dose (mg/kg)	n	Clearance (mL/kg-d)[a]	Volume of distribution (mL/kg)[a]	T1/2[b] (d)
	32	3[c]	9.74 (8.47–11.03)	210.7 (176.9–243.2)	15.0
Population mean	--	--	*7.15 (3.73–10.26)*	*216.5 (149.2–281.4)*	*21.0[d]*
Female rats					
Kim et al. (2016b)	4	5	117.8 (110.7–125.3)	286.9 (264.5–309.6)	1.7
Kim et al. (2018b)	1	5	83.02 (77.22–89.38)	196.0 (117.2–213.6)	1.6
	4	5	106.3 (98.58–113.8)	236.3 (215.5–257.6)	1.5
Huang et al. (2019a)	4	3[c]	50.14 (45.03–55.01)	162.8 (142.9–183.2)	2.3
	16	3[c]	61.36 (55.58–67.17)	187.9 (166.5–208.5)	2.1
	32	3[c]	94.54 (85.43–103.3)	261.9 (231.9–290.2)	1.9
Population mean	--	--	*84.10 (64.72–103.8)*	*224.2 (182.7–266.4)*	*1.8[d]*
Male mice					
Sundström et al. (2012) (all data)	1 & 20	4[c]	3.86 (3.27–4.41)	154.6 (122.6–185.5)	27.8
Female mice					
Sundström et al. (2012) (all data)	1 & 20	4[c]	3.18 (2.83–3.52)	123.0 (104.5–140.6)	26.8[d]
Male monkeys					
Sundström et al. (2012)	10	3	1.39 (0.94–1.83)	282.4 (251.9–314.9)[e]	141
Female monkeys					
Sundström et al. (2012)	10	3	2.12 (1.81–2.44)	228.5 (204.4–252.5)[e]	75
Human					
All males and females below age 12.4 y and above age 50 y	--	577	0.041	228 (women)[f] 278 (men)[f]	3,855 (10.6 y) 4,700 (12.6 y)
Women 12.4-50 years of age			0.074	228 (women)[f]	2,136 (5.9)

[a]Values are mean (study-level 90% credible interval) or population mean (90% credible interval).
[b]$T_{1/2}$ = ([mean] volume of distribution [mL/kg]) × ln (2) / ([mean] clearance [mL/kg-d]).
[c]Number of animals per time point.
[d]Rats displayed a large difference in half-life between sexes that mice did not. This sex-dependence was seen in rats for many PFAS and has been linked to sex-hormone dependent changes in renal transporters (Kudo et al., 2002). It is not fully understood why this phenomenon is different between species.
[e]Sum of central and peripheral compartment volumes from a 2-compartment PK model.
[f]Vd in women assumed equal to the value for female monkeys, Vd in men assumed equal to male monkeys.

While the results for rats showed a fair degree of variability in CL between studies (see Table 3-6), the range in mean values is 1.8-fold for males and 2.3-fold for females is modest and the overall population means were obtained via a Bayesian analysis that addressed the variability both within and among the datasets (see details in Appendix E, Section 1). Hence, these values provided an estimate of the relationship between dose and mean serum concentration levels in rats that appeared to be accurate to within a factor of two, which was set as an acceptable degree of discrepancy between PK model simulations and data in EPA's Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (U.S. EPA, 2018b), and so were considered sufficiently sound for use in cross-species extrapolation.

The assumption that the Vd derived from monkeys is a suitable surrogate for the human Vd introduces some uncertainty to the calculated human half-life. However, Chiu et al. (2022) obtained a mean (95% CI) Vd of 0.25 (0.15, 0.42) L/kg from their analysis of human data, which is essentially the average of the values from male and female monkeys, 0.287 and 0.213 L/kg, respectively. Hence, the extent of the uncertainty is judged to be minimal. Use of the value from Chiu et al. (2022) would only change some of the estimated clearance values in Table 3-5 by less than 20%, so would have a minimal impact on the geometric mean clearance obtained.

Clearance Versus Glomerular Filtration Rate and Free Fraction in Serum

Some mechanistic insight could be gained by comparing the clearance values shown in Table 3-6 with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding. Davies and Morris (1993) summarized GFR for multiple species. Using 0.25 kg as the species average BW for the rat, the GFR/BW for rats is 7.55 L/kg-day, which is approximately 1,100 and 90 times higher than the population mean clearance estimated in male and female rats, respectively.

Binding to serum proteins plays a likely role in these very large differences. As discussed above in the context of distribution, PFHxS binds to albumin with high affinity and it is the major carrier of PFHxS in blood (Forsthuber et al., 2020; Bischel et al., 2010; Weiss et al., 2009). This binding may play a role in the limiting the rate of the renal excretion of PFHxS, in addition to the role played by renal transporters. Kim et al. (2018b) measured reported PFHxS free fractions (f_{free}) of 0.00076 and 0.00069 in male and female rat plasma. Using these values, GFR× f_{free} = 5.7 and 5.2 mL/kg-day in male and female rats. This alternative estimate of clearance for male rats is close to the population mean in Table 3-6 (7.15 mL/kg-day), which could be interpreted as showing minimal renal resorption in males. However, for female rats GFR× f_{free} is more than order of magnitude lower than the population mean clearance of 84.1 mL/kg-day. Section 3.1.5 provided further discussion of the fact that the PBPK model of Kim et al. (2018b), which assumed that tissue distribution was similarly limited by the free fraction, underpredicted the observed short-term distribution of PFHxS in rats. Hence, while it is expected that serum protein binding limits renal excretion (and tissue distribution) to some extent, the reduction appears to be less than predicted by assuming that clearance is strictly limited to the equilibrium free fraction. As noted above, Robinson and Rapoport (1986) used a mathematical model that incorporates the kinetics of plasma binding and release in order to describe uptake of drugs by the brain, supporting this conclusion. Alternately, there could be an error in the measured free fraction.

More qualitatively, the fact that the measured free fraction is similar in male versus female rats indicates that it cannot explain the large sex difference in empirical clearance, and hence that sex differences in renal resorption are likely to be a factor.

3.1.7. Model Evaluation Conclusion and Extrapolation Approach

The clearance in rats is sufficiently slow that PFHxS is expected to accumulate throughout the course of the 28-day NTP bioassay (NTP, 2019) in male rats and for about 10 days in female rats, as illustrated in Appendix E, Section 2. For this reason, the preferred approach would be to perform an interspecies dose extrapolation that accounts for the time-dependence of the internal dose (i.e., bioaccumulation). Further, given the slow clearance of PFHxS in male rats, the growth of rats during these toxicity studies could be a significant factor as increases in BW are expected dilute the body burden from earlier exposures. Therefore, a computational model for a single-compartment PK model was developed to describe the accumulation and elimination of PFHxS during these experiments, with time-dependence in BW based on the empirical data for BW. Details of the model and its evaluation against serum concentration data from NTP (2019) were provided in Appendix E, Section 2. While the period of accumulation was much longer for male rats, female rats were modeled in the same way as males for consistency. However, application of the single-compartment PK model revealed that this simple approach was not suitable for PFHxS due to an observed nonlinear relationship between dose and plasma concentration, which the single-compartment model was not able to replicate.

As noted in the Summary of Human PFHxS Excretion section, uncertainties also exist in the potential extrapolation of such a model to developmental or other early-lifestage effects. Even though the results for the one-compartment PK model indicated that the model may be adequate for low-dose extrapolation of dosimetry in adult animals, the failure of this model (see Appendix E, Section 2) and the issues identified with the published PBPK models (see Section 3.1.5) demonstrated an incomplete understanding of PFHxS pharmacokinetics. Additional research, which may be extensive, is needed to resolve the existing inconsistencies between the various models and the data. Thus, a reliable PK model for PFHxS is not considered to be in the realm of available science. Further, use of the empirical one-compartment PK model for some endpoints and a of data-derived extrapolation factor (DDEF) for others would create inconsistency in the extrapolation approach. This inconsistency would hinder the comparison between different candidate points of departure and the failure of the model in some instances lowers the confidence in model predictions, even for dose ranges where the model appears to be performing well. Therefore, a PK model was not used for dosimetric extrapolation.

Approach for Animal-Human Extrapolation of PFHxS Dosimetry

After evaluation of three published PBPK models and a one-compartment PK model for PFHxS, it was determined that none of these options could reliably predict PFHxS dosimetry. An alternative to use of PK (or PBPK) models for dosimetric extrapolation is use of data-derived extrapolation factors (DDEFs). As stated in EPA's guidance for DDEFs (U.S. EPA, 2014), use of these factors "maximize the use of available data and improve the scientific support for a risk assessment." As discussed above in the Evaluation of Pharmacokinetic Modeling and Summary of Human PFHxS Excretion sections, the estimated population average values of total CL for male and female rats and for humans were considered sufficiently sound for use in such extrapolation, while use of $BW^{3/4}$ scaling (the least preferred option; see U.S. EPA (2011b)) could lead to over-prediction of HEDs by as much as three orders of magnitude. Therefore, DDEFs calculated from the clearance values listed in Table 3-5 and Table 3-6, were used as the next preferred option. Specifically, the ratio of human clearance to clearance in the animal species and sex in which a given POD was identified was used to estimate the HED for that POD. For example, to extrapolate from a POD from the NTP bioassay for an endpoint in male rats to humans,

$$HED = POD \times CL_H/CL_{rat,m},$$

where CL_H is the clearance in humans for the appropriate population, $CL_{rat,m}$ is the clearance in male rats and $CL_H/CL_{rat,m}$ is the DDEF. This calculation assumed the same fraction absorbed or bioavailability in human and rats, which is taken to be 100% as described in Section 3.1. In particular, the computational PK analysis summarized in Section 3.1.6 found that the published PK data showed serum AUC after oral exposures were higher than serum AUCs after matching IV exposures for several key studies rather than results consistent with less than 100% oral bioavailability.

For gestational effects, the clearance in the female animal (dam) was assumed to determine dosimetry to the fetus. However, for effects observed in rat pups at PND 22, the clearance for the same sex adult rat was used.

While menstruation does not occur during pregnancy and may not resume until after weaning of the child, as described in the subsections *Trend in Pregnancy* and *Breast Milk* in 3.1.2 Distribution, studies of longitudinal changes in during and after pregnancy show maternal serum levels remaining fairly constant or constant or declining through this lifestage. This likely occurs because the long half-life of PFHxS results in slow accumulation as well as elimination, while the increase in total body mass during pregnancy (including the fetus and placenta) is expected to result in a dilution of the body burden as the PFHxS distributes into those growing tissues. Therefore, the serum levels in the pregnant and postpartum woman are expected to be consistent with her serum levels at the start of pregnancy, which are determined by her total clearance prior to pregnancy, including menstrual fluid loss. Thus, HEDs for developmental endpoints that occur in-utero such as reduced birthweight or are based on measures of maternal serum concentration will be calculated using the higher clearance estimated for women of childbearing age (12.4–50 years) in Table 3-6.

However, this additional clearance clearly does not occur in young children, and as described in Summary of Human PFHxS Elimination in Section 3.1.4, there may be differences in PK among human lifestages that cannot be quantified because of a lack of empirical PK data during childhood. While effects in adults do not involve extrapolation across lifestages, the degree of accumulation of PFHxS in rats during a 28-day bioassay could be less than the accumulation during a comparable portion (4%) of the human life span. Therefore, HEDs for effects observed in experimental animals more than a few days after birth, where dosimetry in the pups or human child may be a significant factor, and immune effects correlated with serum concentrations measured 5 years after birth, for which the exposure and clearance of the offspring are significant factors, have been calculated using the population-average CL_H from Table 3-6.

The key assumption made in calculating a DDEF for a given endpoint evaluated was that for effects observed in adult male and female rats, the CL and F_{abs} for the corresponding rat sex from Table 3-6 were used to calculate the DDEF. Table 3-7 shows the resulting DDEFs.

Table 3-7. Data-derived extrapolation factor (DDEF) calculations

Sex and species of observation (lifestage)	CLA (mL/kg-d)	DDEF[a]
Male rats (adult and male pups > PND 7)	7.15	5.73×10^{-3}
Female rats (adult and female pups > PND 7), non-reproductive/developmental effects	84.1	4.88×10^{-4}
Female rats (adult), reproductive effects and effects in pups < PND 7	84.1	8.80×10^{-4}

[a]DDEF = (CL_H/CL_A) with CL_H = 0.041 mL/kg-d for effects in all males and females outside of reproductive age, except for those occurring in-utero or correlated with maternal serum levels during or after pregnancy. For reproductive effects in females and developmental effects associated with maternal serum levels, CL_H = 0.074 mL/kg-d was used. These DDEF values assume equal oral bioavailability in rats and humans. Rat CL values from Table 3-6. No data exist showing that CL in juveniles is different from adults.

When an internal dose POD, specifically a serum concentration, is obtained from human epidemiological studies, the HED will likewise be calculated as:

$$HED = POD_{int} \times CL_{H,}$$

using the geometric mean estimate for human clearance from Table 3-5, CL_H = 0.041 mL/kg/d = 4.1×10^{-5} L/kg-day for effects associated with serum levels in children (e.g., immune effects associated with serum levels measured at age 5) and 0.074 mL/kg-day = 7.4×10^{-5} L/kg-day for developmental effects associated with maternal serum levels.

Uncertainty in HED Calculations

The ranges in population mean parameter Table 3-6 can be used as a measure of uncertainty in the CL for male and female rats. The upper end of the 90% credible intervals is only 43% higher than the mean for male rats and 23% higher than the mean for female rats, indicating that concentrations during the bioassays were unlikely to be much lower than effectively estimated using the DDEF, hence that the corresponding HEDs were also judged unlikely to be more than 1.5-fold lower. Applying the DDEF, however, effectively assumed the rats were at steady state, when this was not likely the case, especially for male rats used in the NTP bioassay (NTP, 2018a), which could lead to an over-prediction of the HED_{POD}. The non-menstrual clearance value used for humans was approximately two-fold higher the lowest from among those reported by or estimated from multiple studies of PFHxS dosimetry in humans. Only a modest correction for fecal absorption (using the ratio of fecal/urinary elimination observed in rats after IV dosing) was applied. Hence, the average human clearance is unlikely to be more than two-fold lower than the value used for HED calculation. The relative values of non-menstrual and menstrual clearance correlate strongly with differences between PFHxS serum levels found in the U.S. population (NHANES data) (Jain and Ducatman, 2022), reducing the qualitative uncertainty. While uncertainties in the extrapolation to developmental exposure and dosimetry in children remain, there are currently no data to indicate that these are greater than is accounted for by application of the standard human interindividual uncertainty factor (UF_H), of which a factor of 3 is typically attributed to pharmacokinetic differences across individuals.

3.2. NONCANCER HEALTH EFFECTS

For each potential health effect discussed below, the synthesis describes the evidence base of available studies. Arrays or tables summarizing endpoint results across studies within each evidence stream are also provided. The effect levels presented in these arrays and tables are based on statistical significance[5] or biological significance, or both. Examples relevant to interpretations of biological significance include consideration of the directionality of effect (e.g., statistically significantly decreased cholesterol/triglycerides is of unclear toxicological relevance), tissue-specific magnitude of effect (e.g., statistically nonsignificant increase of ≥10% in liver weight may be considered biologically significant), and dose-dependence (e.g., a significant finding at a single, lower dose level but not at multiple, higher dose levels may be interpreted as potentially spurious). For this section, evidence to inform organ-/system-specific effects of PFHxS in animals following

[2]Throughout the assessment, the phrase “statistical significance” indicates a *p*-value < 0.05, unless otherwise noted.

developmental exposure are discussed in the individual organ-/system-specific sections (e.g., liver effects after developmental exposure are discussed in the hepatic effects section and so on, although they are generally cross-referenced to the Developmental Effects section; Section 3.2.3). Evidence on other developmental effects (e.g., fetal growth) is only discussed in the Developmental Effects section. Lastly, overt toxicity was not observed at any of the highest doses tested in any of the available studies (in contrast to data available for some of the other PFAS being assessed by the IRIS Program), and thus the potential for overt toxicity to complicate interpretation of the health effect-specific PFHxS evidence is not a factor discussed in any of the following sections.

3.2.1. Thyroid Effects

Under normal physiologic conditions, neurons in the hypothalamus release thyroid releasing hormone (TRH) to stimulate epithelial cells of the anterior pituitary gland to release thyroid stimulating hormone (TSH) (Irizarry, 2014). TSH plays a number of important metabolic functions including stimulation of the thyroid gland to release thyroxine (T4), which is converted to triiodothyronine (T3). When increased T3 and T4 serum levels exceed a blood concentration threshold, secretion of TRH from the hypothalamus is inhibited via a negative feedback loop (Irizarry, 2014; Pilo et al., 1990). In adults, T3 and T4 play important metabolic functions; for example, decreases in T3 and T4 serum levels, a condition known as hypothyroidism, result in increased weight gain, fatigue, and dry skin, as well as effects on the memory and a difficulty to concentrate. Conversely, increased levels of T3 and T4, otherwise known as hyperthyroidism, result in increased rate of metabolism, weight loss and increased heart rate (Mullur et al., 2014). During fetal development and throughout early childhood, thyroid hormones play an important role in somatic growth and development. Thyroid hormones have been shown to play a critical role in neurogenesis, neuronal migration, and synaptogenesis, as well as shifting neuronal cells from a proliferative state to a differentiation state and myelination (Gilbert et al., 2016). In humans, alterations of prenatal maternal T4 have been linked to declines in cognitive function in children (Korevaar et al., 2016; Haddow et al., 1999). Importantly, changes in prenatal and maternal T4 have been shown to be biologically important in the absence of changes in TSH reviewed in (Vansell, 2022; Moog et al., 2017; Stagnaro-Green and Rovet, 2016; Dong et al., 2015; Navarro et al., 2014; Rovet, 2014; Patel et al., 2011; Berbel et al., 2010; Morreale de Escobar et al., 2008; Cuevas et al., 2005; Rovet, 2005; Zoeller and Rovet, 2004; Hood and Klaassen, 2000; Hood et al., 1999a; Hood et al., 1999b).

Human Studies

Thirty-nine studies (reported in 44 publications) have investigated the relationship between PFHxS exposure and thyroid hormones and/or thyroid disease in humans. All of the available human studies examined the association between PFHxS exposure measured in blood and thyroid hormones (see Figure 3-5).

There were multiple outcome-specific considerations that were influential on the study evaluations. First, for outcome ascertainment, collection of blood during a fasting state and at the same time of day for all participants (or adjustment for time of collection) is preferred for measurement of thyroid hormones to avoid misclassification due to diurnal variation (van Kerkhof et al., 2015). Studies that did not consider these factors (e.g., by study design or adjustment) were not excluded but were considered deficient for the outcome ascertainment domain. This is expected to result in nondifferential outcome misclassification, and thus, bias toward the null on average. For participant selection, it was considered important to account for current thyroid disease and/or use of thyroid medications; studies that did not consider these factors by exclusion or another method were considered deficient for the participant selection domain. Concurrent measurement of exposure with the outcome was considered acceptable for this outcome since thyroid hormones can be up- or downregulated relatively quickly in relation to the long half-life of PFHxS (half-life of T3 and T4 are in the order of hours/days, respectively (Leboff et al., 1982) versus years for PFHxS (Li et al., 2018); see Section 3.1.3); thus, exposure measurement ratings were not downgraded for timing of measurement. All of the available studies analyzed PFHxS in serum or plasma using appropriate methods as described in the systematic review protocol (see Appendix A). Thyroid hormones were analyzed using standard methods (e.g., immunoassays, HPLC-MS/MS) in all studies. The *medium* confidence studies generally were not downgraded for participant selection, but most did not account for time of day of blood collection and fasting, which is considered likely to result in nondifferential outcome misclassification (expected to be toward the null on average) for thyroid hormone measures. The *low* confidence studies were generally downgraded for both the participant selection issues and outcome ascertainment issues described above, though Liu et al. (2018) did not account for thyroid medication use but was unique in the set of available studies in that data were collected prospectively, and the analysis was based on change in outcome, so there was less concern for the lack of adjustment impacting the results.

In summary, 26 studies were *medium* confidence (Cakmak et al., 2022; Gallo et al., 2022; Li et al., 2021b; Sarzo et al., 2021; Aimuzi et al., 2020; Kim et al., 2020a; Lebeaux et al., 2020; Liang et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Inoue et al., 2019; Reardon et al., 2019; Blake et al., 2018; Dufour et al., 2018; Kang et al., 2018; Liu et al., 2018; Preston et al., 2018; Berg et al., 2017; Crawford et al., 2017; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Wang et al., 2014; Webster et al., 2014; Wang et al., 2013; Wen et al., 2013) and ten were *low* confidence (Liu et al., 2021b; Itoh et al., 2019; Heffernan et al., 2018; Khalil et al., 2018; Zhang et al., 2018b; Li et al., 2017c; Lewis et al., 2015; Ji et al., 2012; Chan et al., 2011; Bloom et al., 2010). Three studies were *uninformative* in study evaluation (Seo et al., 2018; Kim et al., 2016a; Kim et al., 2011a). Sensitivity was a concern across studies due to narrow exposure contrasts in several studies (see sensitivity domain in Figure 3-4), combined with the expected bias toward the null due to outcome misclassification. Thus, null results are difficult to interpret. The *medium* confidence studies were the focus of evidence synthesis; *low* confidence studies did not undergo data extraction but were still considered for consistency in the direction of association. The domain ratings, populations, and thyroid measures for each study are presented in Figure 3-5.

Figure 3-5. Study evaluation results for epidemiology studies of PFHxS and thyroid effects. Full details available by clicking H AWC link. Multiple publications of the same study: Preston et al. (2018) also includes Preston et al. (2020).

G: good; A: adequate; D: deficient; CD: critically deficient; Un: uninformative.

The results for the association between PFHxS exposure and thyroid effects in *medium* confidence studies are presented in Tables 3-8 and 3-9. Twenty-eight studies examined associations with thyroid hormones in adults, including 13 focused on pregnant women (see Table 3-8). For T4, out of 27 studies, the results are mixed. In the 15 *medium* confidence studies, a few statistically significant associations were reported (positive associations in both sexes in Cakmak et al. (2022), positive association in women but inverse in men in Wen et al. (2013), positive association in men >50 years of age in Li et al. (2021b), positive association in pregnant women in Aimuzi et al. (2020), and inverse association in pregnant women in Reardon et al. (2019). Other non-significant results were also in both directions or showed no association. The *low* confidence studies were also inconsistent in direction of association for T4. Many of the inverse associations had small magnitudes of effect and some estimates, particularly for total T4, were imprecise, both of which decrease certainty in the evidence. There is no clear pattern by exposure level or population. Nineteen studies examined associations with T3. In the 12 *medium* confidence studies, most reported no association with the exception of three studies (Aimuzi et al., 2020; Crawford et al., 2017; Wen et al., 2013) in women that reported higher levels of T3 with higher exposure to PFHxS (statistically significant in latter two studies). Twenty-seven studies reported on TSH, and of the 16 *medium* confidence studies, one reported statistically significant higher TSH with higher exposure (Reardon et al., 2019) and one study reported a statistically significant inverse association (Aimuzi et al., 2020), both in pregnant women, but the remaining studies reported no clear association.

Table 3-8. Associations between PFHxS exposure and thyroid hormone levels in *medium* confidence studies of adults.

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	T4	T3	TSH
General population, adults						
Cakmak et al. (2022)	CHMS cross-sectional study (2007–2011), Canada, 6,045 participants (all ages)	1.5 (GM)	Percent change for GM equivalent increase	Total T4 **0.9 (0.1, 1.8)***	NR	-1.1 (-4.9, 2.9)
Crawford et al. (2017)	Time to Conceive cross-sectional study (2008–2009), U.S., 99 women	1.6 (GM)	β (p-value) for log-unit increase	Total T4 -0.15 (0.5) Free T4 0.01 (0.8)	Total T3 2.8 (0.2)	-0.03 (0.7)
Wen et al. (2013)	NHANES cross-sectional study (2007–2010), U.S., 1,181 adults (672 men, 509 women)	2.0 (GM)	β (95% CI) for ln-unit increase	Total T4 Women **0.26 (0.11, 0.41)*** Men -0.03 (-0.18, 0.11) Free T4 Women 0.003 (-0.02, 0.03) Men **-0.02 (-0.03, -0.003)***	Total T3 Women **4.07 (2.23, 5.92)*** Men -0.08 (-1.70, 1.54) Free T3 Women 0.003 (-0.02, 0.03) Men 0.005 (-0.003, 0.01)	Women -0.02 (-0.13, 0.09) Men 0.02 (-0.06, 0.52)
Blake et al. (2018)	Fernald Community Cohort (1990–2008), U.S., 210 adults (81 men, 129 women)	2.7 (1.7–4.1)	Percent change for IQR increase	Total T4 1.74 (-1.73, 5.33)	NR	1.97 (-7.73, 12.7)
Liu et al. (2018)	POUNDS Lost trial of weight loss treatment (2004–2007) 621 adults (237 men, 384 women)	3.1 (2.3–4.4)	Spearman correlation coefficients for change in hormone	0–6 months 0.04 6–24 months -0.02	0–6 months 0.01 6–24 months -0.05	NR
Gallo et al. (2022)	Veneto cross-sectional study in high exposure area (2017), Italy, 14,888 adults	6.5 (3–12)	Percent change for IQR increase	NR	NR	Women 1.1 (-1.8, 4) Men -5.5 (-11, 0.3)
Li et al. (2021b)	Ronneby cross-sectional study in high exposure area (2014–2015), Sweden, 2,687 participants (all ages)	93 in women age 20–50 yrs	Percent change	Free T4 Women 20–50 yrs 0.43 (-0.08, 0.94) Women >50 yrs 0.01 (-0.57, 0.6) Men 20–50 yrs 0.51 (-0.14, 1.16) Men >50 yrs **0.73 (0.02, 1.45)***	Free T3 Women 20–50 yrs 0.08 (-0.41, 0.57) Women >50 yrs 0.05 (-0.47, 0.57) Men 20–50 yrs 0.29 (-0.29, 0.88) Men >50 yrs 0.26 (-0.36, 0.89)	Women 20–50 yrs -0.47 (-2.52, 1.62) Women >50 yrs 0.63 (-1.88, 3.2) Men 20–50 yrs -0.37 (-2.7, 2.01) Men >50 yrs -0.14 (-2.79, 2.58)

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	T4	T3	TSH
Pregnant women						
Yang et al. (2016b)	Beijing Prenatal Exposure cross-sectional study (2013) 157 mother-infant pairs	0.5	Spearman correlation coefficients	Total T4: 0.08 Free T4: 0.04	Total T3: 0.08 Free T3: 0.12	-0.15
Wang et al. (2013)	Cross-sectional analysis within Norwegian Mother and Child Cohort Study (2003–2004), Norway, 903 pregnant women	0.6 (0.4–0.8)	β (95% CI) for ln-unit increase	NR	NR	0.01 (-0.04, 0.07)
Aimuzi et al. (2020)	Cross-sectional analysis within Shanghai Birth Cohort (2013–2016), China, 1,885 pregnant women	0.6 (0.4–0.7)	β (95% CI) for ln-unit increase	Free T4 **0.12 (0.02, 0.22)***	Free T3 **0.2 (0.05, 0.34)***	**-0.12 (-0.22, -0.01)***
Sarzo et al. (2021)	Cross-sectional analysis within INMA (2003–2008), Spain, 919 pregnant women	0.6 (0.4–0.9)	Percent change for doubling (95% CI)	Free T4 -1.6 (-7.56, 4.75)	Total T3 0.52 (-6.05, 7.54)	6.09 (-0.71, 13.4)
Wang et al. (2014)	Taiwan Maternal and Infant Cohort Study (2000–2001), Taiwan, 285 pregnant women and 116 neonates	0.8 (0.3–1.4)	β (95% CI) for unit increase	Total T4 -0.13 (-0.32, 0.06) Free T4 -0.01 (-0.02, 0.003)	Total T3 -0.002 (-0.01, 0.001)	0.11 (-0.002, 0.21)
Webster et al. (2014)	CHirP cohort (2007–2008), Canada, 152 women	1.0 (0.7–1.7)	β (95% CI) for IQR increase	Free T4 -0.02 (-0.1, 0.07)	NR	0.01 (-0.05, 0.07)
Reardon et al. (2019)	Alberta Pregnancy Outcomes and Nutrition cohort (2009–2012), 494 women	1.0	β (95% CI) for unit increase	Free T4 **-0.01 (-0.01, -0.001)***	Free T3 Not significant	**0.14 (0.04, 0.25)***
Inoue et al. (2019)	Cross-sectional analysis within Danish National Birth Cohort (1996–2002), Denmark, 1,366 pregnant women	1.1 (0.8–1.4)	Absolute Percent difference (95% CI) per IQR increase	Free T4 -0.3 (-1.6, 1)	NR	1.7 (-4.4, 8.1)
Lebeaux et al. (2020)	Health Outcome and Measures of the Environment cohort (2003–2006), 355 mother-infant pairs	1.6 (1.5)	β (95% CI) for doubling	Total T4 -0.01 (-0.04, 0.02) Free T4 0.02 (-0.01, 0.05)	Total T3 -0.01 (-0.04, 0.02) Free T3 -0.02 (-0.04, 0)	-0.06 (-0.23, 0.11)

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	T4	T3	TSH
Preston et al. (2018)	Project Viva cohort (1999–2002), U.S., 732 pregnant women and 480 neonates	2.4 (1.6–3.8)	β (95% CI) for IQR increase	Total T4 -0.05 (-0.14, 0.04) Free T4 -0.60 (-1.39, 0.19)	NR	2.89 (-2.12, 8.17)

**p* <0.05.
GM: geometric mean.
One medium confidence study (Berg et al., 2017) is not included because quantitative results were only reported for significant associations.

Six studies examined associations with thyroid hormones in children and/or adolescents, in addition to studies of adults that included adolescents or all ages without stratifying results, which were described above. All six studies (five *medium* confidence and one *low* confidence) reported null associations between PFHxS exposure and thyroid hormones (Gallo et al., 2022; Li et al., 2021b; Kim et al., 2020a; Caron-Beaudoin et al., 2019; Kang et al., 2018; Khalil et al., 2018)

Eleven studies (9 *medium* confidence) examined associations with thyroid hormones in infants. For T4, 10 studies were available, including 9 of *medium* confidence. One study with the highest exposure levels (Preston et al., 2018) reported statistically significant lower levels of total T4, driven by the association in boys, with an exposure-response gradient across quartiles. The remaining studies reported no association. Nine studies examined associations with T3. One *low* confidence study (Shah-Kulkarni et al., 2016) reported statistically significant higher levels of T3 with higher PFHxS exposure in girls and no association in boys, while Aimuzi et al. (2019) reported statistically significant inverse associations, strongest in boys. The remaining studies reported no association. Ten studies examined the association between TSH and PFHxS exposure. There were lower levels of TSH with higher PFHxS exposure in one *low* confidence study (Shah-Kulkarni et al., 2016), and higher levels of TSH in one study (Wang et al., 2014) though neither was statistically significant, and the confidence intervals were wide. The remaining studies reported no association.

Table 3-9. Associations between PFHxS exposure and thyroid hormone levels in *medium* confidence studies of infants.

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	T4	T3	TSH
Guo et al. (2021)	Sheyang Mini Birth Cohort Study (2009–2010), China, 490 infants	0.1 (0.1–0.1)	β (95% CI) for ln-unit increase	Total T4 0.04 (-0.006, 0.09) Free T4 0.02 (-0.007, 0.05)	Total T3 0.04 (-0.003, 0.09) Free T3 0.02 (-0.02, 0.05)	-0.10 (-0.23, 0.03)
Dufour et al. (2018)	University Hospital of Liege cohort (2013–2016) 214 mother-infant pairs	0.2	β (p-value) for detected vs not detected	NR	NR	(0.9) Girls 0.09 (0.5) Boys -0.06 (0.5)
Aimuzi et al. (2019)	Cross-sectional analysis from Shanghai Obesity and Allergy Cohort Study (2012–2013), 568 infants	0.2 (0.1–0.3)	β (95% CI) for ln-unit increase	Free T4 0.06 (-0.06, 0.18) Girls 0.03 (-0.14, 0.2) Boys 0.1 (-0.07, 0.26)	Free T3 **-0.04 (-0.09, -0.001)*** Girls **-0.08 (-0.14, -0.02)*** Boys **-0.02 (-0.16, -0.03)***	-0.03 (-0.06, 0.004) Girls -0.02 (-0.07, 0.02) Boys -0.04 (-0.08, 0.01)
Yang et al. (2016b)	Beijing Prenatal Exposure cross-sectional study (2013) 157 mother-infant pairs	0.5	Spearman correlation coefficients	Total T4: -0.005 Free T4: 0.01	Total T3: -0.07 Free T3: -0.03	0.08
Wang et al. (2014)	Taiwan Maternal and Infant Cohort Study (2000–2001), Taiwan, 116 infants	0.8 (0.3–1.4)	β (95% CI) for unit increase	Total T4 0.002 (-0.50, 0.50) Free T4 -0.03 (-0.10, 0.04)	Total T3 -0.001 (-0.007, 0.004)	0.49 (-1.45, 2.43)
Lebeaux et al. (2020)	Health Outcome and Measures of the Environment cohort (2003–2006), 355 mother-infant pairs	1.6 (1.5)	β (95% CI) for doubling	Total T4 0.02 (-0.01, 0.06) Free T4 -0.01 (-0.04, 0.02)	Total T3 -0.02 (-0.08, 0.03) Free T3 -0.02 (-0.05, 0.02)	0.05 (-0.05, 0.16)
Preston et al. (2018)	Project Viva cohort (1999–2002), U.S., 480 infants	2.4 (1.6–3.8)	β (95% CI) for IQR increase	-0.15 (-0.38, 0.08) Girls 0.07 (-0.23, 0.37) Boys **-0.46 (-0.83, -0.1)***	NR	NR
Liang et al. (2020)	Cross-sectional analysis within Shanghai-Minhang cohort (2012), China, 300 infants	2.7 (2.0–3.4)	β (95% CI) for ln-unit increase	Total T4 -0.59 (-7.94, 6.76) Free T4 -0.32 (-0.87, 0.22)	Total T3 0 (-0.05, 0.04) Free T3 0.02 (-0.08, 0.13)	0.43 (-1.02, 1.88)

*p <0.05.
One medium confidence study (Berg et al., 2017) is not included because quantitative results were only reported for significant associations.

In addition, five studies (four *medium* confidence) (Gallo et al., 2022; Kim et al., 2020a; Dufour et al., 2018; Wen et al., 2013; Chan et al., 2011) reported on the association between PFHxS and dichotomous hyper- and hypothyroidism outcomes defined by the authors using set cutpoints. In Wen et al. (2013), a *medium* confidence study, there were greater odds of subclinical hypothyroidism in men (OR 1.57, 95% CI 0.76, 3.25) and women (OR 3.10, 95% CI 1.22, 7.86), and subclinical hyperthyroidism in women (OR 2.27, 95% CI 1.07, 4.80) and lower odds of subclinical hyperthyroidism in men (OR 0.56, 95% CI 0.24, 1.2). Subclinical hypothyroidism was defined as TSH >5.43 mIU/L, and subclinical hyperthyroidism was defined as TSH < 0.24 mIU/L (both limited to those without diagnosed thyroid disease). Also in adults, Dufour et al. (2018) reported higher odds (though not statistically significant) of hypothyroidism in pregnant women and Gallo et al. (2022) did not report increases in thyroid disease or medication use. In the low confidence study (Chan et al., 2011), hypothyroxinemia in pregnant women was defined as normal TSH concentrations with no evidence of hyperthyroidism (0.15–≤4 mU/L) and free T4 in the lowest 10th percentile (≤8.8 pmol/L) of the study sample). They found higher odds of hypothyroxinemia with higher PFHxS exposure (OR 1.12, 95% CI 0.89, 1.41). In children and adolescents, Kim et al. (2020a) reported lower odds of subclinical hypothyroidism with higher exposure and Gallo et al. (2022) reported no association.

Thyroid effects summary

Overall, the evidence for the association between PFHxS exposure and thyroid effects is inconsistent. Some studies do indicate an association between thyroid hormones or subclinical thyroid disease and PFHxS exposure, but this direction is not consistent across studies and the associations with PFHxS exposure in most studies were null. There is also not clear coherence across outcomes, with indications of associations with both hyper- and hypothyroidism and unclear coherence of the direction of association between TSH and the other hormones. However, almost all of the available studies were deficient in outcome ascertainment due to lack of consideration of timing of sample collection. As discussed above, this is likely to result in nondifferential outcome misclassification, which also is expected to bias results toward the null on average, although the studies without this issue also reported null findings. Given these concerns, the findings across this set of studies are difficult to interpret.

Animal Studies

The toxicity evidence base for PFHxS-induced endocrine outcomes consists of three multigenerational publications (two studies) in SD or Wistar rats (Ramhøj et al., 2020; Chang et al., 2018; Ramhøj et al., 2018), one developmental study in ICR mice (Chang et al., 2018), and one short-term (28 day) study in SD rats (NTP, 2018a). All studies treated the animals orally to PFHxS via gavage. Endocrine-related outcomes evaluated by these studies included: thyroid hormones, histopathology, and endocrine organ weights including thyroid, parathyroid, and adrenal gland weight. Potential PFHxS effects on male and female reproductive organs (e.g., testes and ovaries) and reproductive hormones (e.g., testosterone and estradiol) that also encompass part of the endocrine system are discussed in Male Reproductive Effects and Female reproductive Effects sections.

Evaluation of the available animal studies showed that these were generally well conducted for most endocrine-related endpoints. The available studies examined PFHxS endocrine toxicity effects using doses that ranged between 0 and 10 mg/kg-day in mice (Chang et al., 2018); 0 and 25 mg/kg-day in rats with the exception of NTP (2018a), for which a range of 0–50 mg/kg-day in female rats and 0–10 mg/kg-day in male rats was used. These ranges account for the pharmacokinetic (PK) sex differences that have been observed in rats, for which PFHxS appears to have a lower mean half-life in female rats versus their male counterparts (1.72 and 26.9 days, respectively, after oral dosing (Kim et al., 2016b)). No overt toxicity was observed at any of the highest doses tested in any of the available studies. Two *high* confidence studies, Chang et al. (2018) and NTP (2018a), examined PFHxS effects on histopathology endpoints; three *high* confidence studies (Chang et al., 2018; NTP, 2018a; Butenhoff et al., 2009) examined PFHxS effects on thyroid gland weight. Lastly, two *high* confidence studies (NTP, 2018a; Butenhoff et al., 2009) also measured adrenal gland weights. A summary of the study evaluations for each endpoint are presented in Figures 3-6, 3-12, and 3-13; additional details can be obtained from HAWC.

Thyroid hormones

Four studies (three *high* and one *low* confidence; see Figure 3-6, below) examined the effects of PFHxS on levels of thyroid hormones, T3, T4, and/or TSH. One *high* confidence study, NTP (2018a) examined effects on serum concentrations of TSH, T3, and total and free T4 in adult animals. The other two *high* confidence studies examined effects of PFHxS on serum T4 (Ramhøj et al., 2018), T3 and TSH (Ramhøj et al., 2020) in exposed dams and their offspring (exposed via lactation) through PND 22. Lastly, the fourth study was *low* confidence in which Chang et al. (2018) reported using a developmental study design that followed established guidelines for such studies (OECD 422 Testing guidelines). However, the reported study design ignored essential components of the OECD 422 developmental toxicity screening guidelines. A necessary requirement of the OECD guidelines is that serum T4 be measured as part of developmental toxicity studies. The study authors did not measure T4 serum levels, under the rationale that T4 is an “inactive hormone” and elected to measure TSH serum levels instead. It has been established that serum TSH measures are not good indicators of potential endocrine disruption (OECD, 2016; Stoker et al., 2006; Crofton, 2004).

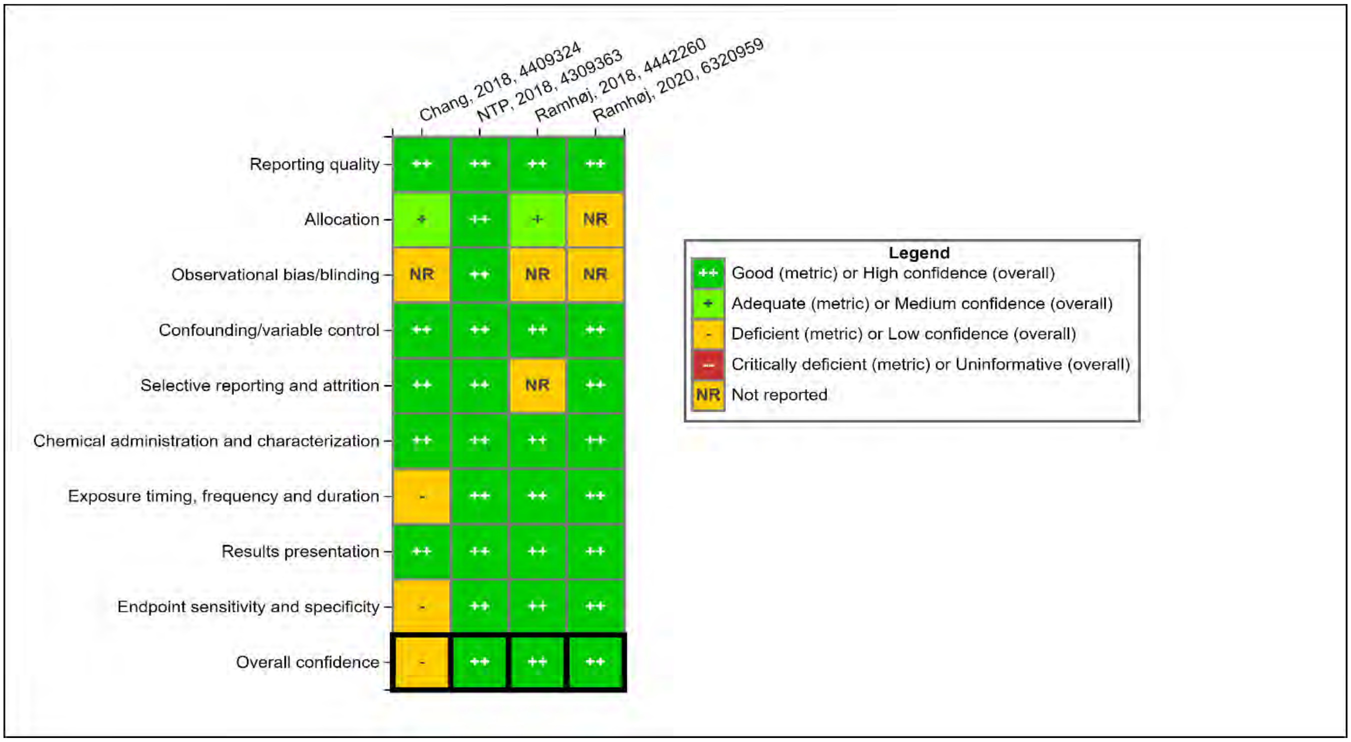


Figure 3-6. Study evaluation results for measures of thyroid hormone levels in PFHxS animal toxicity studies. Full details available by clicking HAWC link.

NTP (2018a) measured free and total T4 serum levels in Sprague Dawley and Ramhøj et al. (2018) measured total T4 serum levels in Wistar rats (see Figures 3-7 and 3-8). NTP observed a statistically significant, dose-dependent decrease ($p < 0.01$) of free and total T4 levels starting at the lowest experimental dose (0.625 mg/kg-day) in male rats (up to 60% and 78% decrease in free and total T4 respectively); free T4 and total T4 were significantly decreased beginning at 12.5 mg/kg-day and 6.25 mg/kg-day, respectively, in female rats ($p < 0.01$, up to 32% and 38 % decrease in free and total T4 respectively). However, serum total T4 levels are a more sensitive and reliable measure of T4 due to sensitivity limitations in the available assays used to measure free T4. Ramhøj et al. (2018) reported similar findings in Wistar rat dams, with statistically significant, dose-dependent decreases in serum total T4 at 5 mg/kg-day and above in dams at PND 22 after exposure from gestational day 7 (GND 7) through postnatal day 16/17 (Ramhøj et al., 2018) (-26% decrease at 5 mg/kg-day dose and up to −71% decrease at 25 mg/kg-day dose). Comparable observations were made in the pups born to the PFHxS-exposed dams in Ramhøj et al. (2018), with statistically significant decreases in total T4 levels in serum collected from PND22 pups at ≥5 mg/kg-day ($p < 0.001$, up to a 71% decrease in total T4 at 25 mg/kg-day dose and 38% decrease in total T4 at 5 mg/kg-day dose). No overt toxicity was observed at any of the highest doses tested in any of the available studies. Effects occurred at lower concentrations of PFHxS in male rats than their female counterparts indicating that males could be more susceptible to PFHxS effects than females (see Figure 3-7). However, a more likely explanation is that these observations, at least in part, can be explained by the differences in PFHxS pharmacokinetics that exist between male and female rats. Sex differences in plasma half-life and tissue distribution have been observed for PFHxS, wherein PFHxS-exposed male rats have a longer plasma half-life (20.7–26.9 days) versus their female counterparts (0.9–1.7 days) (Kim et al., 2016b).

Two studies, NTP (2018a) and Ramhøj et al. (2020), measured T3 in serum. NTP (2018a) observed a statistically significant and dose-dependent decrease ($p < 0.05$) in serum T3 levels in male, but not female, SD rats at ≥0.625 mg/kg-day ($p < 0.01$); Ramhøj et al. (2020) in a similar study design as Ramhøj et al. (2018), reported a significant decrease in serum T3 in Wistar rat dams at the highest tested dose: 25 mg/kg-day at PND 22 after exposure from gestational day 7 (GND 7) through postnatal day 16/17 ($p < 0.001$, 19% decrease). Comparable observations were also made in the pups born from the exposed dams at PD16/17 in which a significant decrease in serum T3 was observed in pups of both sexes at the highest dose: 25 mg/kg-day ($p < 0.001$, 16% decrease).

Lastly, three studies, NTP (2018a), Chang et al. (2018) and Ramhøj et al. (2020) investigated PFHxS effects on TSH levels. None of these studies observed changes in TSH serum levels in male or female CD1 mice, Sprague Dawley rats or Wistar rats in response to PFHxS exposure.

Taken together, and as noted in the study results reported by NTP and the combined Ramhøj studies (Ramhøj et al., 2020; Ramhøj et al., 2018), these results support that PFHxS exposure in rats has the ability to adversely decrease the endocrine hormones, T4 and T3, in the absence of observed effects on TSH.

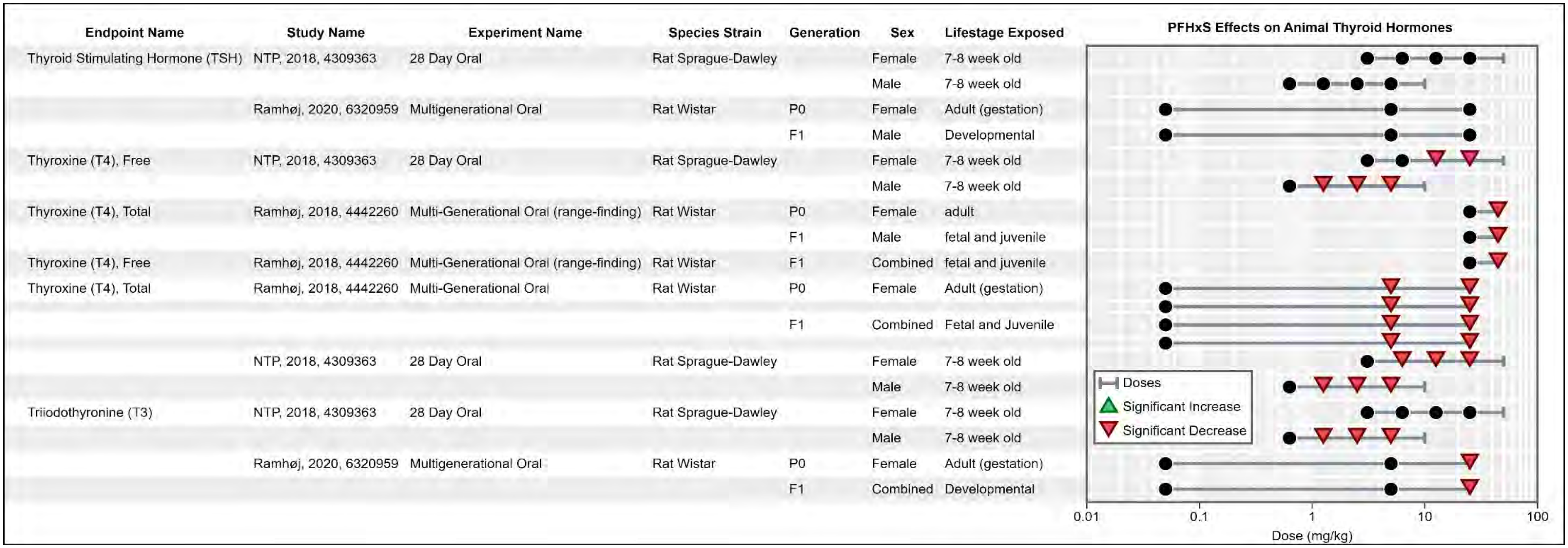


Figure 3-7. Summary of thyroid hormone measures in animal studies. Figure displays the three *high* confidence studies included in the analysis; the sole *low* confidence study, **Chang et al. (2018)** was omitted from the analysis. Full details available by clicking HAWC link. Details on study confidence may be found in Figure 3-6.

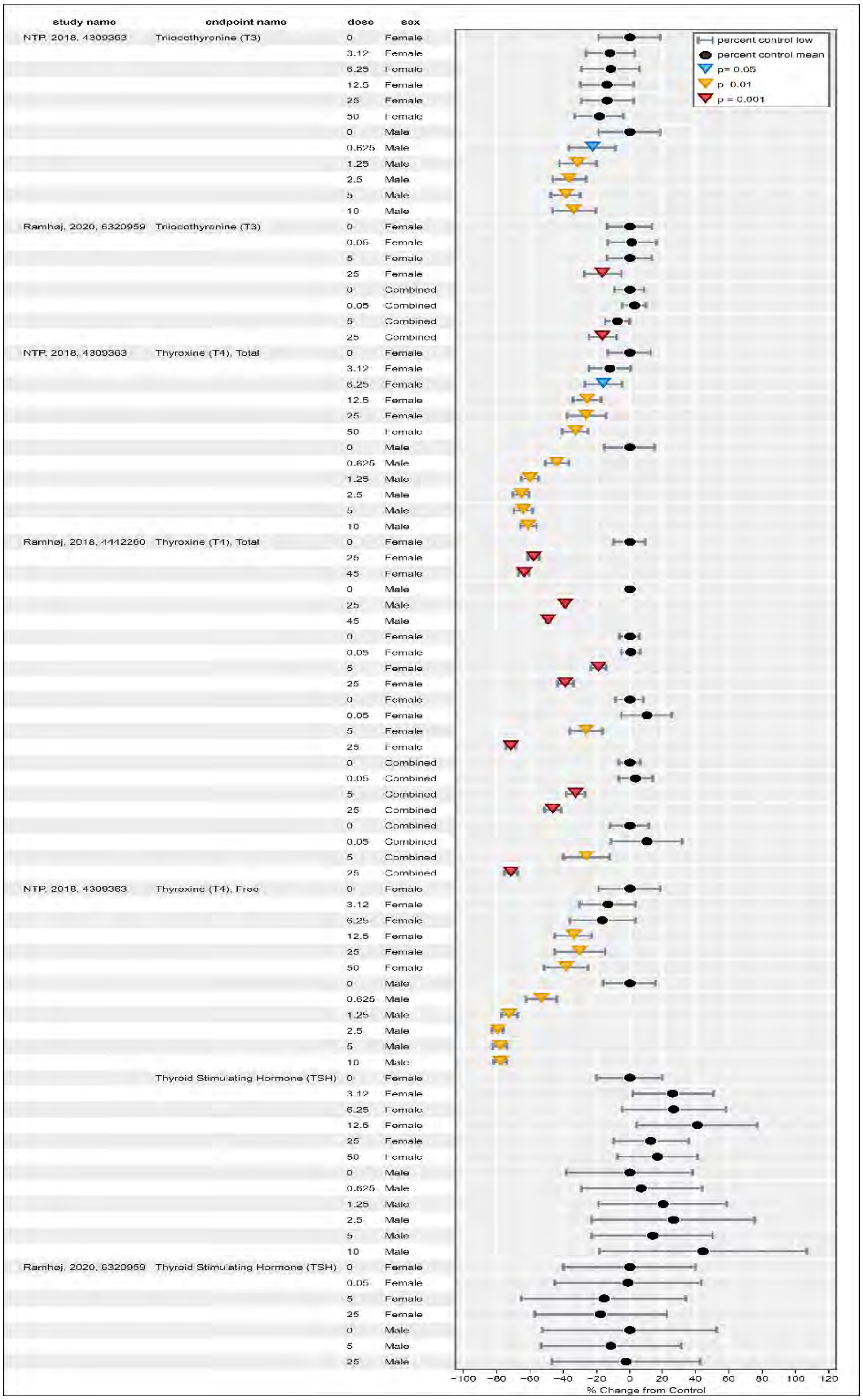


Figure 3-8. Percent change in thyroid hormone levels following PFHxS exposure in the available animal toxicology studies. For details see HAWC link.

Histopathology

Three *high* confidence studies evaluated nonneoplastic histopathologic lesions in endocrine tissues in response to PFHxS exposure (Ramhøj et al., 2020; NTP, 2018a; Butenhoff et al., 2009) (see Figure 3-9). NTP (2018a) evaluated various organs in the endocrine system including the adrenal cortex, adrenal medulla, parathyroid gland, pituitary gland, and the thyroid gland in adult male and female rats exposed to PFHxS for 28 days. NTP (2018a) observed no histological lesions in any of the endocrine tissues they evaluated and made no observations of hyperplasia or hypertrophy in the thyroids at doses up to 10 mg/kg-day in male rats or 50 mg/kg-day in female rats. However, a 44-day study by Butenhoff et al. (2009) observed increased incidences of hypertrophy and hyperplasia (characterized as “minimal”) of thyroid follicular epithelial cells in adult male rats that were exposed to 3.0 mg/kg-day PFHxS (40% incidence) and an increase in “moderate” hypertrophy and hyperplasia at 10 mg/kg-day PFHxS (70% incidence) for up to 44 days (minimal hypertrophy/hyperplasia (20% incidence) was observed in control animals). The study authors attributed the pathological changes in the thyroid to changes in enzyme induction in the liver (see Serum Biomarkers of Liver Function in Section 3.2.5) that have been shown by others (Sanders et al., 1988) to result in a compensatory increase in T4 clearance that may elicit increases in TSH hormone levels or no compensatory TSH responses. The role of TSH in the progression of thyroid hyperplasia and hypertrophy were highlighted in Noyes et al. (2019). In the proposed Adverse Outcome Pathway (AOP) by Noyes et al. (2019), the authors illustrate that increased serum TSH may lead to thyroid hyperplasia and hypertrophy. However, Butenhoff et al. (2009) did not measure thyroid hormone levels as part of their experimental analysis, so this hypothesis was not tested. Lastly, Ramhøj et al. (2020) reported that in Wistar rat dams exposed to PFHxS at doses ranging from 0.05 to 25 m/kg-day from gestational day 7 (GND 7) through postnatal day 16/17, no PFHxS effects on thyroid histopathology were observed. The authors reported that the thyroid glands corresponding to the high dose (25 mg/kg-day) male pups showed “small histological changes;” however, these changes were within the normal range and were no longer evident on PD 22. The authors did not observe hypertrophy or hyperplasia at any time point in either the exposed dams or their offspring (Ramhøj et al., 2020).

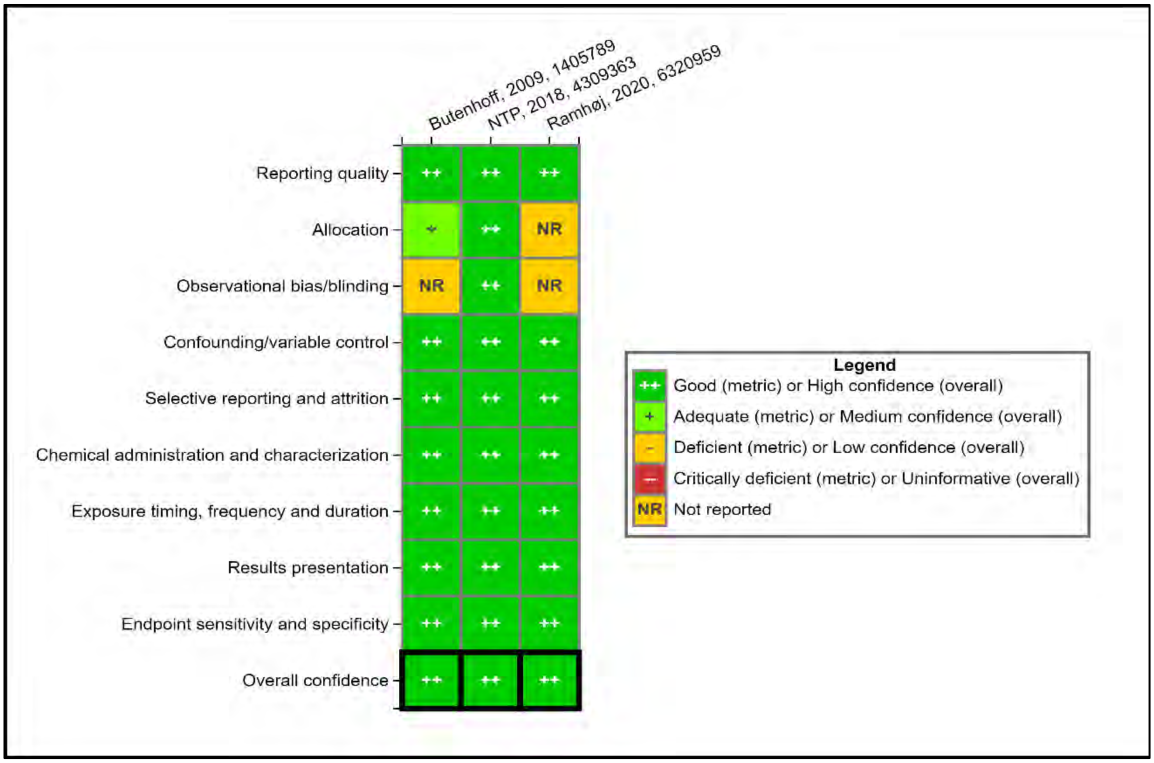


Figure 3-9. Study evaluation results for endocrine histopathology outcomes in PFHxS animal toxicity studies. Full details available by clicking HAWC link.

Organ weights

Three studies evaluated the effect of PFHxS exposure on thyroid gland weights (Ramhøj et al., 2020; Chang et al., 2018; NTP, 2018a) (see Figure 3-10; Figure 3-11). Chang et al. (2018) and NTP (2018a) observed no significant effects in adult CD1 male or female mice or in adult male or female Sprague Dawley rats at the PFHxS doses administered in these studies (see Figure 3-11). However, Ramhøj et al. (2020) observed a statistically significant ($p < 0.05$) decrease in absolute thyroid weights (relative weights were not reported) starting at 5 mg/kg bw-day that continued into the highest dose tested (25 mg/kg bw-day) in PD 22 female Wistar pups exposed to PFHxS starting at GD7 (5 mg/kg bw-day $p < 0.05$, 17% decrease; 25 mg/kg bw-day $p < 0.01$; 23% decrease) (see Figure 3-11). The differences in experimental designs across these studies make it difficult to compare the results and thus the importance of the findings reported by Ramhøj et al. (2020) is unclear.

Two studies, Butenhoff et al. (2009) and Chang et al. (2018) evaluated the effects of PFHxS on adrenal gland weights in SD rats. Butenhoff et al. (2009) reported no effect on absolute or relative adrenal weight resulting from 0, 0.3, 1.3, or 10 PFHxS mg/kg-day for 44 days. NTP observed statistically significant increase in absolute adrenal weights in female rats (at ≥12.5 mg/kg-day; 15% increase) and an increase in relative adrenal gland weight at 50 mg/kg-day (9% increase $p < 0.01$) in female rats. NTP also reported decreases in both absolute (at ≥5 mg/kg-day; -13%;

p < 0.05) and relative adrenal weights (at ≥2.5 mg/kg-day; -17%; $p < 0.05$) in male rats. It is unclear why there were opposing responses across sexes in the NTP study that were not observed in the Butenhoff et al. (2009) (see Figure 3-11); however, these observations could be due to the pharmacokinetic differences between male and female animals coupled with differences in study design between the two studies.

Overall, the organ weight changes are mixed and cannot be readily interpreted.

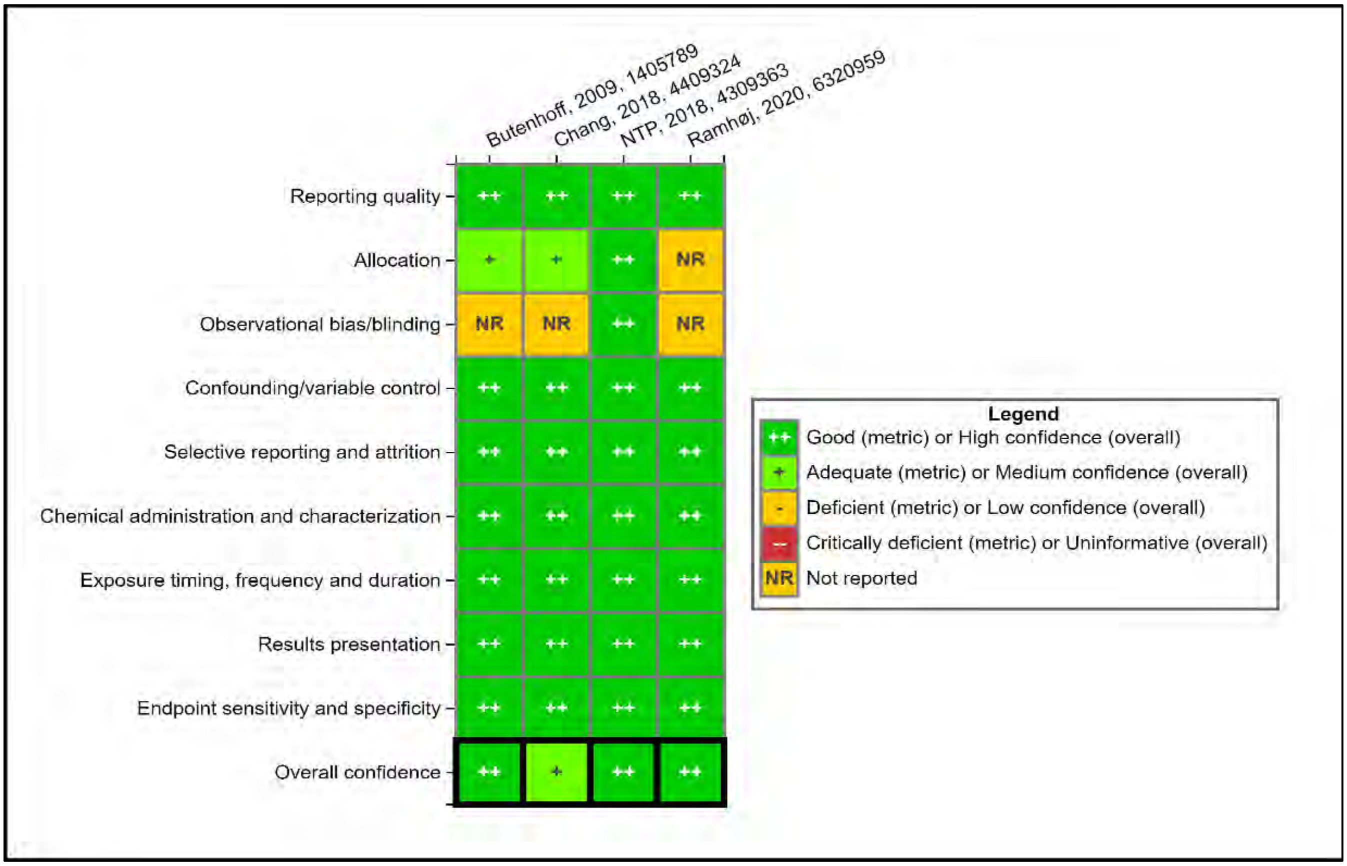


Figure 3-10. Study evaluation results for endocrine organ weights in PFHxS animal toxicity studies. Full details available by clicking HAWC link.

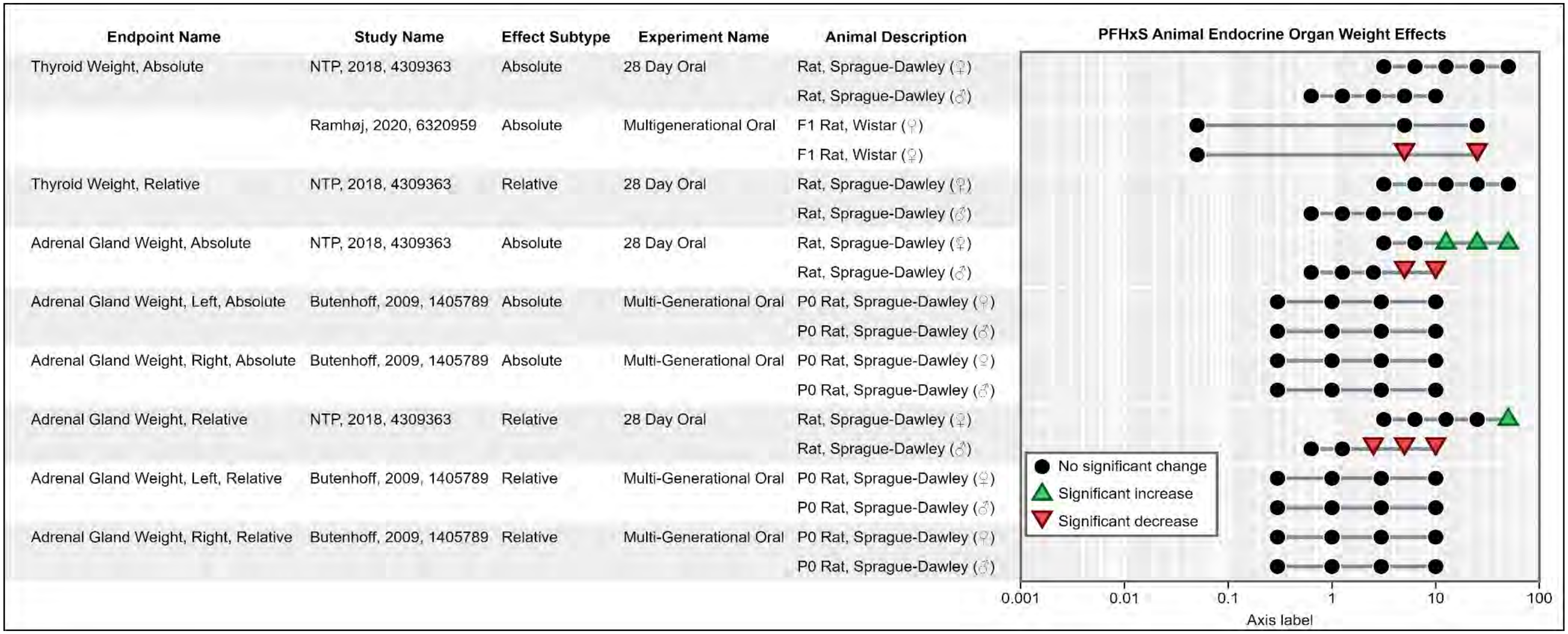


Figure 3-11. Summary of endocrine organ weight effects in animal studies. Figure displays the *medium* and *high* confidence studies. Full details available by clicking HAWC link.

Mechanistic Evidence and Supplemental Information

The available thyroid hormones data in rodents showed strong effects on T4 and T3 after short-term exposure, although no effects were observed on TSH; however, a pattern of decreased T4 without pronounced (or detectable) changes in TSH is consistent with hypothyroxinemia and has been observed in some analyses of other PFAS, including several long-chain (Kim et al., 2018a) and short-chain (U.S. EPA, 2022, 2021a, b) PFAS. During pregnancy and early development, perturbations in thyroid function can have impacts on normal growth and neurodevelopment in the offspring (Stagnaro-Green and Rovet, 2016; Zoeller and Rovet, 2004). Low thyroid hormone status is also likely associated with effects in numerous other organ systems, including the heart, bone, lung, and intestine (Bassett et al., 2007; Mochizuki et al., 2007; Wexler and Sharretts, 2007; Bizzarro and Gross, 2004).

Mechanistic studies on the endocrine effects of PFHxS are scarce, with only one study conducted in a mammalian test system. Long et al. (2013) explored the effects of PFHxS along with other PFAS on thyroid hormone signaling and the aryl hydrocarbon receptor (AhR) using the T3-dependent rat pituitary cell line, GH3. The authors found that PFHxS inhibited GH3 cell proliferation in a dose-dependent manner. Additionally, the authors found that PFHxS—along with three other PFAS (PFOS, PFNA, and PFUnA)—antagonized GH3 cell proliferation in response to exogenous T3 treatment. The authors speculated that PFHxS may compete with T3 for binding to thyroid hormone receptor (TR) or other cofactors to inhibit cell proliferation; however, specific experiments testing this hypothesis were not conducted.

Other studies in nonmammalian systems (e.g., avian neuronal cells and chicken embryos) have shown that PFHxS alters mRNA levels of thyroid hormone-responsive genes, including transthyretin (TTR) (Cassone et al., 2012; Vongphachan et al., 2011). TTR is a transport protein that is secreted into the blood by the liver and by the choroid plexus into the cerebrospinal fluid. TTR binds to thyroid hormones such as T4 and T3 in the serum and in the cerebrospinal fluid. Due to its low affinity for thyroid hormones TTR readily disassociates from these and is therefore responsible for the immediate delivery of T3 and T4 to various extrahepatic tissues and potentially into the brain (Palha, 2002). Decreases in TTR may lead to decreases in T4 transport (Refetoff, 2015). Additionally, TTR plays a key role in thyroid hormone storage and transport during fetal development. PFHxS-induced decreases in TTR mRNA have been shown in nonmammalian systems, and the above mechanism would in part assist in elucidating the mechanisms underlying the in vivo observations pertaining to PFHxS-induced decreases T3 and T4. However, TTR binds only a small portion of the circulating thyroid hormones (15%–20%) (Refetoff, 2015), and confirmatory studies in model systems more relevant to humans would be needed to understand the potential role of PFHxS-induced alterations to thyroid hormone-responsive genes in humans.

Data from the ToxCast Dashboards Endocrine Disruptor Screening Program (EDSP21) (https://comptox.epa.gov/dashboard/chemical-lists/EDSPUOC) reveal that K+PFHxS was active in a total of only 2 out of 57 endocrine-related assays (with both positive hits at PFHxS levels nearing the cytotoxicity limit). A summary of the assay results from the EDSP21 project may be found in Appendix C, Section 3. Briefly, out of 27 estrogen receptor assays, K+PFHxS was active in one, the ATG_ERE_CIS_up induction assay with an AC50 at 96.96 μM (see Figure 3-12). K+PFHxS was not active in any of the 16 androgen receptor assays. K+PFHxS was active in one out of 13 assays associated with perturbation of thyroid hormone signaling, synthesis, or metabolism, namely the NIS-RAIU_inhibition assay with an AC50 of 18.68 μM. It should be noted that the current panel of bioactivity assays interrogating thyroid hormone dynamics is predominately targeted at receptor-dependent agonism/antagonism, which is only one of several pathways by which the mammalian HPT-axis may be perturbed by PFAS (Noyes et al., 2019). K+PFHxS was not active in any of the three steroidogenesis assays in the database. Overall, although not conclusive, PFHxS exhibited little in vitro endocrine activity in these assays (>96% of assays were inactive).

Overall, the mechanistic information is scarce and inconclusive, and therefore does not provide clear support for or against endocrine (thyroid)-modulating activity of PFHxS.

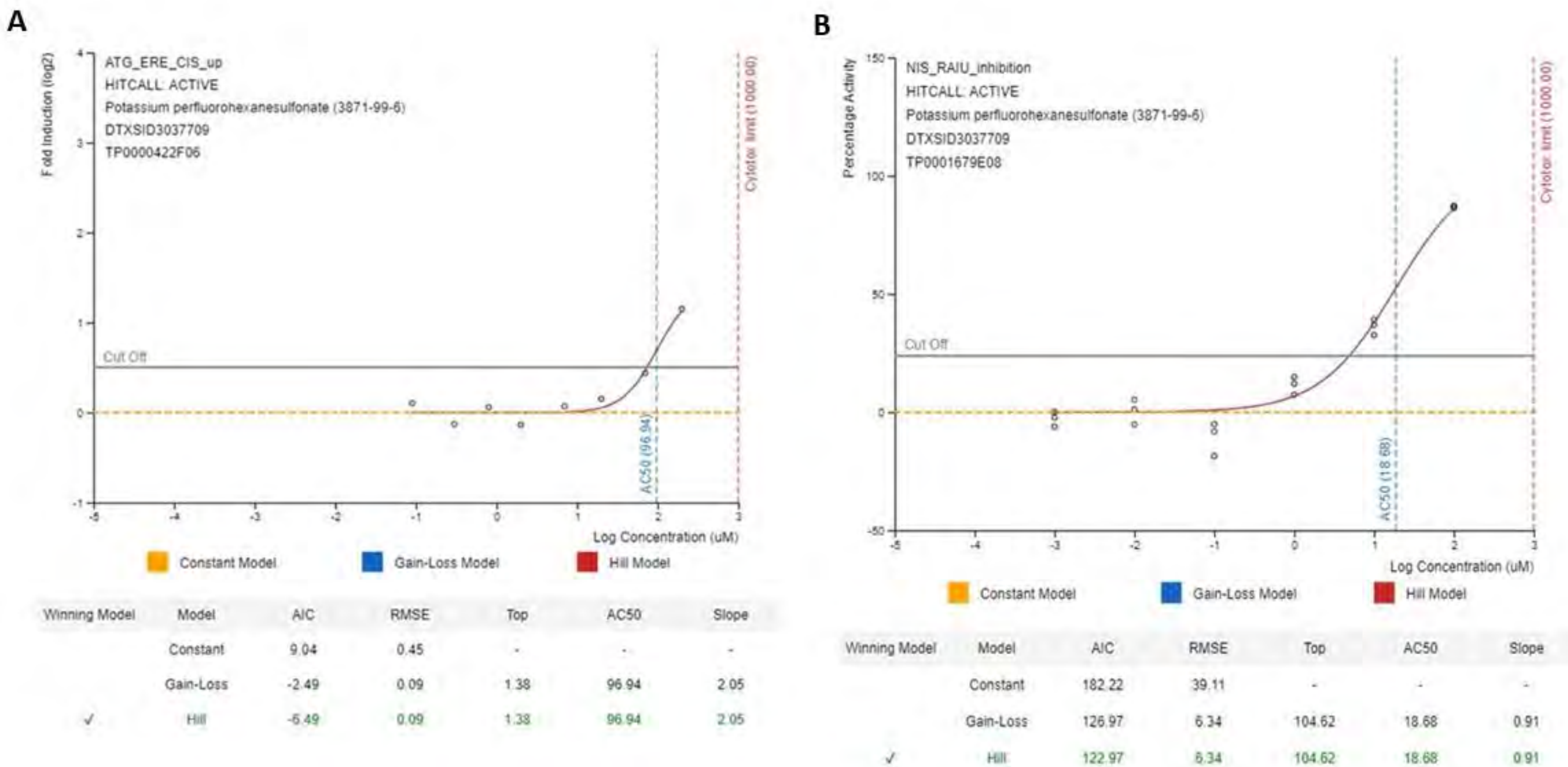


Figure 3-12. EDSP21 results of PFHxS active assays: A: ATG_ERE_CIS_up induction assay performed in HepG2 cells; B: NIS_RAIU_inhibition assay performed in HEK293T cells. Assay details available in Appendix C, Section 3.

Evidence Integration

Human studies provide conflicting evidence as to the potential effects of PFHxS on thyroid outcomes (e.g., thyroid hormone levels). Although a few studies did suggest an association between increasing PFHxS exposure levels and decreased circulating thyroid hormones (i.e., T4) or subclinical thyroid disease, the associations were not consistent across studies (most studies were null); the inconsistent findings could not be explained by differences in study design, confidence, or other factors such as population, and there was no clear coherence across outcomes. The available human evidence on PFHxS effects on the thyroid is *indeterminate*.

Evidence of thyroid toxicity resulting from PFHxS exposure in animal models exposed in short-term and multigenerational studies showed dose-dependent effects on thyroid hormone (TH) levels, most notably consistent decreases in serum T4 levels in rats (untested in mice) (NTP, 2018a; Ramhøj et al., 2018). Coherent and consistent decreases in T3 in rats were also observed across studies, whereas TSH was unchanged. Thyroid organ weights and thyroid histopathology were inconsistently or only weakly affected across studies (e.g., increased incidence of thyroid hypertrophy and mild hyperplasia in one study and decreased thyroid weight in another, with otherwise null results), suggesting that the TH decreases are probably not attributable to effects of PFHxS on thyroid gland function. However, the available evidence from exposed rodents shows a consistent, dose-dependent disruption of thyroid hormone homeostasis, characterized by decreased T4 and T3 serum levels concurrent with unaffected, normal levels of TSH is consistent with hypothyroxinemia and also consistent with what has been observed in other PFAS including PFBS, PFHxA, PFBA and PFOA. The observed TH decreases occurring in exposed adult animals and indirectly (through the dams) exposed offspring were of a large magnitude of effect and occurred even at PFHxS exposure levels as low as 0.625 mg/kg-day in male rats. This finding is consistent with the published proposed thyroid disruption Adverse Outcome Pathway (AOP) by Noyes et al. (2019) and publication by Zoeller and Crofton (2005), in which the authors illustrated that endocrine disruption in humans and rodents possess analogous key events and adverse outcomes perhaps due to conserved biology across species (see additional discussion below). Decreased thyroid hormone levels are judged relevant to human health, given the many similarities in the production, regulation, and functioning of thyroid hormones between rodents and humans (Vansell, 2022; Stagnaro-Green and Rovet, 2016; Dong et al., 2015; Navarro et al., 2014; Rovet, 2014; Berbel et al., 2010; Morreale de Escobar et al., 2008; Cuevas et al., 2005; Rovet, 2005; Zoeller and Rovet, 2004; Hood and Klaassen, 2000; Hood et al., 1999a; Hood et al., 1999b). Taken together, the available animal evidence on endocrine effects, which is primarily based on the observed supporting decreases in thyroid hormone levels after PFHxS exposure, is considered *moderate*.

Mechanistic studies examining the endocrine disrupting effects of PFHxS are scarce. In the single mammalian study, Long et al. (2013), PFHxS, similar to other tested PFAS, inhibited cell growth but not proliferation in the T3-dependent rat pituitary cell line, GH3. However, while this study suggests the possibility that PFHxS might compete with THs, these data alone are insufficient to provide support for biological plausibility.

The currently available **evidence indicates** that PFHxS exposure likely causes thyroid effects in humans given sufficient exposure conditions[6] (see Table 3-10). This conclusion is based primarily on consistent and coherent decreases in thyroid hormone levels across short-term and multigenerational studies in rats exposed to PFHxS levels ≥2.5 mg/kg-day (with males being more sensitive). The pattern of available evidence in rats indicates that PFHxS, like other PFAS (U.S. EPA, 2021a; Coperchini et al., 2017) leads to a disruption of thyroid hormone homeostasis in a pattern similar to hypothyroxinemia. Noyes et al. (2019) along with Zoeller and Crofton (2005) illustrated that endocrine disruption in humans and rodents possess analogous key events and adverse outcomes perhaps due to conserved biology across species, and thus these effects are considered adverse and relevant to humans. These TH decreases could have detrimental effects on susceptible populations as T3 and T4 are critical in brain development and bone growth during early childhood and adolescence (Crofton, 2004). However, at present, few epidemiological studies and toxicological studies have addressed PFHxS-induced effects in these populations, highlighting an important data gap.

[6] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-10. Evidence profile table for PFHxS thyroid effects

<table>
<tr><th colspan="5">Evidence Stream Summary and Interpretation</th><th>Evidence Integration Summary Judgement</th></tr>
<tr><td colspan="6">Evidence from studies of exposed humans (see Human Thyroid Section)</td></tr>
<tr><td>Studies and confidence</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Summary and key findings</td><td>Evidence stream judgment</td><td rowspan="6">⊕⊕⊙
Evidence Indicates (likely)

Primary basis:
Moderate animal evidence for decreased T4 and T3 in adult and juvenile rats

Human relevance:
Effects in rats are considered relevant to humans due to conserved biology across species (see Evidence Integration section.)

Cross-stream coherence:
NA; human evidence indeterminate

Susceptible Populations and lifestages:
Young individuals exposed to PFHxS during gestation and early childhood may be susceptible populations.</td></tr>
<tr><td>Thyroid Measures & Disease
Twenty-six medium confidence studies
Ten low confidence</td><td>• No factors noted</td><td>• Unexplained inconsistency</td><td>Some human studies report an inverse association between thyroid hormones and PFHxS exposure, but most studies reported null findings.</td><td>⊙⊙⊙
Indeterminate</td></tr>
<tr><td colspan="5">Evidence from in vivo animal studies (see Animal Thyroid Section)</td></tr>
<tr><td>Studies and confidence</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Summary and key findings</td><td>Evidence stream judgment</td></tr>
<tr><td>Thyroid Hormones
Three high confidence studies in rats
• 28-d
• Multigenerational</td><td>• Consistent and coherent decreases of T4 and T3 in adult and juvenile rats in the absence of effects on TSH
• Large Magnitude of effect (up to 70%)
• Dose response in studies</td><td>• No factors noted</td><td>Studies in rats (2 for T3 and 3 for T4) reported significant decreases in TH levels in both male and female rats (for T4), or just male rats (for T3), generally after PFHxS exposure at ≥2.5 mg/kg-d.</td><td>⊕⊕⊕
Moderate
Based on decreased T4 and T3</td></tr>
</table>

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgement
Histopathology Three *high* confidence studies in rats • 28- and 42-d • Multigenerational	• No factors noted	• No factors noted	Increased incidence of thyroid hypertrophy and hyperplasia in male rats in one study.		
Organ Weights Three *high* confidence studies in rats and one *medium* confidence study in mice	• Concerning *magnitude of effect* (up to 23% decrease) in female pups in one study	• Unexplained *inconsistency* (across studies for thyroid weights and across sexes for adrenal weights)	Decreased absolute thyroid weight in female F1 pups at PD22 (one study); Increased absolute adrenal gland weight in female rats and decreased absolute adrenal gland weight in male rats (one study); Increased relative adrenal gland weight in female rats (highest dose only) and decreased a relative adrenal gland weight in male rats (one study).		

3.2.2. Immune Effects

Human Studies

Epidemiology studies examining immune effects of PFHxS exposure include studies on antibody response, infectious diseases, and hypersensitivity-related outcomes, which includes asthma, allergies, and atopic dermatitis. The health effects results were grouped across studies to develop conclusions on the same or related outcomes for the main categories of immune response according to immunotoxicity guidance from the World Health Organization/ International Programme on Chemical Safety (IPCS, 2012): (1) immunosuppression, (2) sensitization or allergic response, and (3) autoimmunity. Evidence for potential immune effects was considered within these three categories because of common and related mechanisms within each category. Within each category, health effects data were considered in the order of most to least informative for immunotoxicity risk assessment (IPCS, 2012). Specifically, clinical studies on disease or immune function assays are considered most informative, then general/observational immune assays (lymphocyte phenotyping or cytokines), and finally endpoints such as hematology (i.e., blood leukocyte counts) are least informative. Outcomes related to immunosuppression were considered within two subcategories: antibody response and infectious disease. Several different outcomes, such as asthma and food allergies, were included in the sensitization and allergic response category. No studies were identified that evaluated outcomes related to autoimmunity.

Immunosuppression

Antibody response outcomes

The production of antigen-specific antibodies in response to an immune challenge (e.g., vaccination in humans or injection with sheep red blood cells in rodents) is a well-accepted measure of immune function included in risk assessment guidelines and animal testing requirements for immunotoxicity (IPCS, 2012; ICH Expert Working Group, 2005; U.S. EPA, 1998; IPCS, 1996). The production, release, and increase in circulating levels of antigen-specific antibodies are important for protection against infectious agents and preventing or reducing severity of influenza, respiratory infection, colds, and other diseases as part of the humoral immune response. Reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious disease.

Evaluations for studies of antibody responses following vaccination as reported in ten epidemiological studies (reported in 11 publications) are summarized in Figure 3-13. Among these studies, there were analyses of several vaccinations: diphtheria (six studies), tetanus (seven studies), measles (three studies), rubella (two studies), mumps (one study), Haemophilus influenzae Type B (two studies), hepatitis (one study), and FluMist (one study). There were four prospective birth cohorts, including three in the Faroe Islands and one in Norway (Granum et al., 2013), and one cohort of children beginning in their first year of life in Guinea-Bissau

(Timmermann et al., 2020). The three Faroe Islands studies included non-overlapping populations enrolled at separate times, all *medium* confidence, one with enrollment in 1997–2000 and subsequent follow-up to age 7 (Grandjean et al., 2012) and age 13 (Grandjean et al., 2017a), one with enrollment in 2007–2009 and follow-up to age 5 (Grandjean et al., 2017b), and one with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021). These cohorts are thus considered independent of each other. Some analyses in Grandjean et al. (2017b) combined new data from the cohort born in 2007-2009 with new follow-up data from the cohort born in 1997–2000 (Grandjean et al., 2012); these are labeled in the results table. Given that the etiologic window for immune effects of PFAS exposure is not known, these studies in the Faroe Islands have the benefit of assessing multiple windows of exposure (maternal, multiple points in childhood) as well as following outcomes over time. For example, exposures measured during infancy could have reflected residual maternal antibodies, but the half-life of maternal antibodies is short and residual antibodies would not be expected to exist beyond infancy and would not exist in the children at age five years. Similarly, vaccine boosters likely changed these children's antibody concentrations over time, but such changes were not expected to be related to PFHxS concentration. Having multiple windows of exposure in this study allowed for comparisons of effects. In children, there were also two *medium* confidence cross-sectional studies in the U.S. and Greenland (Timmermann et al., 2021; Stein et al., 2016b) and one *low* confidence (due to expected residual confounding) cross-sectional study in Germany (Abraham et al., 2020). In adults, there were two additional *low* confidence studies, a short-term cohort (with exposure measured at vaccination and follow-up 30 days later) in the United States (Stein et al., 2016a) and a cross-sectional study in Denmark (Kielsen et al., 2016). These studies were *low* confidence due to concerns for potential selection bias and confounding.

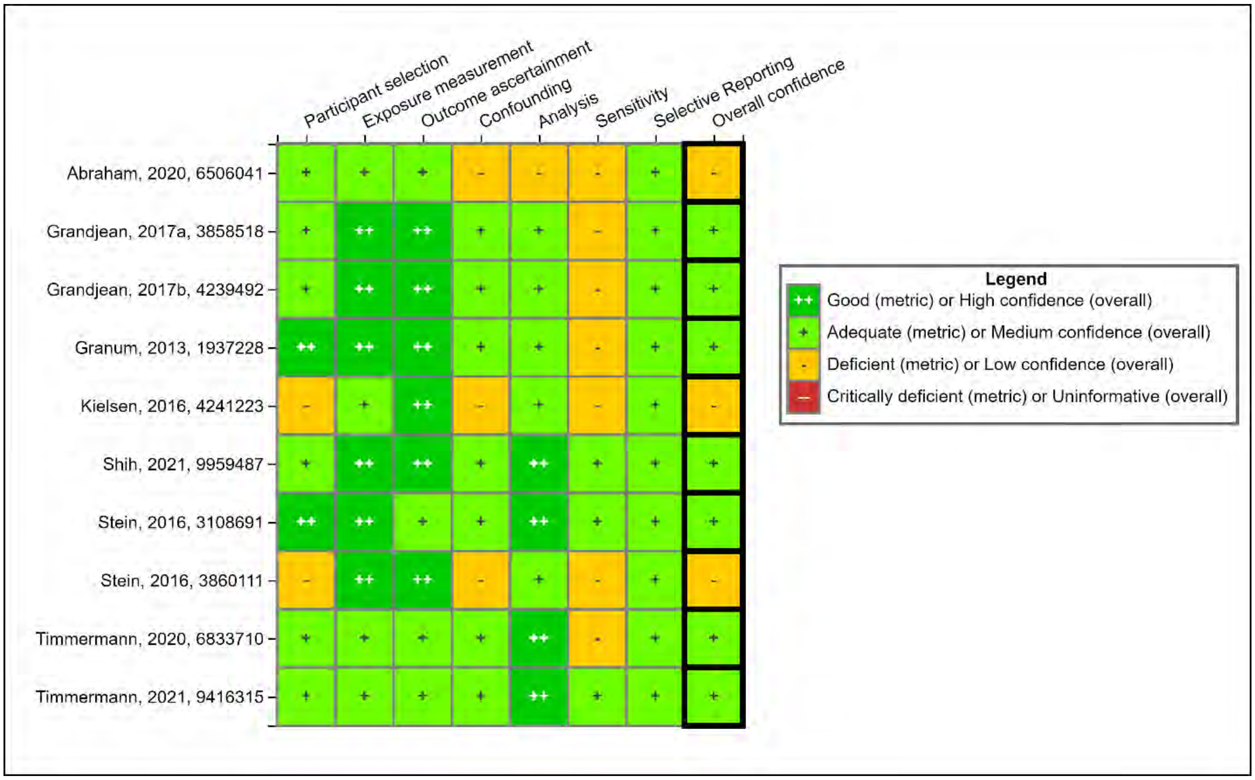


Figure 3-13. Summary of evaluation of epidemiology studies of PFHxS and antibody response immunosuppression effects. For additional details see HAWC link.

There are outcome-specific ratings for these domains. Multiple publications of the same data are presented on the heat map as one study. Grandjean et al. (2017a) also includes Grandjean et al. (2012).

The results for this set of studies are shown in Tables 3-11 (children) and 3-12 (adults). Although results were mostly not statistically significant, a general inverse trend was apparent, particularly among studies of children. Of the six *medium* confidence studies in children, three (Grandjean et al., 2017a; Stein et al., 2016b; Granum et al., 2013) observed a statistically significant inverse association for at least one vaccine type while the other three also reported inverse associations in some analyses (Timmermann et al., 2021; Timmermann et al., 2020; Grandjean et al., 2017b). Antibody levels were measured in the blood of individuals of several age groups (and therefore different lengths of time since their initial vaccination or booster vaccination) and compared with serum PFHxS concentrations also measured at different ages. All the studies in children reported an association between higher concentrations of PFHxS and lower anti-vaccine antibody levels in at least some exposure-outcome analysis pairs. These associations were statistically significant for tetanus vaccination in children at ages 5 and 7 with childhood exposure measurement Grandjean et al. (2012) and for rubella vaccination in Granum et al. (2013) and Stein et al. (2016b). There are some results in the opposite direction for sub-analyses of the Faroe Island cohorts and in Timmermann et al. (2021). In Timmermann et al. (2020), an inverse association was observed in children who had received only one measles vaccination, but a positive association was observed in children who had received two vaccinations. Neither of these results were statistically significant, but the exposure contrast in this study was limited, which may have influenced their ability to detect a statistically significant effect. No biological rationale has been identified as to whether one exposure time period is more predictive of an overall immune response which might explain the few inconsistent results. Only one study (Timmermann et al., 2021) examined the odds ratio for not being protected against diphtheria (antibody concentrations < 0.1 IU/mL), which has clearer clinical significance than continuous changes in antibody levels, and they reported an OR of 6.44 (95% 1.51, 27.36) among children with known vaccination records (adjusted for area of residence, consistent with continuous antibody results).

In adults, the birth cohort with follow-up to young adulthood (Shih et al., 2021) reported inconsistent results across exposure measurement timing windows. Results were similarly inconsistent for antibodies to Hepatitis A and B (not shown). One *low* confidence study reported an inverse association for diphtheria and tetanus vaccination (Kielsen et al., 2016). The single study of FluMist reported no immunosuppression (Stein et al., 2016a).

It is plausible that the observed associations with PFHxS exposure could be explained by confounding across PFAS. Exposure levels to other PFAS in the Faroe Islands populations were considerably higher (blood concentrations of PFOS 17 ng/mL, PFOA 4 ng/mL, PFHxS 0.6 ng/mL) at age 5 years in Grandjean et al. (2012), and there was a moderately-high correlation between PFHxS with PFOS and PFOA (r = 0.57 and 0.53, respectively). The authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018) in which PFHxS effect estimates from a piecewise-linear model were adjusted for PFOS and PFOA and there was only limited attenuation of the observed effects of PFHxS indicating that there was still an independent effect of PFHxS(see Appendix D, Table D-1). These two PFAS were the most important to control for given that they were the most highly correlated with PFHxS and present at the highest concentrations in the population. The other available studies did not perform multipollutant modeling. In Stein et al. (2016b), correlations between PFHxS and PFOS and PFOA were moderate-high (r = 0.6 and 0.45, respectively), while in the other studies of antibody response, specific correlations for each pair of PFAS were not provided, so it is difficult to determine the potential for highly correlated PFAS to confound the effect estimates. Still, seeing PFHxS associated with the outcome in multiple studies, each of which have different exposure conditions and thus different inter-PFAS correlations, reduces the likelihood that confounding is the explanation. Overall, while it is not possible to rule out confounding across PFAS, the available evidence supports that it is unlikely to completely explain the observed effects, based primarily on the multipollutant modeling results of the Faroe Islands studies (Budtz-Jørgensen and Grandjean, 2018). Other sources of potential confounding, including possible co-exposures such as PCBs, were controlled appropriately.

Despite the imprecision of many of the individual exposure-outcome analysis pairs, the findings are generally consistent with an association between PFHxS exposure and immunosuppression. Of the 37 antibody-to-PFHxS-exposure analyses provided in Table 3-11, 26 support a finding of decrease in antibodies with higher PFHxS concentration. While some were less than a 1% decrease in antibody concentration per doubling of PFHxS concentration, the majority were greater than 5% and several were greater than 10%. While there is not clear clinical adversity for these fairly small changes in antibody levels for a healthy individual, by lowering the immune response of the entire population, it is likely that a subset of people will be shifted into clinically relevant immune suppression and that people with pre-existing immunosuppression will be more severely affected. This combined with the elevated odds for lack of protection from diphtheria in Timmermann et al. (2021) support that this is a relevant health effect resulting from PFHxS exposure. The variability in the results, including a few null and positive associations, could be related to differences in sample sizes, individual variation, vaccine type, and differences in timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available evidence. The inverse associations were observed despite limited sensitivity resulting from narrow exposure contrast in some studies. While multiple of the available studies are in a fairly specific population (i.e., Faroe Islands), this is the highest quality evidence available and the results are directly relevant to humans in general, particularly given the similar exposure levels to the general U.S. population. There is not evidence that differences in dietary habits (e.g., marine diet) or social determinants of health in this population can explain the results. In summary, some uncertainty remains resulting from variability in the response by age of exposure and outcome measures as well as from vaccination (initial and boosters), and also due to the potential for confounding across PFAS discussed above; but overall, the available evidence provides support for an association between increased serum levels of PFHxS and decreased antibody production following routine vaccinations in children and adults.

Table 3-11. Summary of PFHxS and data on antibody response to vaccines in children

Reference, N, confidence	PFHxS Exposure timing and concentration in serum	Outcome measure timing	Effect estimate as specified	Effect estimate as specified [a]
			Diphtheria vaccine (% change in antibodies with increase in PFHxS)	Tetanus vaccine (% change in antibodies with increase in PFHxS)[a]
Grandjean et al. (2012), N = 380–537, *medium*	Maternal; mean (IQR): 4.4 (2.2–8.4) ng/mL	Children (age 5), prebooster	−6.4 (−16.0 to 4.3)	−6.3 (−15.1 to 3.4)
		Children (age 5), postbooster	−3.7 (−14.1 to 7.9)	6.3 (−8.4 to 23.2)
		Children (age 7)	−0.5 (−13.1 to 14.0)	4.5 (−9.6 to 20.6)

Reference, N, confidence	PFHxS Exposure timing and concentration in serum	Outcome measure timing	Effect estimate as specified	Effect estimate as specified [a]
Grandjean et al. (2017a) 1997–2000 cohort	Children (age 5); mean (IQR): 0.6 (0.5–0.9) ng/mL	Children (age 5), prebooster	5.0 (−8.9 to 21.0)	−6.3 (−17.6 to 6.5)
		Children (age 5), postbooster	−9.1 (−18.7 to 1.7)	**−19.0 (−29.8 to −6.6)**
		Children (age 7)	−9.8 (−22.3 to 4.9)	**−19.7 (−31.6 to −5.7)**
	Children (age 7); mean (IQR): 0.5 (0.4–0.7) ng/mL	Children (age 13)	−10.2 (−25.7 to 8.5)	14.8 (−13.3 to 52.2)
	Children (age 13); mean (IQR): 0.4 (0.3–0.5) ng/mL	Children (age 13)	−5.5 (−22.9 to 15.8)	8.7 (−18.5 to 45.0)
Grandjean et al. (2017b) [b], N = 349, *medium* 2007–2009 cohort (unless specified)	At birth, not reported	Children (age 5), prebooster	−3.33 (−15.28 to 10.30)	−11.31 (−21.72 to 0.49)
	Infant (18 m); median (IQR): 0.2 (0.1–0.4) ng/mL	Children (age 5), prebooster	2007–2009 cohort 7.85 (−0.38 to 16.76) 1997–2000 cohort −12.42 (−55.25 to 71.43)	2007–2009 cohort −2.616 (−10.08 to 5.47) 1997–2000 cohort −5.18 (−51.71 to 86.19)
	Children (age 5); median (IQR):0.3 (0.2–0.4) ng/mL	Children (age 5), prebooster	4.26 (−15.12 to 28.08)	−4.432 (−21.26 to 15.99)
Granum et al. (2013), N = 49, *medium*	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	Children (age 3)	n/a	0.07 (−0.03 to 0.18)
Granum et al. (2013), N = 50, medium	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	Children (age 3)	−0.48 (−4.64 to 3.67)	n/a
Timmermann et al. (2021), N = 314, *medium*	Children (age 7-12)	Children (age 7-12)	Adjusted for time since vaccine booster, breastfeeding duration 48 (1, 115) Additionally adjusted for area of residence -40 (-64, 1)	Adjusted for time since vaccine booster, breastfeeding duration 28 (-6, 73) Additionally adjusted for area of residence -28 (-53, 10)
	Maternal		-53 (-87, 73)	-1 (-72, 245)
			Measles vaccine β (95%)[a]	**Rubella vaccine** β (95%)[a]
Timmermann et al. (2020), N = 237, medium	Children (<1 yr) 0.1 (0.1–0.1)	Children (<1 yr)	-5 (-23, 18)	NR
		Children (2 yrs)	After 1 vaccine (control group) -11 (-34, 19) After 2 vaccines (intervention group) 10 (-18, 48)	NR
Granum et al. (2013), N = 50, *medium*	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	Children (age 3)	−0.04 (−0.30 to 0.22)	**−0.38 (−0.66 to −0.11)**

Reference, N, confidence	PFHxS Exposure timing and concentration in serum	Outcome measure timing	Effect estimate as specified	Effect estimate as specified [a]
Stein et al. (2016b), N = 1,101–1,190, *medium*	Children (age 12–19); mean: 2.5 ng/mL	Children (age 12–19)	−2.8 (−10.1 to 5.21) (seropositive)	**−6.0 (−9.6 to −2.2)** (seropositive)
			Hib vaccine β (95%)[a]	**Mumps vaccine** β (95%)[a]
Granum et al. (2013), N = 50, medium	Maternal 0–3 d post-delivery; median: 0.7ng/mL	Children (age 3)	−0.48 (−4.64 to 3.67	n/a
Stein et al. (2016b), N = 1,101–1,190, *medium*	Children (age 12–19); mean: 2.5 ng/mL	Children (age 12–19)	n/a	−2.3 (−5.5 to 0.9)

[a]Linear regression (β or % change in antibody per twofold increase of PFHxS). Numbers in parentheses are 95% confidence intervals.
[b]Results for Faroe Islands Cohort 5 (2007–2009) unless otherwise stated.
Bold font indicates $p < 0.05$.
One study did not report quantitative results. Abraham et al. (2020) stated in text that there were no significant correlations of levels of PFHxS with levels of the vaccine antibodies for Hib, tetanus, or diphtheria.

Table 3-12. Summary of PFHxS and data on antibody response to vaccines in adults

Reference, N, confidence	Exposure timing and concentration	Outcome measure timing	Diphtheria vaccine β (95%)[a]	Tetanus vaccine β (95%)[a]	FluMist (A H1N1) vaccine Seroconversion RR (95% CI)
Shih et al. (2021), Faroe Islands, N = 281, medium	Cord blood; median (IQR) 0.2 (0.2)	Adults (age 28)	Total: 13.57 (-2.4, 32.15)) Women: 12.94 (-6.42, 36.32) Men: 14.72 (-10.98, 47.82)	Total: 0.63 (-10.86, 13.6) Women: 0.58 (-13.47, 16.91) Men: 0.74 (-17.78, 23.43)	n/a
	Children (age 7); 0.9 (0.4)		Total: 1.96 (-18.98, 28.31) Women: -18.74 (-43.42, 16.68) Men: 17.48 (-11.86, 56.59)	Total: 3.23 (-13.22, 22.79) Women: -8.27 (-30.54, 21.15) Men: 11.01 (-10.78, 38.13)	
	Children (age 14); 0.6 (0.4)		Total: -7.62 (-37.93, 37.48) Women: -8.03 (-47.08, 59.84) Men: -7.20 (-47.17, 62.98)	Total: -10.24 (-35.99, 25.87) Women: -17.92 (-48.63, 31.14) Men: -1.37 (-39.02, 59.53)	
	Adults (age 22); 0.5 (0.4)		Total: -8.44 (-27.27, 15.27) Women: -15.68 (-36.26, 11.55) Men: 8.32 (-27.37, 61.54)	Total: -3.47 (-19.88, 16.3) Women: -10.25 (-28.45, 12.57) Men: 11.85 (-18.98, 54.4)	
Kielsen et al. (2016), N = 12, low	Adult (10 d post vaccination); median (IQR): 0.4 (0.3–0.7) ng/mL	Adult – change from 4 d to 10 d postvaccination	−13.31 (−25.07, 0.29)	−4.35 (−13.72 to 6.04)	n/a
Stein et al. (2016a), N = 75, low	Adult (18–49 yrs old), d of vaccination; mean: 1.1 ng/mL	Adult (18–49 yrs old), 30 d postvaccination	n/a	n/a	by hemaglutinin inhibition: T2: 1.2 (0.2, 6.5) T3: 3.1 (0.8, 12.7) by immuno-histochemistry: T2: 1.1 (0.4, 2.9) T3: 1.7 (0.6, 4.8)

[a]Linear regression (β or % change in antibody per two-fold increase of PFHxS). Numbers in parentheses are 95% confidence intervals.
Bold font indicates $p < 0.05$.

Infectious disease

Direct measures of infectious disease incidence or severity such as respiratory tract infections, pneumonia or otitis media are useful for evaluating potential immunotoxicity in humans. Increases in incidence or severity of infectious disease can be a direct consequence of impaired immune function whether the specific functional deficit has been identified or not. Given the clear adversity of most infectious diseases, they are generally considered good measures for how immunosuppression can affect individuals and communities. Physician diagnosis is the most specific way to assess infectious diseases, but these are usually only available for severe diseases and are less likely for diseases where treatment is not sought. Self-reported incidence or severity of disease may be less reliable but may be the only way to assess diseases such as the common cold or gastroenteritis which while less adverse, are more common and can thus provide information about immunosuppression and susceptibility to more severe infections. In general, symptoms of infection alone are not considered reliable measures of disease because of their lack of specificity. Antibody levels in response to infection are also included in this section (differentiated from antibody levels in response to vaccination, described above); the utility of these measures depends on the study design and population due to various factors such as potential confounding and prevalence of infection.

Ten studies examined infectious disease occurrence in children, including eight prospective birth cohorts one cohort with exposure measurement in childhood, and one cohort examining antibody response to Hand, Foot, and Mouth Disease (HFMD) infection in the first three months of life. In addition, two studies examined infectious disease occurrence in adults, including a cross-sectional study of COVID-19 illness severity (Grandjean et al., 2020) and a cross-sectional study of antibody levels in response to several persistent infections (Bulka et al., 2021).

Study evaluations are summarized in Figure 3-14. Of the studies in children, four studies in Japan (Goudarzi et al., 2017), Spain (Manzano-Salgado et al., 2019), Denmark (Dalsager et al., 2021a), and China (Wang et al., 2022) were *medium* confidence, and the remaining studies were *low* confidence (Kvalem et al., 2020; Impinen et al., 2019; Zeng et al., 2019b; Impinen et al., 2018; Dalsager et al., 2016; Granum et al., 2013). The *low* confidence birth cohorts were rated as "deficient" in outcome ascertainment due to relying on parental self-report of incidence of common infections or symptoms, with no validation of the measures. However, because the parents are unlikely to know their child's exposure level, this misclassification is likely to be nondifferential with respect to exposure. In contrast, the *medium* confidence studies assessed physician-diagnosed conditions and were limited to more severe illnesses (otitis media, pneumonia, varicella, and respiratory syncytial viral infection), which likely have better parental recall. Zeng et al. (2019b) was *low* confidence because the outcome is difficult to interpret in infants and there are concerns for confounding by timing of HFMD infection as well as other limitations. The two studies in adults were both considered *medium* confidence. Grandjean et al. (2020) used biobank samples and national registry data in Denmark to examine severity of COVID-19 illness severity. There was some concern for selection bias in this study due to the expectation that biobank samples were more likely to be available for individuals with chronic health concerns. In addition, severity of COVID-19 is not a direct measure of immune suppression as other factors may contribute to illness severity.

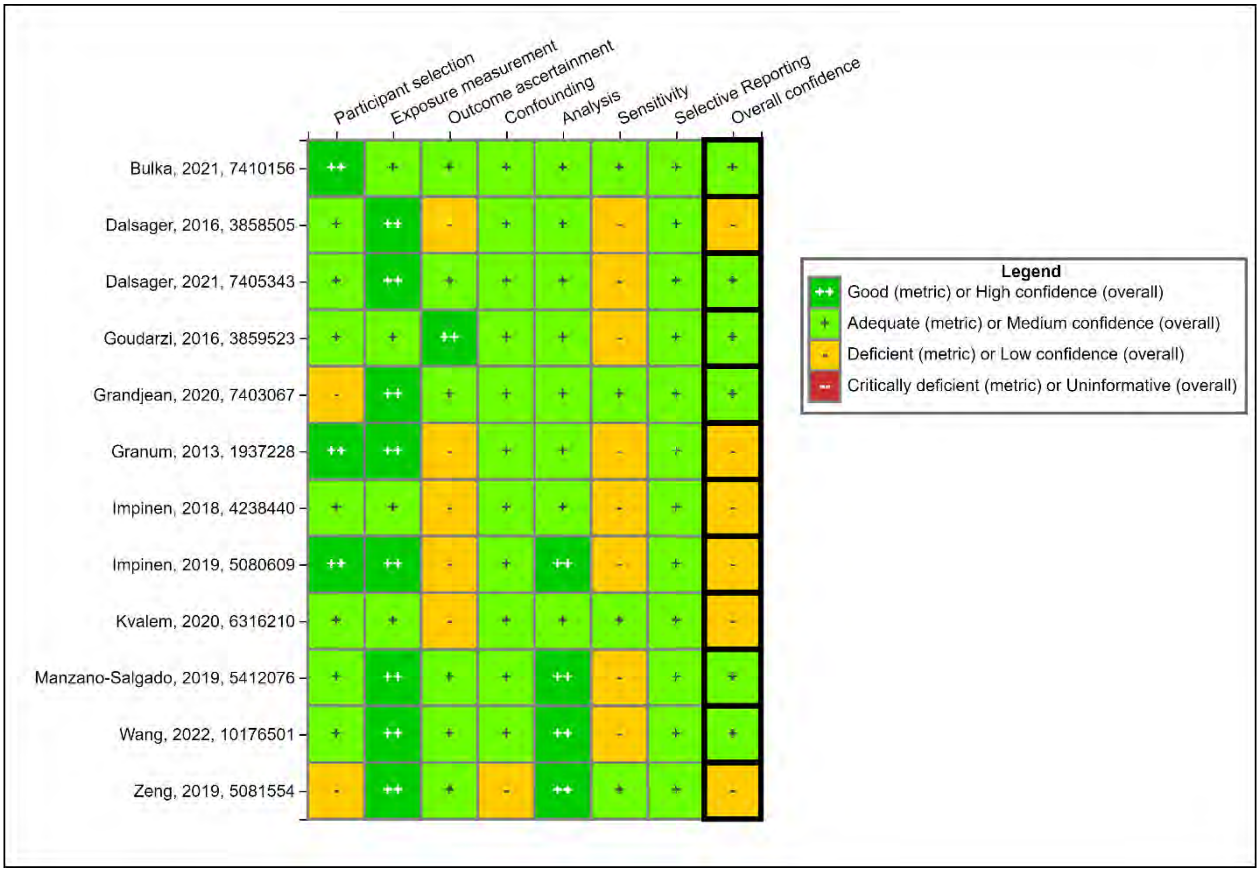


Figure 3-14. Summary of evaluation of epidemiology studies of PFHxS and infectious disease immunosuppression effects. For additional details see HAWC link.

Two studies (Impinen et al., 2018; Granum et al., 2013) were sub-samples of the Norwegian Mother and Child (MoBa) cohort. The cohort sub-samples for these publications were different, so their study evaluations and results are reported independently, but it is possible that there is some overlap in the participants. Two studies (Dalsager et al., 2021a; Dalsager et al., 2016) were both analyses of the Odense Child Cohort. They were evaluated separately due to their different samples and outcome measurement methods but were not considered fully independent samples.

In children, higher odds of infectious disease with higher PFHxS levels were reported in two of the four *medium* confidence studies (Wang et al., 2022; Goudarzi et al., 2017) and three of the six *low* confidence studies (Impinen et al., 2019; Dalsager et al., 2016; Granum et al., 2013) (see Table 3-11). Wang et al. (2022) reported higher odds (though not statistically significant) of upper and lower respiratory infection and diarrhea with higher exposure. (Goudarzi et al., 2017) reported higher odds of total infectious disease from birth to age 4, but only in girls, and a significant trend was observed, but the association was nonmonotonic across quartiles. No clear explanation for why these results might vary by sex is available, and none of the other studies of immunosuppression analyzed the results stratified by sex. Impinen et al. (2019) also reported higher odds of gastroenteritis (statistically significant from birth to age 3), but not common cold or otitis media. Dalsager et al. (2016) reported higher odds of diarrhea and fever ($p > 0.05$), but not cough or nasal discharge. Another *medium* confidence study (Manzano-Salgado et al., 2019) reported an association in the same direction, but the effect estimate was small and imprecise. Two other *low* confidence studies did not observe an association between maternal PFHxS concentrations and infections. In adults and adolescents, one study found higher persistent pathogen burden with higher exposure (Bulka et al., 2021). In contrast, there an inverse association between PFHxS exposure and COVID-19 illness severity. Overall, many of the studies had limited sensitivity due to narrow exposure contrast, but there was no apparent relationship between higher study exposure levels and observed associations. Given the inconsistency across studies, there is considerable uncertainty in this outcome. The associations observed in some studies provide some limited support for (and coherence with) the evidence of immunosuppression observed in the antibody response studies.

Table 3-13. Summary of PFHxS and selected data on infectious disease in humans

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFHxS results
Total infectious disease[a]	Dalsager et al. (2021a), medium	Maternal; median: 0.4	From birth to age 4	HR (95% CI) 1.02 (0.90, 1.16)
	Goudarzi et al. (2017) medium	Maternal; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 4	Adj OR (95% CI) Total: Q2 1.03 (0.764, 1.41) Q3 1.23 (0.905, 1.69) Q4 0.957 (0.703, 1.30) Trend $p = 0.928$
				Male: Q2 0.780 (0.508, 1.19) Q3 0.947 (0.614, 1.45) Q4 0.708 (0.461, 1.08) Trend $p = 0.223$
				Female: Q2 1.46 (0.938, 2.29) Q3 1.81 (1.14, 2.88) Q4 1.55 (0.976, 2.45) Trend $p = 0.045$
Lower respiratory tract infection[b]	Impinen et al. (2018) low	Cord blood	From birth to age 10	Adj β (95% CI) 0.04 (−0.01, 0.09)
	Dalsager et al. (2021a), medium	Maternal; median: 0.4	From birth to age 4	HR (95% CI) 1.01 (0.78, 1.32)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFHxS results
	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4-0.8)	Through Age 1	OR (95% CI) 10.62 (0.65, 173.7) IRR (95% CI) 1.81 (0.27, 12.19)
	Manzano-Salgado et al. (2019) medium	Maternal (1st trimester), median (IQR): 0.6 (0.4–0.8) ng/mL	Age 1.5–7	1.07 (0.96, 1.18)
	Impinen et al. (2019) low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj RR (95% CI): 1.15 (1.06, 1.24)
			Age 6–7	0.92 (0.70, 1.21)
	Kvalem et al. (2020) low	Child age 10; median (IQR): 1.3 (0.9)	Age 10–16	Adj RR (95% CI) 0.98 (0.95, 1.02)
			Age 16 (last 12 m)	0.93 (0.74, 1.18)
Gastroenteritis (No. episodes/ frequency)	Granum et al. (2013), low	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	From birth to age 3	Adj β (95% CI) 3rd yr: 0.33 (−0.05, 0.71) All 3 yrs: 0.35 (0.10, 0.61)
	Dalsager et al. (2021a), medium	Maternal; median: 0.4	From birth to age 4	HR (95% CI) 0.85 (0.50, 1.43)
	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj (RR): 0.98 (0.96, 1.02)
			Age 6–7	1.27 (1.18, 1.38)
Diarrhea	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3	OR for proportion of d with symptoms (under/above median) Low exposure: Ref Medium: 1.16 (0.66, 2.02) High: 1.39 (0.77, 2.51) IRR for number of d with symptoms Low exposure: Ref Medium: 1.18 (0.64, 2.19) High: 1.71 (0.92, 3.16)
	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4-0.8)	Through age 1	OR (95% CI) 1.17 (0.20, 6.83) IRR (95% CI) 1.27 (0.50, 3.20)
Common cold (No. episodes/ frequency)	Impinen et al. (2018), low	Cord blood; median (IQR): 0.2 (0.2–0.3) ng/mL	From birth to age 2	Adj β (95% CI) −0.01 (−0.04, 0.02)
	Granum et al. (2013), low	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	From birth to age 3	Adj β (95% CI) c 3rd year: 0.24 (−0.03, 0.51) All 3 yrs: 0.15 (−0.02, 0.32)
	Dalsager et al. (2021a), medium	Maternal; median: 0.4	From birth to age 4	HR (95% CI) for upper respiratory infections 1.01 (0.83, 1.21)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFHxS results
	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4-0.8)	Through Age 1	OR (95% CI) 1.49 (0.28, 7.97) IRR (95% CI) 1.16 (0.60, 2.26)
	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj RR (95% CI): 1.01 (1.00, 1.03)
	Kvalem et al. (2020) medium	Child age 10; median (IQR): 1.3 (0.9)	Age 10–16	Adj OR (95% CI): Reference 1–2 colds 3–5 colds: 0.99 (0.93, 1.04) >5: 0.97 (0.93, 1.03)
			Age 16 (last 12 m)	Adj OR (95% CI) Reference 0 colds 1–2 colds:0.98 (0.96, 1.00) ≥3: 0.97 (0.94, 1.00)
Cough	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3	OR for proportion of d with symptoms (under/above median) Low exposure: Ref Medium: 1.04 (0.60, 1.79) High: 0.97 (0.54,1.73) IRR for number of d with symptoms Low exposure: Ref Medium: 1.14 (0.87, 1.48) High: 1.00 (0.76, 1.31)
Ear infection	Granum et al. (2013), low	Maternal 0–3 d post-delivery; median: 0.3 ng/mL	From birth to age 3	No significant association with otitis media (data not shown)
	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj RR (95% CI): 1.09 (1.04, 1.14)
			Age 6–7	1.08 (0.93, 1.25)
Throat infection	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj RR (95% CI): 1.10 (1.02, 1.18) (no association with streptococcus throat infection)
Pseudocroup	Impinen et al. (2019), low	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 3	Adj RR (95% CI): 1.20 (1.11, 1.30)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFHxS results
Fever	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3	OR for proportion of d with symptoms (under/above median) Low exposure: Ref Medium: 0.99 (0.58, 1.71) High: 1.29 (0.72, 2.28) IRR for number of d with symptoms Low exposure: Ref Medium: 1.07 (0.80, 1.42) High: 1.20 (0.89, 1.62)
Hand Foot and Mouth Disease Virus Antibodies	Zeng et al. (2019b), low	Cord; median (IQR): 4.0 (2.3–5.4)	Birth and age 3 mo	OR (95% CI) for HFMD antibody concentration below clinically protective level Cord blood: 1.08 (0.74, 1.60) 3 mo: 1.00 (0.71, 1.43)
COVID-19 illness severity	Grandjean et al. (2020), medium	Biobank prior to illness; median (IQR): 0.5 (0.3–0.7)	Adulthood	OR (95% CI) for 1 unit increase Increased severity based on hospitalization, admission to intensive care and/or death 0.52 (0.29, 0.93)*
Pathogen burden of persistent infections based on antibodies	Bulka et al. (2021)	Mean: 1.5	Ages 12–49 yrs	Relative difference (95% CI) per doubling 12-19 yrs: 1.11 (1.06, 1.15)* 20-49 yrs: 1.02 (1.00, 1.05)* For individual pathogens, only Toxocara spp had positive association

Bolded values are statistically significant.
[a]Includes Otitis media, pneumonia, RS virus, Varicella.
[b]Lower respiratory tract infections include bronchitis, bronchiolitis, and pneumonia.
[c]Bivariate model was statistically significant (p = 0.036) for all 3 years.

Sensitization or allergic response

Another major category of immune response is the evaluation of sensitization-related or allergic responses resulting from exaggerated immune reactions (e.g., allergies or allergic asthma) to foreign agents (IPCS, 2012). A chemical may be either a direct sensitizer (i.e., promote a specific IgE-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. For example, chemical exposure could promote a physiological response resulting in a propensity for sensitization to other allergens (pet fur, dust, pollen, etc.). Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. While these responses are mediated by circulating factors such as T-cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of measurements of the health effects such as allergies or asthma and skin prick tests. Observational tests such as measures of total IgE levels measure indicators of sensitivity and allergic responses but are not a direct measurement of the response. The section is organized by the different types of measurements, starting with functional measures as the most informative.

Thirteen studies (reported in 19 publications) examined hypersensitivity outcomes in children. The study evaluations are summarized in Figure 3-15. Two of the included studies were subsamples of the Norwegian Mother and Child (MoBa) cohort that were analyzed independently (Impinen et al., 2019; Granum et al., 2013). In addition, three publications of NHANES data are grouped together as one study because there is significant overlap in the NHANES years included in the analysis samples (Buser and Scinicariello, 2016; Stein et al., 2016b; Humblet et al., 2014); another publication examined a different year range of NHANES data and was considered separately (Jackson-Browne et al., 2020). Ten studies were prospective birth cohorts, with exposure measured during gestation or in cord blood. These studies were performed in China (Chen et al., 2018a), Japan (Goudarzi et al., 2016; Okada et al., 2014), Norway (Impinen et al., 2019; Impinen et al., 2018; Granum et al., 2013), Greenland and Ukraine (Smit et al., 2015), Spain (Manzano-Salgado et al., 2019), Denmark (Beck et al., 2019), and the Faroe Islands (Timmermann et al., 2017). In addition to the cohort studies, there was a case-control study of asthma in Taiwan reported in multiple publications (Zhou et al., 2017b; Zhu et al., 2016; Dong et al., 2013), a cohort of children with exposure measured at age 10 (Kvalem et al., 2020), and the analyses of NHANES data, which is cross-sectional. All the studies were considered *medium* confidence.,

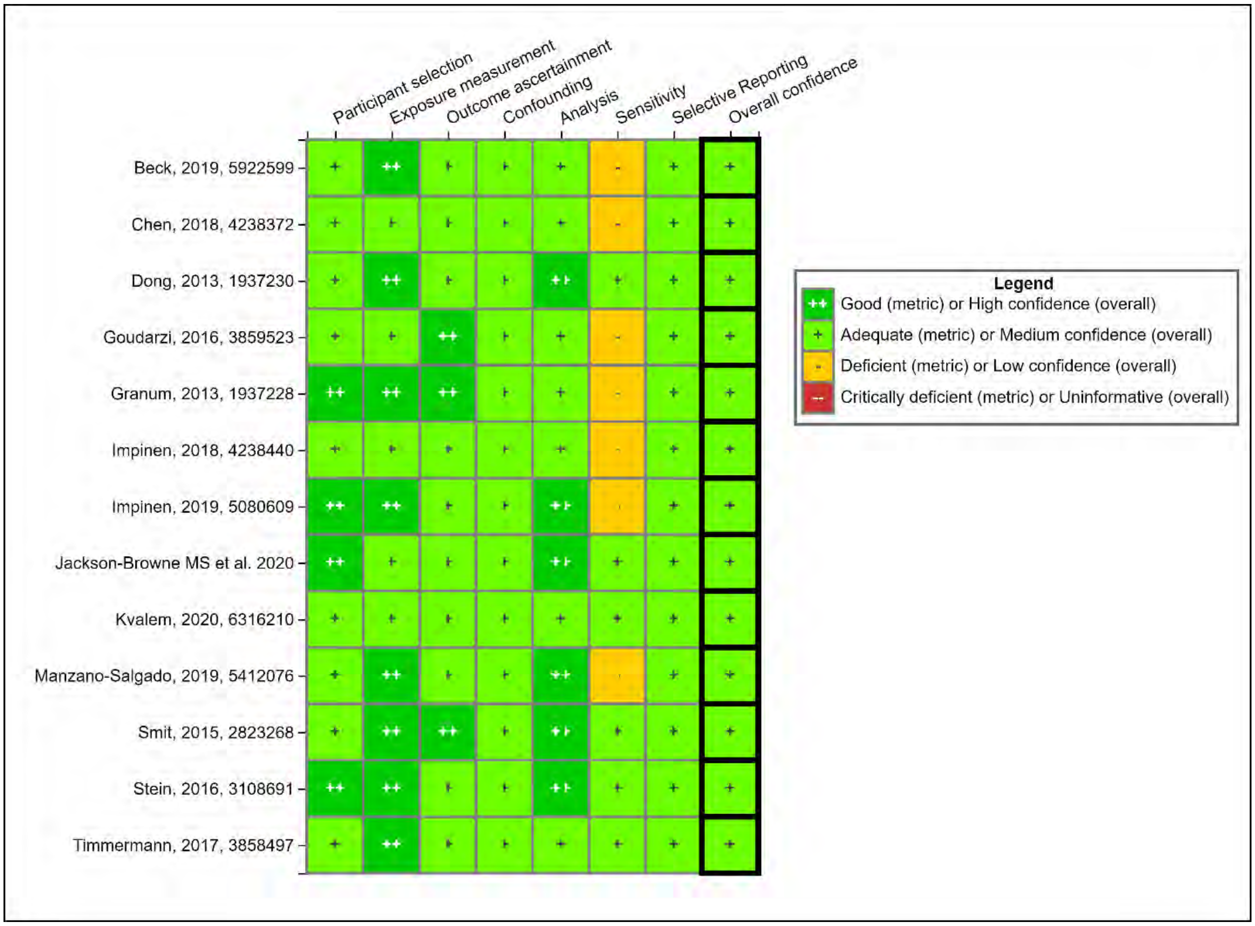


Figure 3-15. Summary of evaluation of epidemiology studies of PFHxS and hypersensitivity effects (e.g., asthma, allergies, and atopic dermatitis). For additional details see HAWC link.

Multiple publications of the same data are presented on the heat map as one study. Goudarzi et al. (2016) also includes Okada et al. (2014). Stein et al. (2016b) also includes Buser and Scinicariello (2016) and Humblet et al. (2014).

Asthma

Twelve studies evaluated different measures related to asthma diagnosis and symptoms in relation to PFHxS exposure (see Table 3-12. All studies were *medium* confidence. One study examined asthma incidence (i.e., diagnosis within the past year, with cases identified from two hospitals), which is the most specific measure available across studies, but which may result in under-ascertainment because only severe cases are identified. The remaining studies examined asthma prevalence using validated questionnaires, either “current” asthma (generally experiencing symptoms in the past year with asthma diagnosis) or “ever” asthma (asthma diagnosis at any time during their life). These measures are less specific than asthma incidence and the relevant etiologic period is less clear.

Four studies examined "current" asthma and 11 studies examined "ever" asthma. Looking at current asthma, one study (Impinen et al., 2019) out of four reported higher odds, although this was not statistically significant. Three studies also reported a positive association with "ever" asthma, but with inconsistency within each study. Zeng et al. (2019a) reported a strong positive, but very imprecise, association in boys, and an imprecise inverse association in girls, while in Beck et al. (2019), a strong positive association ($p < 0.05$) was observed in girls for doctor-diagnosed asthma, but there was no sex-interaction with self-reported asthma. In Timmermann et al. (2017), a positive association was observed only in a small subgroup (4%, 22 children) of the study population that did not receive MMR vaccination and may be due to chance. The remaining studies showed no association with ever asthma.

The single study (reported in multiple publications) of asthma incidence (the most specific outcome measurement available) reported higher odds of asthma in children 10–15 years of age with higher PFHxS exposure with an exposure-response gradient observed across quartiles in the overall population (Dong et al., 2013). The association was stronger in girls than in boys (Zhu et al., 2016), although there was no significant interaction with sex hormone levels (Zhou et al., 2017b). The association was strong (OR >3 in highest quartile of exposure), and the outcome measurement is likely to suffer from less outcome misclassification than would measures of asthma prevalence in the other available studies. This *medium* confidence study in Taiwan also had PFHxS exposure levels that were among the highest of the available studies, while several studies with null results had exposure levels with narrow exposure contrast across participants, which may have reduced sensitivity. While there is considerable uncertainty due to inconsistency in the results across studies, the null results are not interpreted as contradictory to the positive findings given the better sensitivity and specificity (and relatively higher exposure levels) in Dong et al. (2013).

Allergies/Allergic sensitization

Five studies, all *medium* confidence, evaluated allergies and allergic sensitization outcomes (see Table 3-12). Two studies examined food allergies. Buser and Scinicariello (2016), an NHANES analysis, reported higher odds of allergy in the second and fourth quartiles, with statistical significance in the fourth quartile. Impinen et al. (2019) observed slightly higher, but not statistically significant odds of current food allergies with higher exposure. Impinen et al. (2019) also found higher, but not significant, odds of inhaled allergies. Four studies examined allergic sensitization, and one study observed higher odds of elevated IgE with higher exposure, although this was not monotonic as the highest odds were in the third quartile (Buser and Scinicariello, 2016). The other NHANES analysis (Stein et al., 2016b) and three other studies did not report higher odds of sensitization with higher exposure.

Dermal allergic measures – eczema

Nine studies evaluated eczema (see Table 3-12). While the studies used different terminology including eczema, atopic eczema, and atopic dermatitis, most assessed presence of an itchy rash that was coming and going for at least 6 months using the International Study of Asthma and Allergies in Childhood questionnaire. Three studies examined physician-diagnosed atopic eczema, also collected using a questionnaire (Impinen et al., 2019; Impinen et al., 2018; Granum et al., 2013), and Kvalem et al. (2020) used a different questionnaire for self-reported eczema. These dermal response conditions can represent hypersensitivity to an antigen exposure from any route. Two *medium* confidence studies reported higher odds of eczema with higher PFHxS exposure (Chen et al., 2018a; Timmermann et al., 2017), both statistically significant (in girls only for Chen et al. (2018a)), while two studies (Kvalem et al., 2020; Okada et al., 2014) reported an inverse association. The remaining five studies reported no association. Exposure levels were highest in Timmermann et al. (2017), but levels in Chen et al. (2018a) were similar to the null studies, and Okada et al. (2014). There is no apparent explanation for the inconsistency across studies on the basis of study design, population, bias, or other factors.

Table 3-14. Summary of PFHxS and data on hypersensitivity in humans.

Reference		Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFHxS OR (95% CI)[a] or as specified
Asthma Incidence				
GBCA	Dong et al. (2013)	Children, current; median (IQR): 1.3 (0.6–2.8) (without asthma)	Children (age 10–15)	**Asthma diagnosed in past year** Q2: 1.54 (0.85, 2.77) **Q3: 2.94 (1.65, 5.25)** **Q4: 3.83 (2.11, 6.93)** **Trend $p < 0.001$**
	Zhou et al. (2017b)		Children (age 10–15)	By Sex Hormone Levels Low Testosterone M: 2.12 (1.34, 3.35) F: 1.62 (1.08, 2.45) High Testosterone M: 1.43 (0.99, 2.07) F: 2.27 (1.29, 3.99) Low Estradiol M: 1.47 (1.00, 2.15) F: 2.39 (1.39, 4.12) High Estradiol M: 1.62 (1.01, 2.60) F: 1.65 (1.07, 2.55) No significant interaction between PFHxS and sex hormone category
	Zhu et al. (2016)		Children (age 10–15)	By Sex Q4 vs Q1 M: 2.97 (1.33, 6.64) F: 5.02 (2.05, 12.30)
Current Asthma				

Reference	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFHxS OR (95% CI)[a] or as specified
Impinen et al. (2019)	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 7	1.21 (0.87, 1.67)
Impinen et al. (2018)	Cord blood; median (IQR): 0.2 (0.2–0.3) ng/mL	From birth to age 10	0.99 (0.82, 1.21)
Kvalem et al. (2020)	Child (age 10); median (IQR): 1.3 (0.9) ng/mL	Child (age 16)	Last 12 months RR: 1.00 (0.98, 1.02)
NHANES Stein et al. (2016b)	Children, current; mean: 2.5 ng/mL	Children (age 12–19)	IQR increase: 0.98 (0.51, 1.87)
Ever Asthma			
Zeng et al. (2019a)	Cord blood median (IQR): 0.2 (0.1-0.2)	Child (age 5)	Ever asthma 2.02 (0.24, 17.24) Girls: 0.48 (0.00, 85.33) Boys: 3.40 (0.18, 65.11)
MoBa: Granum et al. (2013)	Maternal 0–3 day post-delivery; median: 0.3 ng/mL	From birth to age 3	No significant association (data not shown)
MoBa: Impinen et al. (2019)	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 7	0.96 (0.79, 1.18)
Beck et al. (2019)	Maternal, gest week 8-16; median (IQR): 0.4 (0.2–0.5) ng/mL	Child (age 5)	Ever doctor-diagnosed asthma 1.16 (0.78, 1.71) Boys: 0.89 (0.59, 1.34) **Girls: 2.96 (1.26, 6.96)** Ever self-reported asthma (≥ episodes of wheezing lasting more than a day in past 12 months) 1.18 (0.73, 1.90) Boys: 1.33 (0.66, 2.71) Girls: 1.04 (0.55, 1.98)
Manzano-Salgado et al. (2019) medium	Maternal (1st trimester), median (IQR): 0.6 (0.4–0.8) ng/mL	Age 1.5–7	Ever asthma RR: 0.96 (0.74, 1.24)
Jackson-Browne et al. (2020)	Child (age 3–11); mean (IQR): 0.8 (0.5–1.3)	Child (age 3–11)	Ever asthma OR: 1.1 (0.9, 1.3)
Kvalem et al. (2020)	Child (age 10); median (IQR): 1.3 (0.9) ng/mL	Child (age 10)	Ever asthma RR: 0.99 (0.97, 1.01)
		Child (age 10–16)	Asthma between 10 and 16 years RR: 1.00 (0.99, 1.02)

Reference	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFHxS OR (95% CI)[a] or as specified
Smit et al. (2015)	Maternal, mean gestational week 24 or 25; mean (5th-95th): Ukraine: 1.5 (0.5–4.1), Greenland: 2.1 (1.0–5.1)	Children (age 5–9)	0.91 (0.69, 1.18)
Impinen et al. (2018)	Cord blood; median (IQR): 0.2 (0.2–0.3) ng/mL	From birth to age 10	0.94 (0.72, 1.21)
Timmermann et al. (2017)	Maternal, gestational week 34–36; median (IQR): 4.5 (2.2–8.3)	Child (age 5)	0.99 (0.80, 1.22)
		Child (age 13)	0.98 (0.79, 1.20)
	Child (age 5); median (IQR): 0.6 (0.4–0.9)	Child (age 5)	No MMR: 3.57 (0.95, 13.43)[b] Yes MMR: 0.81 (0.58, 1.14) Interaction $p = 0.03$
		Child (age 13)	No MMR: 2.52 (0.77, 8.16)[b] Yes MMR: 0.90 (0.63, 1.27) Interaction $p = 0.10$
	Child (age 13); median (IQR): 0.4 (0.3–0.5)	Child (age 13)	**0.63 (0.41, 0.97)**
NHANES Humblet et al. (2014)	Children, current; median (IQR): 2.0 (1.0, 4.1)	Children (age 12–19)	Continuous: 0.98 (0.88–1.08) T2: 1.07 (0.89, 1.30) T3: 0.92 (0.74, 1.14)
Allergies (Food)			
Impinen et al. (2019)	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 7	Ever: 1.03 (0.82, 1.30) Current: 1.10 (0.86, 1.41)
NHANES Buser and Scinicariello (2016)	Children, current; mean: 2.2 ng/mL	Children (age 12–19)	Q2 1.43 (0.40, 5.14) Q3 0.99 (0.37, 2.65) **Q4 3.06 (1.35, 6.93)** Trend $p = 0.11$
Allergies (Inhaled)			
Impinen et al. (2019)	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 7	Ever: 1.18 (0.93, 1.50) Current: 1.21 (0.81, 1.81)
Allergies (Sensitization)			

Reference		Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFHxS OR (95% CI)[a] or as specified
Impinen et al. (2018)		Cord blood; median (IQR): 0.2 (0.2–0.3) ng/mL	From birth to age 10	Positive SPT or sIgE > 0.35 kU/L 1.01 (0.84, 1.21)
Kvalem et al. (2020)		Child (age 10); median (IQR): 1.3 (0.9) ng/mL	Child (age 10)	Positive skin prick test RR: 1.01 (1.00, 1.02)
			Child (age 16)	Positive skin prick test RR: 1.00 (1.00, 1.01)
Timmermann et al. (2017)		Maternal, gestational week 34–36; median (IQR): 4.5 (2.2–8.3)	Children (age 13)	Positive skin prick test 0.94 (0.79, 1.12)
		Children (age 5)	Children (age 13)	Positive skin prick test 0.95 (0.75, 1.20)
		Child (age 5); median (IQR): 0.6 (0.4–0.9)	Children (age 13)	Positive skin prick test 0.88 (0.64, 1.21)
NHANES	Buser and Scinicariello (2016)	Children, current; mean: 2.2 ng/mL	Children (age 12–19)	Sensitization (any sIgE >0.35 kU/L) Q2 1.11 (0.66, 1.88) Q3 1.46 (0.79, 2.69) Q4 1.17 (0.56, 2.44) Trend $p = 0.72$
	Stein et al. (2016b)	Children, current; mean: 2.5 ng/mL	Children (age 12–19)	Sensitization (any sIgE >0.35 kU/L) IQR increase: 0.92 (0.66, 1.28)
Eczema				
MoBa	Granum et al. (2013)	Maternal 0–3 day post-delivery; median: 0.3 ng/mL	From birth to age 3	Eczema and itchiness or doctor-diagnosed atopic eczema: No significant association (data not shown)
	Impinen et al. (2019)	Maternal mid-pregnancy; median (IQR): 0.7 (0.5–0.9) ng/mL	From birth to age 7	Ever: 1.09 (0.90, 1.31) Current: 1.06 (0.83, 1.36)
Hokkaido	Goudarzi et al. (2016)	Maternal, gestational week 28–32; median (IQR): 0.3 (0.2-0.4)	Children (age 4)	Ever: Q2: 0.953 (0.658, 1.38) Q3: 0.910 (0.623, 1.32) Q4: 0.917 (0.626, 1.34) Trend $p = 0.618$
	Okada et al. (2014)		Children (age 1 or 2)	Ever: Q2 0.82 (0.60, 1.13) **Q3 0.69 (0.50, 0.95)** Q4 0.79 (0.57, 1.08) Trend $p = 0.08$

Reference	Exposure measurement timing and concentration	Hypersensitivity measurement timing	PFHxS OR (95% CI)[a] or as specified
Smit et al. (2015)	Maternal, gestational week 24	Children (age 5–9)	Ever: 1.03 (0.86, 1.24) Current: 0.93 (0.73, 1.20)
Chen et al. (2018a)	Cord blood; median (IQR): 0.2 (0.2–0.2) ng/mL	Children (age 2)	Ever: 1.08 (0.62, 1.85) per log unit increase Q2 1.25 (0.74, 2.12) Q3 1.15 (0.68, 1.94) Q4 1.14 (0.67, 1.94) Trend $p = 0.73$ Females only Q2 1.43 (0.62, 3.30) Q3 1.29 (0.55, 2.99) **Q4 2.30 (1.03, 5.15)** Trend $p = 0.06$
Impinen et al. (2018)	Cord blood; median (IQR): 0.2 (0.2–0.3) ng/mL	From birth to age 10	0–2 years of age 1.06 (0.89, 1.26) Ever in 10 years 1.00 (0.67, 1.49)
Manzano-Salgado et al. (2019)	Maternal (1st trimester), median (IQR): 0.6 (0.4–0.8) ng/mL	Age 1.5-7	Ever eczema RR: 0.95 (0.86, 1.05)
Kvalem et al. (2020)	Child (age 10); median (IQR): 1.3 (0.9) ng/mL	Child (age 10)	Ever doctor diagnosed: RR: 1.00 (0.98, 1.01)
		Child (age 10-16)	Ever between 10 and 16 years **RR: 0.79 (0.34, 0.99)**
		Child (age 16)	Current (last 12 months) RR: 0.78 (0.60, 1.02)
Timmermann et al. (2017)	Maternal, gestational week 34–36; median (IQR): 4.5 (2.2–8.3)	Children (age 13)	**1.32 (1.08, 1.62)**
	Children (age 5)	Children (age 13)	0.92 (0.70–1.22)
	Child (age 5); median (IQR): 0.6 (0.4–0.9)	Children (age 13)	No MMR: 1.27 (0.16, 10.15)[c] Yes MMR: 0.80 (0.53, 1.20) Interaction $p = 0.66$

[a]All estimates are presented as OR (95% CI) for the odds of the outcome per two-fold increase in PFHxS concentration unless otherwise stated.
[b]Results provided broken down by MMR vaccination status; yes (n = 537) or no (n = 22) when provided; some results were not split by MMR vaccination status.
Bold font indicates $p < 0.05$.

Animal Studies

Animal toxicity studies examining the effects of PFHxS on the immune system include two (*high* confidence) short-term oral exposure studies performed in Sprague Dawley rats, (NTP, 2018a; 3M, 2000b) and one (*medium* confidence due to lack of results presentation) subchronic oral exposure study performed in Crl:CD1 mice (Chang et al., 2018); the study details are provided in Table 3-15. It should be noted that none of the studies in the database were immunotoxicity specific studies, but rather short-term or subchronic studies that focused on reproductive endpoints but also measured general immune-related endpoints. IPCS guidance states that a 28-day exposure period, such as those in the three studies in the evidence base, are adequate to elicit an immune response (IPCS, 2012). The immune-relevant endpoints evaluated in these studies include immune hematology (i.e., blood leukocyte counts), histopathology, and organ weights (i.e., bone marrow, lymph nodes, spleen), which may inform sensitization and allergic response and autoimmunity, categories of immunotoxicity described in guidance from the International Programme on Chemical Safety (IPCS, 2012).[7] Studies were separately evaluated for each of these endpoints; however, the overall confidence rating was the same regardless of endpoint (see Figure 3-16; for study details please see Table 3-15 and HAWC).

[7]IPCS guidance notes that "the dataset[s] for most chemicals is unlikely to contain all the data on all the described endpoints" (IPCS, 2012).

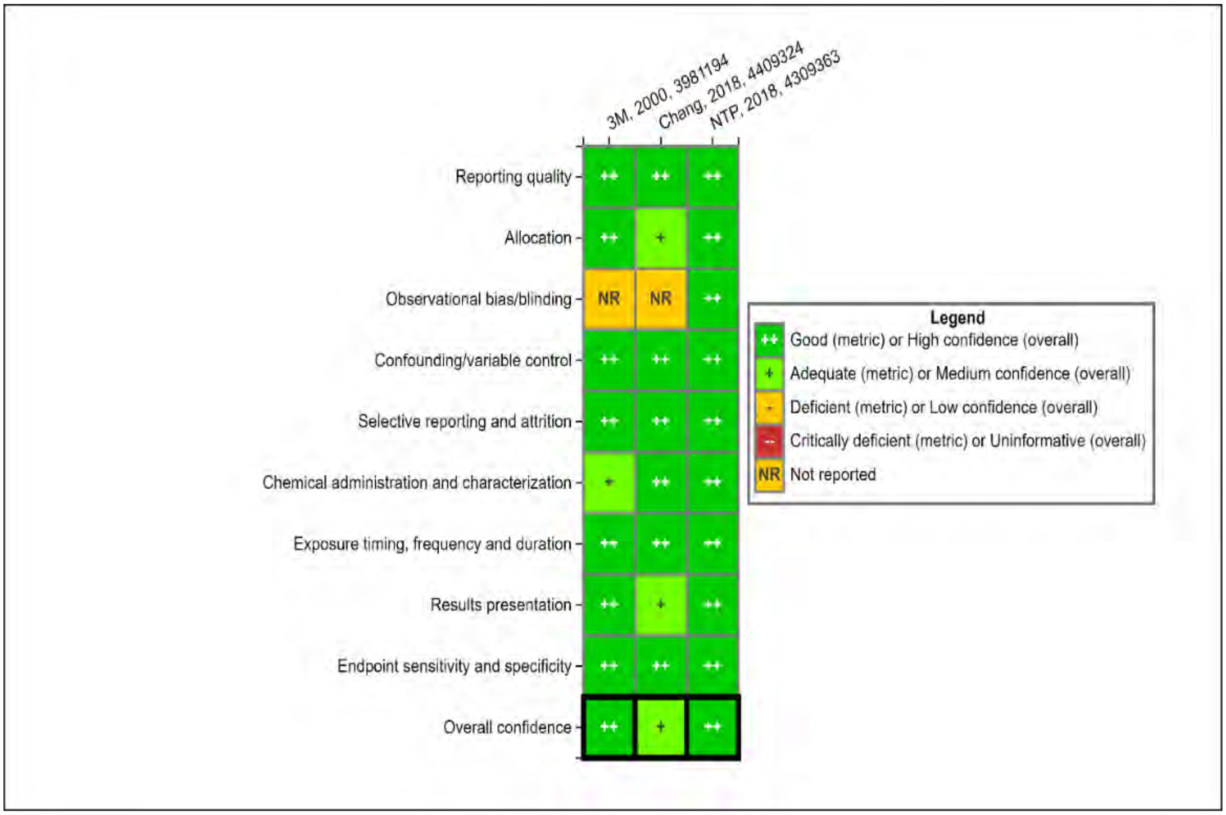


Figure 3-16. Study evaluation results of PFHxS animal toxicity studies with immune-related endpoints. For additional details see HAWC link.

Table 3-15. Animal study details

Study	Experimental model	Exposure route	Exposure doses	Duration	Immune endpoint(s)
3M (2000b)	Male and Female SD rats	Oral Gavage	0, or 10 mg/kg-d	28 d	Total immune cell counts[a] Histopathology, Organ Weights
Chang et al. (2018)	Male and Female CD-1 Mice	Oral Gavage	0, 0.3, 1, or 3 mg/kg-d	F0: Males: dosing started 14 d prior to cohabitation for a total of 42 d until scheduled to be euthanized. Females: dosing started 14 d prior to cohabitation and continuing through mating, gestation, and lactation. F0 dams were euthanized on lactation d 22 (LD22) which was 1 d post-last dose. F1: Mice were exposed in utero and via lactation. After weaning at postnatal d 22, pups were directly dosed with PFHxS for an additional 14 d at the same respective maternal doses.	Total Leukocyte counts[b] Histopathology[c] Organ Weights
NTP (2018a)	Male and Female SD rats	Oral Gavage	Males: 0, 0.625, 1.25, 2.5, 5 or 10 mg/kg-d Females: 0, 3.12, 6.25, 12.5, 25 or 50 mg/kg-d	28 d	Total immune cell counts Histopathology Organ Weights

[a]Total immune cell count included detailed counts of immune cells, e.g., basophil, eosinophil counts.
[b]Total leukocyte count does not include detailed counts of immune cells.
[c]Data not shown.

Immune hematology

A summary of the immune hematology outcomes can be found in Figure 3-17. Briefly, of the three studies that examined immune outcomes, two 3M (2000b) and NTP (2018a) performed a complete detailed analysis of blood leukocyte counts including basophils, eosinophils, leukocytes, lymphocytes, monocytes, and neutrophils, while Chang et al. (2018) reported only total blood leukocyte counts. 3M (2000b) and Chang et al. (2018) reported no statistically significant changes in white blood cell counts in response to PFHxS exposure while NTP observed a statistically significant decrease ($p < 0.05$) in eosinophil counts at the 10 mg/kg-day dose in male but not in female SD rats. However, there were no other statistically significant changes in immune hematology parameters, and the inconsistency in findings across the two rat studies is not explained by dose or duration of exposure, or rat strain.

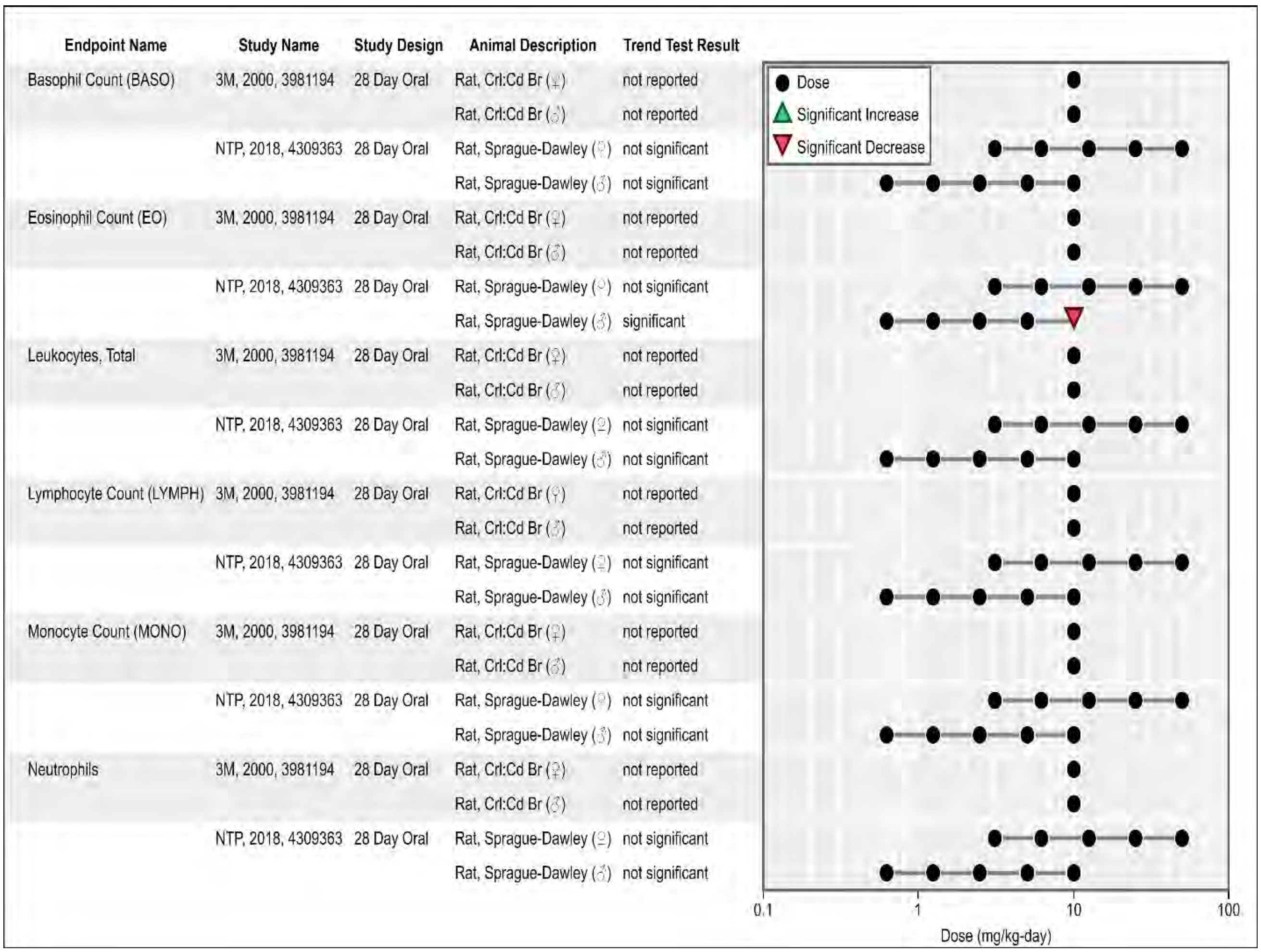


Figure 3-17. Summary of PFHxS immune hematology results. Figure displays the high and *medium* confidence studies included in the analysis. For additional details see HAWC link.

Histopathology

All three studies, 3M (2000b), NTP (2018a), and Chang et al. (2018), performed histological analyses of immune organs and tissues, including bone marrow, lymph nodes, spleen, and thymus. All three studies reported that they found no PFHxS-related histological abnormalities in the immune organs and tissues that they examined although specific results were not reported.

Organ weights

All three studies, 3M (2000b), NTP (2018a), and Chang et al. (2018), measured thymus and spleen weights of control and exposed animals, and no PFHxS-related effects were observed.

Mechanistic Evidence and Supplemental Information

Most of the mechanistic evidence available relates most closely to potential sensitization or allergic response outcomes. Specifically, five studies examined mechanistic endpoints related to hypersensitization in the human studies. None of the five studies reported significant associations between PFHxS and IgE (Timmermann et al., 2017; Stein et al., 2016b; Zhu et al., 2016; Ashley-Martin et al., 2015; Dong et al., 2013). Among asthmatics in the Taiwan population where an association was observed with asthma, increases in eosinophilic cationic protein concentration were significantly associated ($p = 0.004$) with increasing PFHxS concentration (Dong et al., 2013). In addition, one study examined cord blood gene expression in relation to PFHxS levels and found that gene changes associated with PFHxS tracked very well with a set of 27 gene changes associated with common cold episodes (Pennings et al., 2016); however, changes with PFHxS tracked very poorly with a second set of 26 gene changes associated with rubella titers, and the relevance of these gene changes to immune function in general, or antibody responses in particular, remains unknown. No mechanistic evidence from animal, in vitro, in silico, or other evidence streams was identified.

Evidence Integration

Human studies provide *moderate* evidence for immune system effects following exposure to PFHxS (see Table 3-16). Specifically, increased serum levels of PFHxS correlated with decreased antibody responses were observed in most exposure-outcome timing combinations in multiple *medium* confidence studies, although most results were imprecise (i.e., not statistically significant). While variability in response by age of exposure and outcome measure (vaccine type) as well as timing of vaccinations (initial and boosters) resulted in some uncertainty, decreases (generally between 5% and 10%) in antibody concentration per doubling of PFHxS concentration were observed with reasonable consistency across multiple well-conducted studies. In addition, higher odds of infectious disease or symptoms with higher PFHxS concentrations were observed in four of seven available studies, which is coherent with the immunosuppression observed in antibody response studies. There are remaining sources of uncertainty in the immunosuppression evidence, including potential confounding by other PFAS and imprecision of some effect estimates. The evidence for sensitization or allergic response was generally inconsistent, but there was some evidence of an association with asthma incidence. A strong positive association with doctor-diagnosed asthma within the last year was observed in one *medium* confidence study, and this was considered the most specific outcome measure available across the set of studies. However, unlike the evidence on infectious disease, it is unclear how this finding might relate to the evidence supporting immunosuppression, and without additional support or mechanistic understanding (mechanistic information was predominantly null apart from a biomarker coherent with the development of asthma observed in this same study) it does not support a stronger strength of evidence determination. Other studies of sensitization and allergic response were inconsistent. Studies of autoimmunity were not available.

Animal studies provide *indeterminate* evidence for immune system effects following exposure to PFHxS (see Table 3-16). There were no immunotoxicity-specific animal studies in the database, but rather general toxicity or developmental toxicity studies that included immune-related endpoints. As a result, the immune endpoints evaluated in the animal studies were less sensitive and less informative for hazard identification than the endpoints evaluated in the human studies available in the database. No reliable findings of PFHxS-related immune effects were observed in *high* and *medium* confidence studies in animals exposed to PFHxS.

Taken together, the currently available **evidence indicates** that PFHxS likely causes immune toxicity in humans given sufficient exposure conditions[8]. This conclusion is based on epidemiology evidence of an association between PFHxS exposure and immune effects—specifically, immunosuppression, driven primarily by studies of antibody response following vaccination, with median PFHxS blood concentrations in children of 0.3–2.5 ng/mL. Despite imprecision in the results, the antibody results present a generally consistent pattern of findings that higher prenatal and childhood concentrations of PFHxS were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines, and coherent findings from more limited evidence of associations between PFHxS exposure and higher odds of infectious disease. These associations were observed despite poor study sensitivity. While clinical adversity of fairly small changes in antibody concentrations is not established, one study reported higher odds for lack of protection from diphtheria, and there is potential for a subset of people to be more severely affected. Some uncertainty remains resulting from variability in the response by age of exposure and outcome measures as well as from timing of vaccination (initial and boosters) and the potential for confounding by other PFAS.

[8] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-16. Evidence profile table for PFHxS immune effects

Evidence Stream Summary and Interpretation					**Evidence Integration Summary Judgment**
Evidence from studies of exposed humans (see Immune Human Studies Section)					
Studies and interpretation	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	⊕⊕⊙ **Evidence indicates (likely)**
Antibody Response to Vaccine • 7 medium confidence studies • 3 low confidence studies	• *Consistency* – Evidence is generally consistent in the direction of association across vaccine type, timing of vaccination, and age at antibody response measurement • *Low risk of bias* in studies in children • *Magnitude of effect* – Large effect size observed in most studies despite limited sensitivity	• Potential for residual confounding across PFAS • Imprecision of most findings	Studies in children observed inverse associations between PFHxS exposure and antibody levels following vaccination in at least some analyses. While not all results were statistically significant, the direction of association was generally consistent across studies and timing of exposure and outcome measures.	⊕⊕⊙ **Moderate** Generally consistent evidence for immunosuppression with PFHxS exposure based on lower antibody response in multiple *medium* confidence studies, supported by coherent but limited results for infectious diseases [Note: the evidence of hypersensitivity, based a single well-conducted study of asthma with inconsistent findings across other studies with less robust outcome measures, did not contribute to this judgment].	Based on generally consistent evidence of reduced antibody response to vaccination at median blood concentrations of 0.2–0.6 ng/mL. Human relevance: Evidence comes from epidemiological studies (see Immune Human Studies Section) Cross-stream coherence: NA: animal evidence is indeterminate
Infectious Disease • 6 medium confidence study • 6 low confidence studies	• Despite potential limited sensitivity, six studies observed a significant positive association for at least one outcome	• Unexplained inconsistency • High risk of bias from potential outcome misclassification in low confidence studies	2 *medium* and 3 *low* confidence studies reported higher odds of infectious disease or symptoms with higher PFHxS exposure, including total infectious disease, lower respiratory infection, throat infection,		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
			pseudocroup, and gastroenteritis		
Sensitization or allergic response • 13 medium confidence studies	• *Magnitude of effect* – Large effect size in the only study of asthma incidence • *Exposure-response gradient* observed for asthma incidence in 1 study with the most reliable outcome measure • *Biological plausibility* – mechanistic change coherent with asthma in the only study of asthma incidence	• Potential for residual confounding across PFAS • Unexplained inconsistency – Inconsistent direction of associations across studies for all hypersensitivity outcomes (with predominantly null findings)	1 well-conducted study reported a clear positive association with asthma incidence and eosinophilic cationic protein. Of 11 other studies of asthma, only four reported higher odds of asthma in at least one subpopulation but were based on "current" or "ever" asthma definitions, which are less specific. Results for allergies/allergic sensitization, and dermal allergic measures had inconsistent findings.		
Evidence from In vivo Animal Studies (see Immune Animal Studies Section)				**Evidence stream judgment**	
Hematology • 2 *high* confidence studies • One *medium* confidence study	• Low risk of bias	• Endpoints considered nonspecific and insensitive indicators of immune function	Decreased eosinophil counts in 1 study (NTP, 2018a); however, there were no other statistically significant changes in immune hematology parameters and this finding alone is not considered adverse.	⊙⊙⊙ **Indeterminate** [noting that the immune endpoints evaluated in the available animal studies are considered insensitive or nonspecific indicators of immune function.]	
Histopathology • 2 *high* confidence studies	• Low risk of bias		No PFHxS-induced effects observed for histopathology.		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
• 1 *medium* confidence study					
Organ weights • 2 *high* confidence studies • 1 *medium* confidence study	• Low risk of bias		No PFHxS-induced effects observed for organ weights.		

C: cohort, CC: case control, CS: cross sectional.

3.2.3. Developmental Effects

This section describes studies of PFHxS exposure and potential in utero and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. The latter includes all studies for which exposure is limited to gestation and/or early life. Given that some endpoints examined here, such as spontaneous abortion and preterm birth, could be driven by either female reproductive or developmental toxicity, these endpoints are also discussed in the context of coherence in Section 3.2.7 on Female reproductive effects. As such, this section has some overlap with evidence synthesis and integration summaries for other health systems for which studies evaluated the effects of developmental exposure (see Sections 3.2.5, 3.2.2, 3.2.7, 3.2.8, and on potential Hepatic, Endocrine, and Female and Male Reproductive Effects, respectively).

Human Studies

The epidemiologic studies of possible developmental effects of PFHxS evaluate the following endpoints: fetal and childhood growth restriction, spontaneous abortion, and gestational duration (i.e., preterm birth and gestational age). Given that many of these endpoints could be driven by either female reproductive or developmental toxicity, some are also discussed in the context of coherence in the female reproductive effects section (see Section 3.2.7). The evidence informing specific endpoints is discussed and synthesized below; however, the hazard conclusion was determined at the level of developmental effects for the group of endpoints.

<u>Study evaluation considerations</u>

As detailed in the PFAS Systematic Review Protocol (see Appendix A), multiple outcome-specific considerations informed domain-specific ratings and overall study confidence. For the Confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Some pregnancy hemodynamic factors related to physiological changes during pregnancy were also considered in this domain as potential confounders (e.g., glomerular filtration rate and blood volume changes over the course of pregnancy) because these factors may be related to both PFHxS levels and the developmental effects examined here. Irrespective of study design, more confidence was placed in the epidemiologic studies that adjusted for glomerular filtration rate in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, it was considered in the study quality evaluations and as part of the overall weight of evidence determination (see Appendix C for additional discussion of these issues).

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFHxS in humans (range: 4.7 to 8.5 years; see Section 3.1.4.), samples collected during all three trimesters (and shortly after birth) were considered adequately representative of the most critical in utero exposures for fetal growth and gestational duration measures. Many of the cross-sectional studies relied on umbilical cord measures collected shortly after birth. Exposure measures collected close to or concurrently with outcome ascertainment were considered etiologically relevant and acceptable for these developmental endpoints; thus, exposure measurement ratings were not downgraded for timing of measurement. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where in utero perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in De Boo and Harding (2006) and Perng et al. (2016) and other PFAS syntheses for potential cardiometabolic disorders in Section 3.2.6). There is some evidence that birth weight deficits can be followed by increased weight gain that may occur especially among those with rapid growth catch-up periods during childhood (Perng et al., 2016). Therefore, the primary critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero for study evaluation purposes, and studies of this outcome were downgraded in the exposure domain if exposure data were collected later during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (e.g., for sex-stratified results). In the outcome domain, specific considerations address validation and accuracy of specific endpoints and adequacy of case ascertainment for some dichotomous (i.e., binary) outcomes. For example, birthweight measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, some spontaneous abortion studies were downgraded for participant selection due to incomplete case ascertainment given that some pregnancy losses go unrecognized early in pregnancy including before participants would be enrolled. This incomplete ascertainment, referred to as left truncation, can result in bias toward the null if ascertainment of fetal loss is not associated with PFHxS exposures (i.e., nondifferential). In some situations where there is a true association with PFHxS, differential loss is possible, possibly causing a bias away from the null, and can manifest as an apparent protective effect. Fetal and childhood growth restriction were examined using several endpoints including low birth weight, small for gestational age (SGA), ponderal index [i.e., birth weight grams)/birth length (cm^3) × 100], abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. When sufficient *high* and *medium* confidence evidence is available for a set of related endpoints, the developmental effects synthesis is largely focused on the higher quality endpoints (i.e., classified as good in the outcome domain).

Overall, mean birth weight and birth weight-related measures are considered very accurate and were collected predominately from medical records; therefore, more confidence was placed in these developmental endpoints in the outcome domain judgments. Some of the adverse birth weight endpoints of interest examined here included fetal growth restriction endpoints based on birth weight such as mean birth weight (or variations of this endpoint such as standardized birthweight z-scores), as well as binary measures such as SGA (e.g., lowest decile of birthweight stratified by gestational age and other covariates) and low birth weight (i.e., typically <2,500 grams; 5 pounds, 8 ounces) births. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary to be classified as good for these endpoints in this domain. In contrast, other measures of fetal growth that are subject to greater measurement error (e.g., head circumference and body length measures such as ponderal index) were given a rating of adequate (Shinwell and Shlomo, 2003). These sources of measurement error are expected to be nondifferential with respect to PFHxS exposure status and, therefore, would not typically be a major concern for risk of bias but could impact study sensitivity.

Gestational duration measures were presented as either continuous (i.e., per each gestational week) or binary endpoints such as preterm birth (typically defined as gestational age <37 weeks). The potential for measurement error can complicate accurate estimates of gestational age and may decrease study sensitivity related to some of these endpoints especially when based on recall of last menstrual period alone. However, many of the studies were based on ultrasound measures early in pregnancy, which should increase the accuracy of estimated gestational age and the ability to detect associations that may be present. Studies were downgraded if based solely on last menstrual period and more certainty was anticipated for studies using a combination of measures with comparisons of any differences. Any sources of error in the classification of these endpoints should be nondifferential with respect to PFHxS exposure and, therefore, would not be considered a major concern for risk of bias, but could impact precision and study sensitivity.

Anogenital distance (AGD) is an externally visible marker that has been shown in animal studies to be a sensitive indicator of prenatal androgen exposure (lower androgen levels associated with decreased AGD, and the reverse). It is associated with other reproductive tract abnormalities, including hypospadias and cryptorchidism in human and animal males (Liu et al., 2014; Sathyanarayana et al., 2010; Salazar-Martinez et al., 2004); the potential adverse consequences in females are less well defined. In boys, measures can be taken from the center of the anus to the posterior base of the scrotum (ASD) or from the center of the anus to the cephalad insertion of the penile (APD). In girls, there are two possible measures, the anoclitoris distance (ACD) and the anofourchette distance (AFD). The primary outcome-specific criteria for this outcome are the use of clearly defined protocols for measurement, ideally multiple measures of each distance (averaged), and minimal variability in the age of participants at measurement.

Growth restriction – fetal growth

Developmental Epidemiologic Studies

Sixty-one epidemiological publications (across 58 different studies) examining PFHxS exposures in relation to developmental endpoints were identified in the literature search. Several studies examined multiple endpoints that are captured in separate sub-sections below. This included the following: 12 studies on postnatal growth, 19 studies on gestational duration, 5 on fetal loss, 4 on anogenital distance, 2 studies on birth defects, and 42 publications (across 39 different studies) that examined fetal growth restriction.

Fetal Growth Restriction – Study Background

The heat map of 39 fetal growth restriction studies below does not include three overlapping publications, such as the Woods et al. (2017) publication from the same study population (Health Outcomes and Measures of the Environment cohort) as Shoaff et al. (2018) (see Figures 3-18 and 3-19). For consistency, birth outcomes measures reported in (Manzano-Salgado et al., 2017a) were preferred to in utero growth estimates in the Costa et al. (2019) study from the same Environment and Childhood - Infancia y Medio Ambiente (INMA) birth cohort. The smaller population subset from the Bjerregaard-Olesen et al. (2019) study is from the same Aarhus birth cohort as Bach et al. (2016). Given disparate results shown below in this subset versus the whole cohort for head circumference and birth length, results from the full study population in Bach et al. (2016) are given precedent. However, the Bjerregaard-Olesen et al. (2019) provide additional sex-specific data not examined in Bach et al. (2016). Difference in results for these endpoints are highlighted in the syntheses below but only one study is plotted for each endpoint to aid the evaluation of consistency across studies. Five of the remaining 39 fetal growth studies (Maekawa et al., 2017; Alkhalawi et al., 2016; Lee et al., 2016; Lee et al., 2013; Monroy et al., 2008) are not included in the synthesis further as they were classified as *uninformative* largely due to critical study deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies.

Birth Weight – Background of Studies

As shown in Figure 3-18 and Table 3-17, there were 34 informative studies that examined birth weight measures in relation to PFHxS exposures. This included 13 studies that examined PFHxS in relation to continuous standardized birth weight scores. Ten of these 13 reported standardized measures along with mean birth weight differences in relation to PFHxS. Three (Gardener et al., 2021; Gross et al., 2020; Xiao et al., 2019) of the 13 studies reported only standardized birthweight measures, with Gardener et al. (2021) not plotted below with the others given an atypical, dichotomized effect estimate with different scaling.

Of the 31 epidemiological studies with mean birth weight data, four (Marks et al., 2019a; Ashley-Martin et al., 2017; Lind et al., 2017; Maisonet et al., 2012) only reported sex-specific findings, including a study in boys (Marks et al., 2019a) and girls (Maisonet et al., 2012) from the

ALSPAC study (see Figure 3-19). Fifteen different studies examined mean birth weight differences across the sexes 14 each in boy and girls. Among the 27 studies with results in the overall population, three studies (Eick et al., 2020; Gao et al., 2019; Cao et al., 2018) reported results based only on categorical data.

Figure 3-18. Study evaluation results for 39 epidemiological studies of birth weight and PFHxS. For additional details see HAWC link.

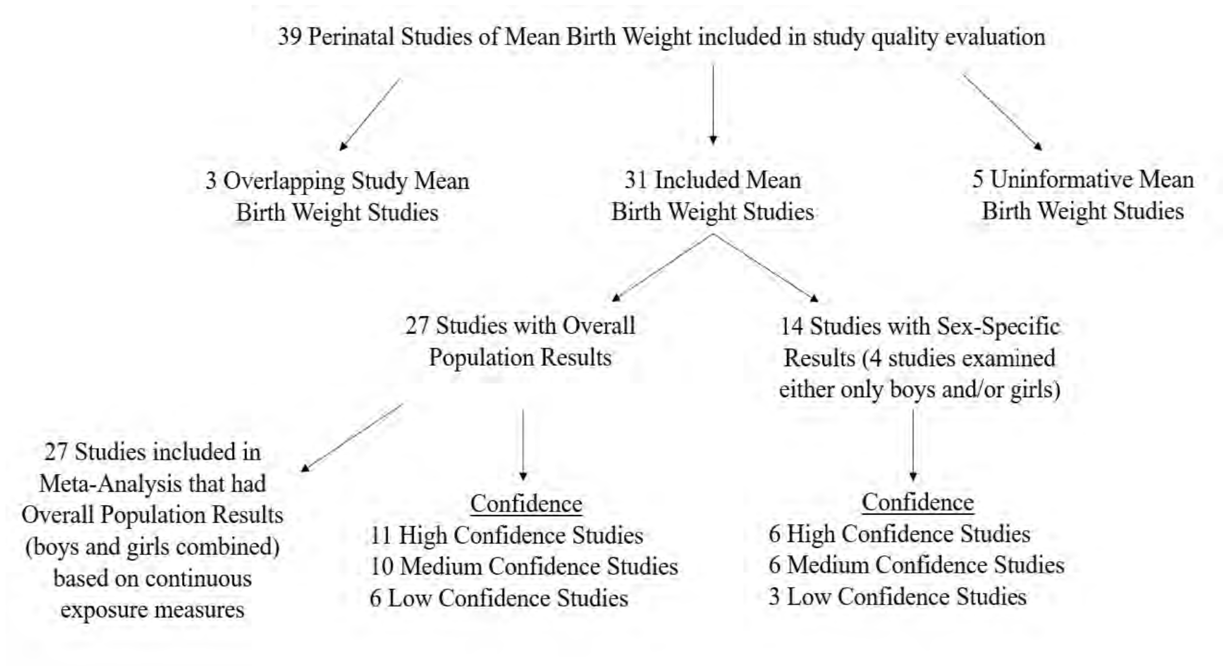


Figure 3-19. Perinatal studies of birth weight measures and subsets included in different evaluations.

Birth weight – Mean Differences – Background

Twenty-five of the included 31 mean birth weight studies were prospective birth cohorts, and six were cross-sectional studies (Xu et al., 2019; Gyllenhammar et al., 2018; Li et al., 2017b; Shi et al., 2017; Callan et al., 2016; Kwon et al., 2016) (see Figures 3-20 and 3-21). Five of these six studies relied on umbilical cord blood measures (Xu et al., 2019; Cao et al., 2018; Li et al., 2017b; Shi et al., 2017; Kwon et al., 2016), and one collected PFHxS blood samples in infants 3 weeks following delivery (Gyllenhammar et al., 2018). Twenty-four studies had maternal blood measures that were sampled during trimesters one (Buck Louis et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017; Manzano-Salgado et al., 2017a), two (Hamm et al., 2010), three (Luo et al., 2021; Yao et al., 2021; Kashino et al., 2020; Gao et al., 2019; Valvi et al., 2017; Callan et al., 2016), or across multiple trimesters (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Wikström et al., 2020; Marks et al., 2019a; Workman et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Starling et al., 2017; Bach et al., 2016; Lenters et al., 2016; Maisonet et al., 2012). The study by Meng et al. (2018) pooled exposure data from two study populations, one that measured PFHxS in umbilical cord blood and one that measured PFHxS in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean birth weight, EPA only examined data from one measure, such as umbilical cord or maternal serum concentrations, and when necessary, relied on other related publications (e.g., Gyllenhammar I (2017)) or additional information or data provided by study authors. When possible, EPA converted effect estimates that were based on continuous PFHxS measures to a 1 ln-unit increase to enhance comparability across studies (see Figures 3-22, 3-23, 3-24). These results employing a common unit of measurement were also used for the birth weight meta-analysis conducted by EPA (see Appendix C for details on the methods employed).

Thirteen of the 31 mean birth weight studies were rated *high* in overall study confidence (Luo et al., 2021; Yao et al., 2021; Eick et al., 2020; Wikström et al., 2020; Buck Louis et al., 2018; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016), while 11 were rated *medium* (Chang et al., 2022; Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018; Li et al., 2017b; Kwon et al., 2016; Lenters et al., 2016; Maisonet et al., 2012; Hamm et al., 2010), and 7 were classified as *low* (Gao et al., 2019; Marks et al., 2019a; Workman et al., 2019; Xu et al., 2019; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016) (see Figure 3-18).

Of the 31 mean birth weight studies detailed in this synthesis, 13 studies (Luo et al., 2021; Wikström et al., 2020; Marks et al., 2019a; Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Li et al., 2017b; Starling et al., 2017; Valvi et al., 2017; Lenters et al., 2016; Maisonet et al., 2012) were considered to have good study sensitivity. Ten studies (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Buck Louis et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016; Kwon et al., 2016; Hamm et al., 2010) were classified as adequate and eight were deficient (Yao et al., 2021; Kashino et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016).

Birth weight – Mean Difference Results (in Grams) in Overall Population

Overall, 14 of the 27 different epidemiological studies that examined associations in the overall population (i.e., both male and female neonates combined) detected some deficits in relation to PFHxS exposures (see Figures 3-20, 3-21, 3-22, and Table 3-17). This included five (Buck Louis et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Bach et al., 2016) out of 11 *high* confidence studies, five (Chang et al., 2022; Hjermitslev et al., 2020; Gyllenhammar et al., 2018; Li et al., 2017b; Kwon et al., 2016) out of 10 *medium* and four (Gao et al., 2019; Xu et al., 2019; Cao et al., 2018; Callan et al., 2016) out of six *low* confidence studies. In contrast, four studies reported increased birth weight with PFHxS exposures while eight other studies were null. For example, the *high* confidence study by Eick et al. (2020) reported non-significant increased birth weight across PFHxS tertiles (β range: 75.7 to 82.2 g) relative to tertile 1. The *medium* confidence study by Chen et al. (2021) reported a small increased mean birth weight based on continuous exposures (β = 27.6 g; 95%CI: −64.7, 119.9 per ln-unit increase) along with mixed results based on categorical PFHxS exposures (β range: −46 to 26 g). The *high* confidence Manzano-Salgado et al. (2017a) study showed consistent but non-monotonic birth weight decreases across all three upper quartiles (β range: −30 to −65 g), but a relatively small deficit per each unit increase (β= − 12.4 g; 95%CI: −46.2, 21.4). The latter results were indicative of deficits seen in the five *high* confidence studies (β range: −12 to −22 g per each ln-unit increase).

Birth weight deficits detected in the five *medium* confidence studies were larger (β range: −30 to −93 g per each ln-unit increase). For example, the *medium* confidence study by Hjermitslev et al. (2020) reported a large birth weight deficit (β= −93 g; 95%CI: −230, 44 per each ln-unit increase). Two other *medium* confidence studies (Gyllenhammar et al., 2018; Kwon et al., 2016) reported birth weight decreases consistent in magnitude (β range: −53 to −60 g per each ln-unit increase). The *medium* confidence study by Chang et al. (2022) reported a non-significant deficit per each ln-unit increase (β= −20 g; 95%CI: −84, 45) but larger results for PFHxS quartiles 2 (β= −36 g; 95%CI: −154, 83) and 4 (β= −54 g; 95%CI: −173, 66). The *medium* confidence study by Kashino et al. (2020) reported a null association with PFHxS and mean birth weight (β= −1.3 g; 95%CI: −26.3, 23.6 per each ln-unit increase). They did show large differences in multiparous participants (β= −81.2 g; −122.3, −40.1 per each ln-unit increase) but not for primiparous participants (β= −2.2 g; −46.2, 41.7 per each ln-unit increase).

Birth weight deficits detected in the two *low* confidence studies were consistent in magnitude (β range: −72 to −76 g per each ln-unit increase). The *low* confidence study by Gao et al. (2019) reported larger decreased birth weight in a non-monotonic fashion across PFHxS tertiles 2 (β= −154.1 g; 95%CI: −332.2, 24.0) and 3 (β= −101.2 g; 95%CI: −275.5, 73.1). Across all confidence levels, only one (Cao et al., 2018) of 11 studies with categorical data in the overall population showed some evidence of exposure-response relationships (β range: −14 to −25 g across tertiles).

Birth Weight- Mean Difference- Overall Population Summary

In the overall population, there were consistent results of deficits across all study confidence levels (5 of 11 *high*, 5 of 10 *medium*, and 4 of 6 *low* confidence studies). However, the five *high* confidence studies showed consistently smaller deficits (β range: −12 to −22 g per each unit increase) compared to the five *medium* (β range: −20 to −93 g) and two *low* (β range: −72 to −76 g) confidence studies. Although the majority of *low* confidence studies observed larger birth weights in association with PFHxS exposure, the estimates were consistently imprecise, and the identified methodological limitations preclude further interpretation in that subset. There was limited evidence of exposure-response relationships based on categorical data, but the magnitude of changes in those studies showing deficits ranged from −25 to −101 grams for the highest quantile (compared to the lowest quantile) were comparable to those results (β range: −12 to −93 grams per each ln-unit increase) based on the continuous exposure expressions shown above.

Limited patterns were evident as study sensitivity, exposure levels and contrasts and other study design elements were not explanatory for null or inverse associations detected across the birth weight studies. The birth weight deficits in the overall population may be influenced by hemodynamic changes during pregnancy related to exposure assessment timing, as only four of the fourteen were based on early biomarker sampling.

Meta-Analysis of Mean Birth Weight Differences

Twenty-eight studies were identified for possible inclusion into a meta-analysis of overall population estimates (see Figure C-1 and more details on the Methods in Appendix C) if they provided results in the overall population or in both sexes which allowed combination to estimate an overall population result. Three studies with PFHxS categorical data only (Eick et al., 2020; Gao et al., 2019; Cao et al., 2018) were not included in the meta-analysis due to the lack of results on a per continuous exposure increase. The remaining 27 studies (from 28 publications) include the other 24 studies identified in the overall population section noted above as well as three additional studies, which reported sex-specific data only on boys and girls individually (Ashley-Martin et al., 2017; Lind et al., 2017). Another cohort (ALSPAC) reported results in girls (Maisonet et al., 2012) in one publication and boys (Marks et al., 2019a) in another and were combined for the meta-analysis.

Following scale conversions and re-expressions (to ln-unit) for some studies by U.S. EPA, the meta-analysis of 27 studies showed negligible between-study heterogeneity (I_2 = 0%), and a small but statistically significant decrease in birthweight (β=−7.7 g; 95% CI: −14.8, −0.5) per each ln-unit PFHxS increase (see Figure 3-20). Statistically significant results comparable in magnitude were also detected when restricted to just *medium* and *high* confidence studies (β=−8.0 g; 95% CI: −15.2, −0.7) and also to 23 studies that provided results based on some logarithmic transformation (β= −6.5 g; 95% CI: −14.8, −0.5).

Mean birth weight deficits were detected only among the 12 *high* (β= −6.8 g; 95% CI: −16.3, 2.8) and 11 *medium* (β=−9.6 g; 95% CI: −20.8, 1.6) confidence studies. The pooled effect in the *low* confidence studies was null (β=−1.5 g; 95% CI: −51.6, 48.7) and based upon far fewer studies (n = 4). Stratified mean birth weight deficits were also different based on studies with later sample timing. The five studies that used umbilical cord samples or maternal samples after birth or pregnancy samples had considerably larger deficits (β= −28.3 g; 95% CI: −69.3, 12.7) compared with the 12 studies with sampling from early pregnancy (β= −7.3 g; 95% CI: −16.0, 1.4) or the ten studies with sampling from mid- to late pregnancy (β= −3.9 g; 95% CI: −17.7, 9.9).

Overall, the meta-analytical data showing a small change in mean birth weight per each ln-unit change (i.e., a 2.7-fold increase in exposure in ng/mL within the range of observed exposures in the study populations) support the main epidemiologic findings detailed above and provide some limited evidence of an adverse effect on birthweight from maternal exposure to PFHxS (see Appendix C for more detail and additional stratified analyses). The median exposure ranged from 0.16 to 10.36 ng/mL across the 27 studies with birth weight data in the meta-analysis. The pooled birth weight estimates expressed here per each unit change are relatively small in magnitude are expressed here per each unit change and could be larger depending on the range of exposures within a particular study population or the range to which it is being extrapolated to. Although a gradient across sample timing was not evident across all time periods, the pooled estimate in the five studies with post-partum sample was much larger. In contrast to the late maternal sampled studies, the associations in the early sampled studies were consistent in magnitude to the pooled estimate across all studies as well as the combined *medium* and *high* confidence studies. Thus, while some uncertainty remains on the potential impact due to pregnancy hemodynamics especially in the later sampled studies, the overall combined results, the early sample timing studies as well as the higher confidence (*medium* and *high* combined) studies do show a small association between mean birthweight and PFHxS.

Table 3-17. Summary of 34 epidemiologic studies of PFHxS exposure and growth restriction measures

Author	Study location, years	Sample size[a]	Median exposure (range) in ng/mL	Birth weight	Birth length	HC	SGA/ LBW
High Confidence Studies							
Ashley-Martin et al. (2017)	Canada, 2008–2011	1,509	1.0 (0.3, 25.0)	∅ Overall + Boys – Girls			
Bach et al. (2016); Bjerregaard-Olesen et al. (2019)	Denmark, 2008–2013	1,507	0.5 (<LOQ, 6.82)	– Overall/ Boys/Girls	– Overall[a] ∅ Boys/Girls	– Overall[b]	
Buck Louis et al. (2018)	USA, 2009–2013	2106	0.71 (N/A)	– Overall	– Overall*	– Overall	
Eick et al. (2020)	USA, 2014–2018	506	0.33	+ Overall/ Boys/Girls			
Gardener et al. (2021)	USA, 2009–2013	354	0.5	↑ All (BWT-z)			
Lind et al. (2017)c	Denmark, 2010–2012	636	0.3 (LOD, 7.3)	– Boys ∅ Girls		–Boys ∅ Girls	
Luo et al. (2021)	China, 2021	224	10.36 (N/A)	∅ Overall	∅ Overall		
Manzano-Salgado et al. (2017a)	Spain, 2003–2008	1,202	0.58 (0.05, 11.01)	– Overall* ∅ Boys/Girls	– Overall*[b] ∅ Boys/Girls	– Overall[ab] ∅ Boys/Girls	∅ SGA Overall/Girls/Boys ∅ LBW Overall/Girls ↑ Boys
Sagiv et al. (2018)	USA, 1999–2002	1,645	2.4 (0.1, 74.5)	∅ Overall			

Author	Study location, years	Sample size[a]	Median exposure (range) in ng/mL	Birth weight	Birth length	HC	SGA/ LBW
Shoaff et al. (2018)	USA, 2003–2006	345	1.5 (0.1-32.5)	– Overall			
Starling et al. (2017)	CO, USA, 2009–2014	598	0.8 (0.1, 10.9)	– Overall			
Valvi et al. (2017)	Denmark, 1997–2000	604	4.54 (N/A)	+ Overall/ Boys/Girls	– Overall/ Boys ∅ Girls	+ Overall*/Boys* ∅ Girls	
Wikström et al. (2020)	Sweden, 2007–2010	1533	1.23 (N/A)	∅ Overall/Boys /Girls			∅ SGA Overall/Boys ↑ SGA Girls
Xiao et al. (2019)	Faroe Islands, 1994–1995	172	0.55 (0.1, 2.8)	– Overall/Boys/ Girls	– Overall/Boys/Girls*	– Overall/Boys/ Girls*	
Yao et al. (2021)	China, 2010–2013	369	0.32	∅ Overall			
Medium Confidence Studies							
Chang et al. (2022)	USA, 2014–2018	370	1.10 (<LOD, 4.80	– Overall			∅ Overall
Chen et al. (2021)	China, 2013–2015	214	0.67 (N/A)	+ Overall	– Overall/Boys ∅ Girls	– Overall	
Gyllenhammar et al. (2018)	Sweden, 1996–2001	381/ 587	0.24 (0.32, 26)	– Overall*/ Boys/Girls	∅ Overall	∅ Overall	
Hamm et al. (2010)	Canada, 2005–2006	252	2.1[e] (<LOD, 43)	+ Overall			↑ SGA

Author	Study location, years	Sample size[a]	Median exposure (range) in ng/mL	Birth weight	Birth length	HC	SGA/ LBW
Hjermitslev et al. (2020)	Greenland, 2010–2011; 2013–2015	266	1.15 (0.21, 7.87)	– Overall/Girls + Boys	∅ Overall + Boys - Girls	–Overall/Girls ∅ Boys	∅ Overall SGA ∅ Overall LBW
Kashino et al. (2020)	Japan, 2003–2009	1,591	0.3 (N/A)	∅ Overall/Boys /Girls	∅ Overall/Boys/Girls	∅ Overall/Girls–Boys	
Kwon et al. (2016)	S. Korea, 2006–2010	268	0.38 (0.11, 1.20)	– Overall			
Lenters et al. (2016)	Ukraine/Poland/ Greenland, 2002–2004	1,321	1.56, 2.28 (0.45, 5.95)[d]	∅ Overall			
Li et al. (2017b)	China, 2013	321	3.87 (ND, 20.15)	– Overall/Boys ∅ Girls			
Maisonet et al. (2012)	United Kingdom, 1991–1992	422	1.6 (0.2-54.8)	– Girls[*a]	– Girls[*a]		
Meng et al. (2018)	Denmark, 1996–2002	2,120	~1 (N/A)	∅ Overall/Girls + Boys			↑LBW ↑VLBW
Low Confidence Studies							
Callan et al. (2016)	W. Australia, 2003–2004	98	0.33 (0.06, 3.3)	– Overall	– Overall	– Overall	
Cao et al. (2018)	China, 2013–2015	337	0.09 0.03-0.31[f]	– Overall[a]/Boys[a] + Girls	– Overall/ Boys ∅ Girls		
Gao et al. (2019)	China, 2015–2016	132	0.24 (N/A)	– Overall	– Overall		

Author	Study location, years	Sample size[a]	Median exposure (range) in ng/mL	Birth weight	Birth length	HC	SGA/ LBW
Gross et al. (2020)	USA, 2014	98	0.108 (N/A)[g]	– Overall/ Boys/Girls			
Marks et al. (2019a)	England, 1991–1992	447	1.9 (0.5, 74.2)	–Boys	– Boys[b]	∅ Boys	
Shi et al. (2017)	China, 2012	170	0.16 (<LOD, 3.05)	+ Overall/ Girls/Boys	+ Overall + Boys* ∅ Girls		
Workman et al. (2019)	Canada, 2010–2011	414	0.44 (<LOQ, 24)	∅ Overall	∅ Overall	+ Overall	
Xu et al. (2019)	China, 2016–2017	98	0.61 (0.30, 1.94)[d]	– Overall	+ Overall	∅ Overall	↑ SGA

Abbreviations: HC = Head circumference; SGA = small for gestational age; LBW = low birth weight; VLBW = very low birth weight; LOQ: level of quantification; LOD: level of detection; ND: non detectable; N/A: not available.

*Denotes statistical significance at $p < 0.05$; Æ represents a null association; + represents a positive association; - represents a negative association; - represents increased odds ratio; ¯ represents decreased odds ratio.

Note: “Adverse effects” are indicated by both increased ORs (-) for dichotomous outcomes and negative associations (-) for the other outcomes.

/ Denotes multiple groups with the same direction of associations.

[a]Exposure-response relationship detected based on categorical data.

[b]Reduction based on categorical data, null results based on continuous data.

[c]High confidence for birth weight and Medium confidence for head circumference.

[d]No range provided but 5th–95th percentiles included.

[e]Arithmetic mean value, no median value available.

[f]No range provided but 10th–90th percentiles included.

[g]Dried Blood spot PFHxS sample collected within 48 hours of birth.

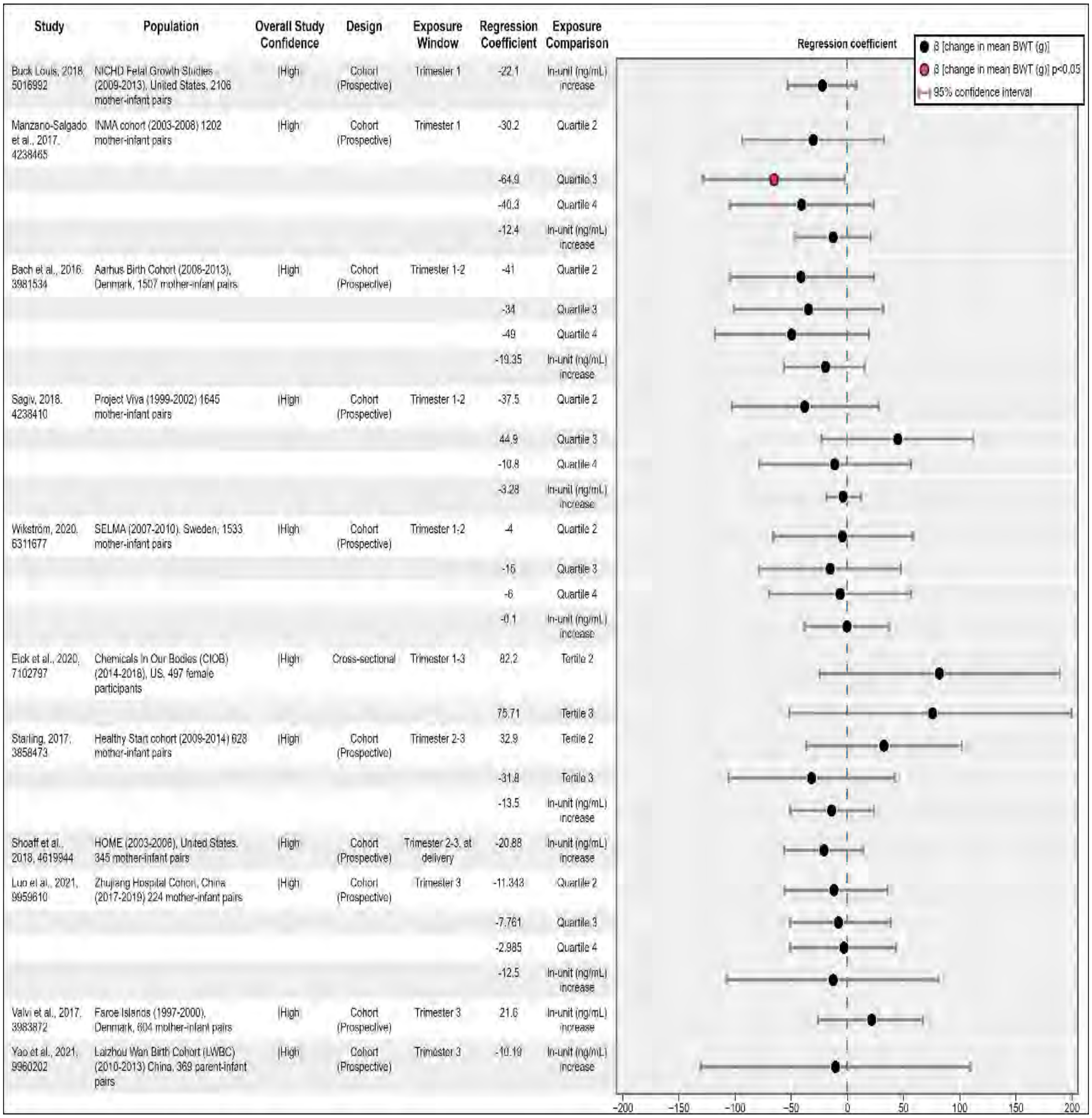


Figure 3-20. Overall population birth weight results for 11 high confidence PFHxS epidemiological studies.[a,b] For additional details see HAWC link.

Abbreviation: BWT = Birth Weight

[a]Studies are sorted first by overall study confidence level, then by exposure window(s) examined.

[b]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

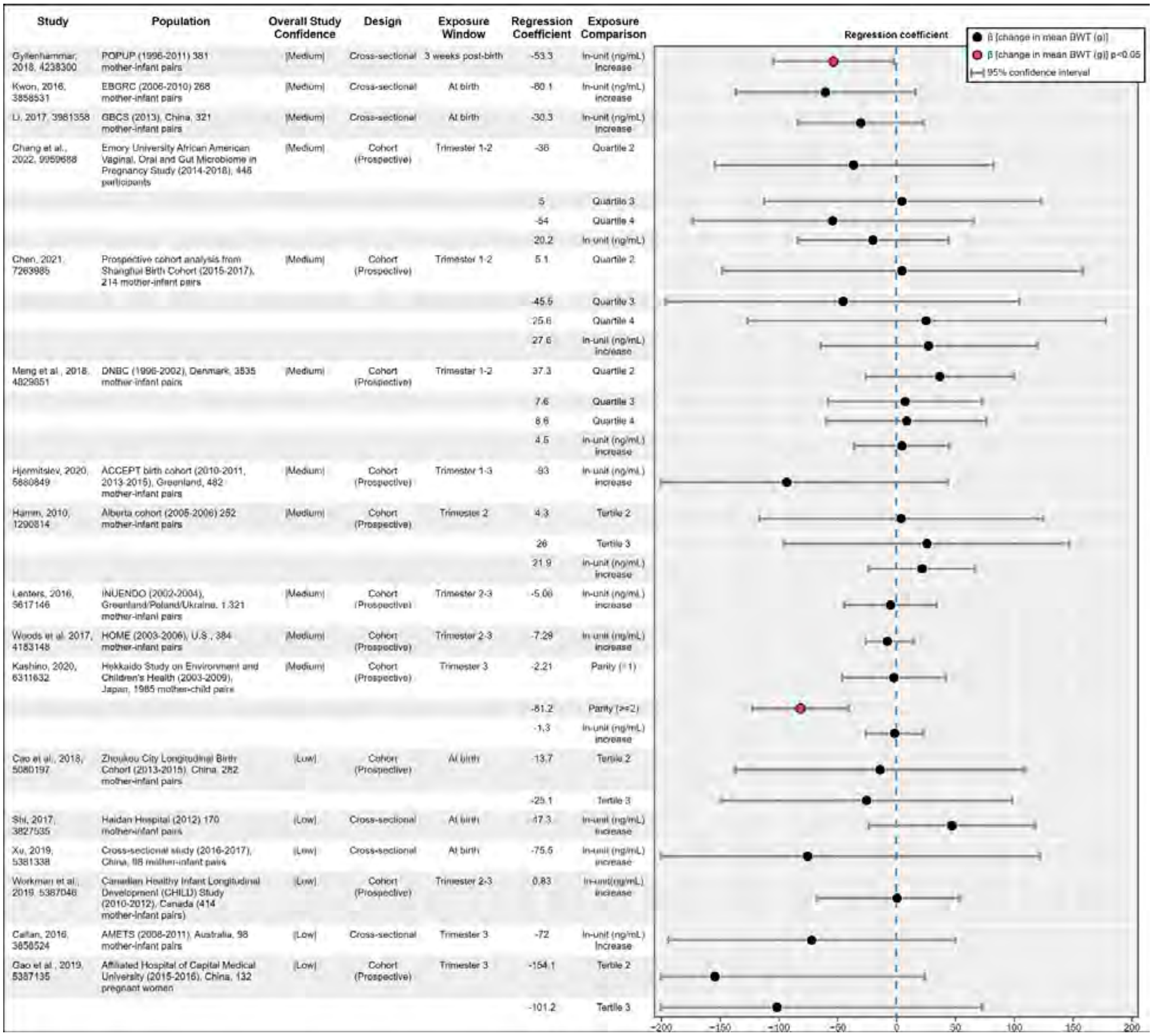


Figure 3-21. Overall population birth weight results for 17 medium and low confidence epidemiological studies. For additional details see HAWC link.

Abbreviation: BWT= Birth Weight

[a]Studies are sorted first by overall study confidence level, then by exposure window(s) examined.

[b](Meng et al., 2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c](Gyllenhammar et al., 2018) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort compared to a smaller sample size of 381 in their 2018 publication.

[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

[e]Some confidence intervals (CIs) truncated, e.g. the entire 95%CIs for these studies are: (Hjermitslev et al., 2020): -230, 44.1; (Xu et al., 2019): -272.7, 121.6; (Gao et al., 2019): Tertile 2: -332.2, 24; Tertile 3: -275.5, 73.1

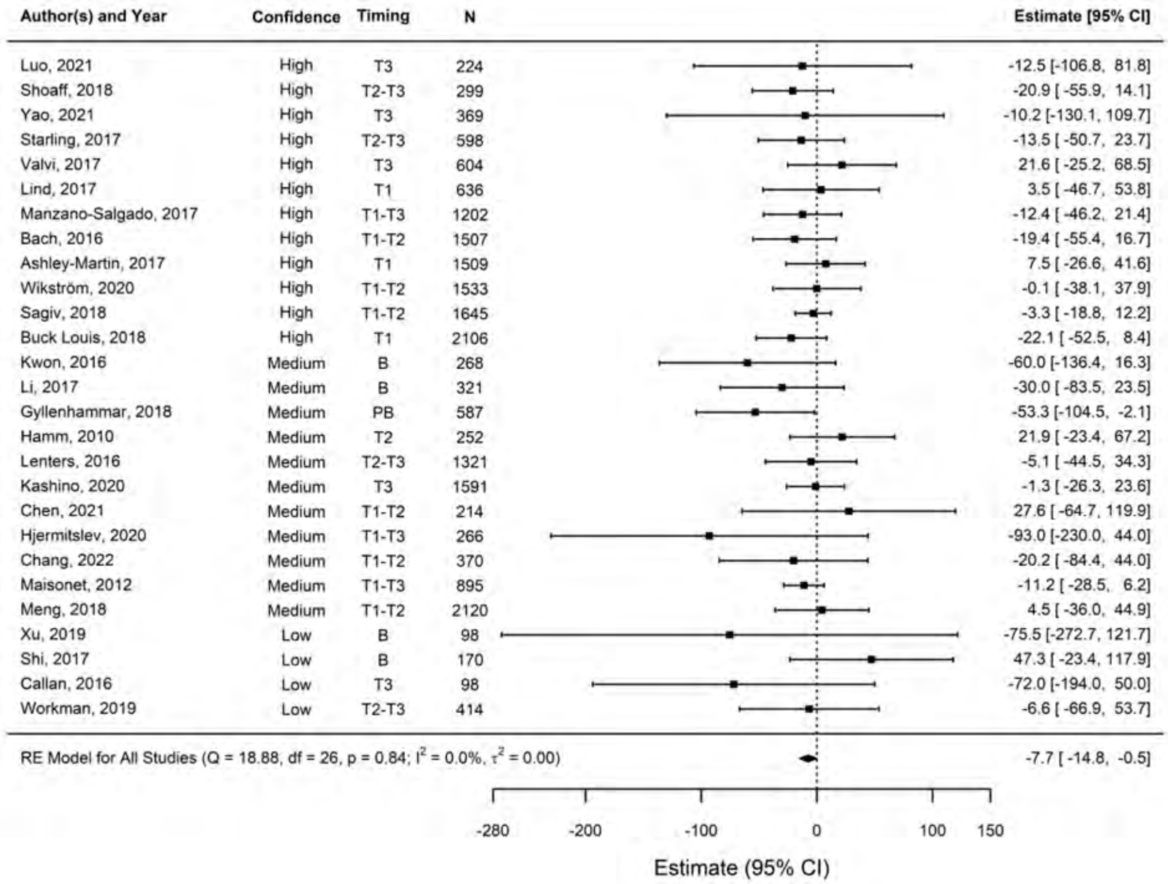


Figure 3-22. Forest plot of 27 studies included for the EPA meta-analysis on changes in mean birth weight per each ln-unit PFHxS increase.

Abbreviations: T1 = first trimester; T1–T2 = first and second trimester, T2 = second trimester; T2–T3 = second and third trimester; T3 = third trimester; B = at birth, PB = post-birth. See Appendix C for more details.

Birth Weight – Mean Differences – Sex-specific Results

Eight of the 14 studies with results showed some birth weight deficits in relation to PFHxS exposures in either or both sexes (see Figures 3-23 and 3-24). In contrast, five studies in boys (β range: 17 to 70 g per ln-unit increase) and three studies in girls (β range: 20 to 70 g per ln-unit increase) showed non-significant increased birth weight. Seven studies in girls were null (Kashino et al., 2020; Wikström et al., 2020; Meng et al., 2018; Ashley-Martin et al., 2017; Li et al., 2017b; Lind et al., 2017; Manzano-Salgado et al., 2017a), while three were null in boys (Kashino et al., 2020; Wikström et al., 2020; Manzano-Salgado et al., 2017a).

Among the eight different studies that showed some evidence of inverse associations, six were in boys and four were in girls. Two (Gyllenhammar et al., 2018; Bach et al., 2016) of the eight different studies reported decrements in both sexes. For example, birth weight deficits ranging from −21 to −34 grams for quartiles 3 and 4 were seen in girls from the *high* confidence Bach et al. (2016) study, but results were null for continuous exposure (per each ln-unit increase). In contrast, results in boys for each ln-unit were −25 g but smaller (β range: −16 to −21 g) based on the upper three quartiles (compared to quartile 1). In the *medium* confidence Gyllenhammar et al. (2018) study, results were stronger in males (β= −71 g; 95%CI: -150, 8 per each ln-unit PFHxS increase) than females (β= −45 g; 95%CI: −139, −47 per each ln-unit PFHxS increase).

Four of the studies noted above showed deficits only in boys (Marks et al., 2019a; Cao et al., 2018; Li et al., 2017b; Lind et al., 2017). Two of the four studies noted above detected deficits in girls only (Hjermitslev et al., 2020; Maisonet et al., 2012). The largest association in girls was seen in the *medium* confidence study by Hjermitslev et al. (2020) (β= −145; 95%CI: −306, 14.7 per each ln-unit increase). The *medium* confidence Maisonet et al. (2012) study showed some evidence of an exposure-response relationship (β range: −9 to −108 grams across PFHxS tertiles). Two (Marks et al., 2019a; Lind et al., 2017) of the seven studies that reported decrements in boys showed incongruent results based on continuous and categorical exposures. For example, they both showed null results for each ln-unit increase but large deficits were seen for exposure categories (β range: −54 to −104 grams across PFHxS quantiles). A large deficit was also seen in the *low* confidence Li et al. (2017b) study (β= −53 g; 95%CI: −127, 20 per each ln-unit increase). The *low* confidence Cao et al. (2018) study showed some evidence of an exposure-response relationship in boys (β range: −30 to −109 g across tertiles). The study by Hjermitslev et al. (2020) was null for their continuous exposure measure and quartile 4, did show some elevated non-significant results for quartiles 2 and 3 (β range: -39 to -51 g).

Birth Weight – Mean Difference – Sex-Specific Summary

Eight different studies showed some birth weight deficits in relation to PFHxS exposures in either or both sexes. Although the magnitude of deficits was larger among girls (β range: −45 to −145 g) per each ln-unit PFHxS increase than boys (β range: −25 to −71 g), more studies showed deficits among boys. Four of these studies showed deficits in girls, while six showed deficits in boys. There were no patterns seen for results across confidence levels among boys, but the deficits seen in girls were limited to *medium* and *high* confidence studies only. Two of the three *low* confidence studies in boys showed adverse results including one with evidence of an exposure-response relationship based on categorical data. Among the five studies with categorical data, one study each in boys and girls had exposure-response relationships that were comparable in magnitude (−108 and −109 g in tertile 3). Those results were coherent with linear birth weight relationships detected in several studies with continuous exposure metrics data as noted above (ranging from –25 to –145 grams per each unit change in PFHxS).

Among these eight sex-specific studies, five had early biomarker samples indicative that pregnancy hemodynamics was not likely an explanatory factor here. No other patterns by other study characteristics were evident in the sex-specific findings including study sensitivity among the null studies. Although the evidence may be somewhat stronger among males, the lack of consistent patterns within and across studies and insufficiently sensitive studies to detect statistically significant sex-specific associations preclude more definitive conclusions from being drawn.

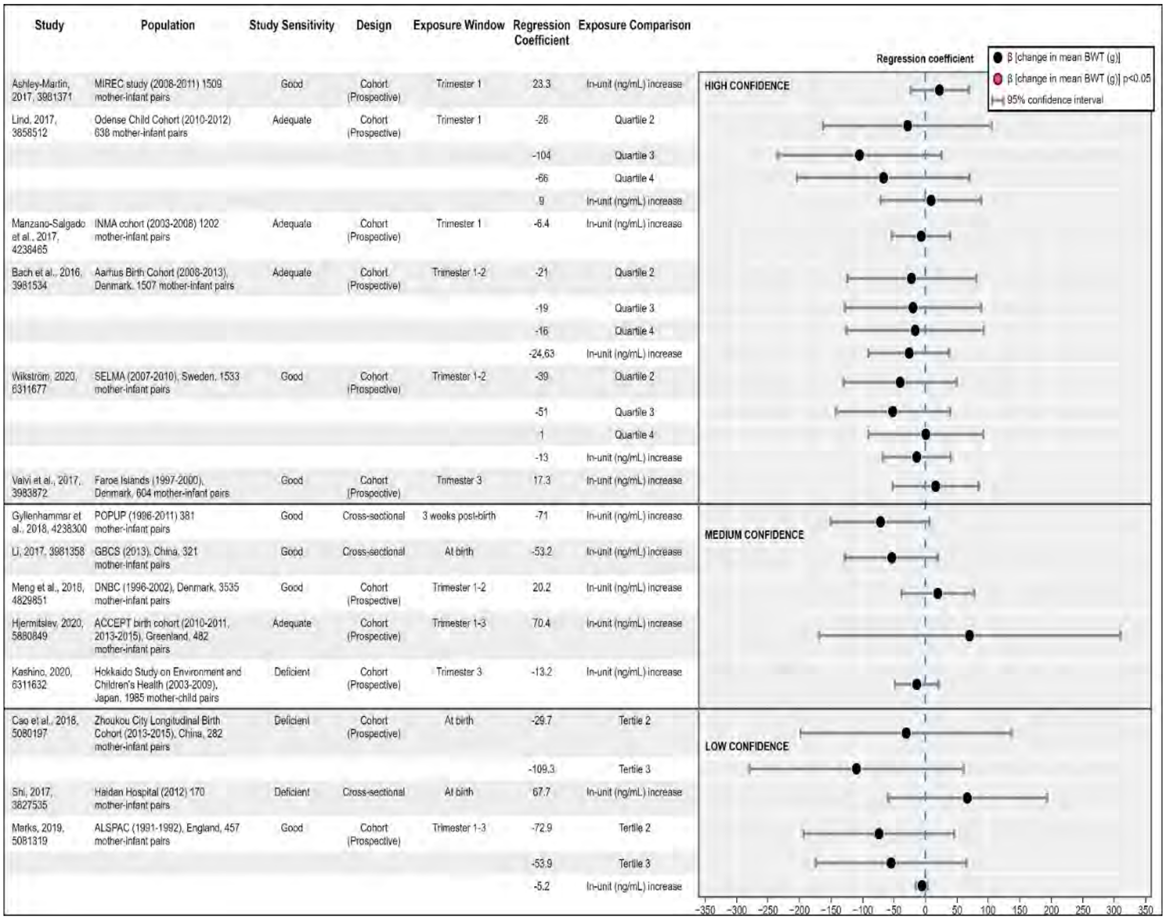


Figure 3-23. Sex-specific male infants only mean birth weight results for 14 PFHxS epidemiological studies.[a,b,c,d] For additional details see HAWC link.

Abbreviations: BWT = Birth Weight
[a]Studies are sorted first by sex, overall study confidence level, then by exposure window(s) examined.
[b](Meng et al., 2018) pooled samples from umbilical cord blood and maternal plasma during first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.
[c](Gyllenhammar et al., 2018) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort compared to a smaller sample size of 381 in their 2018 publication.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

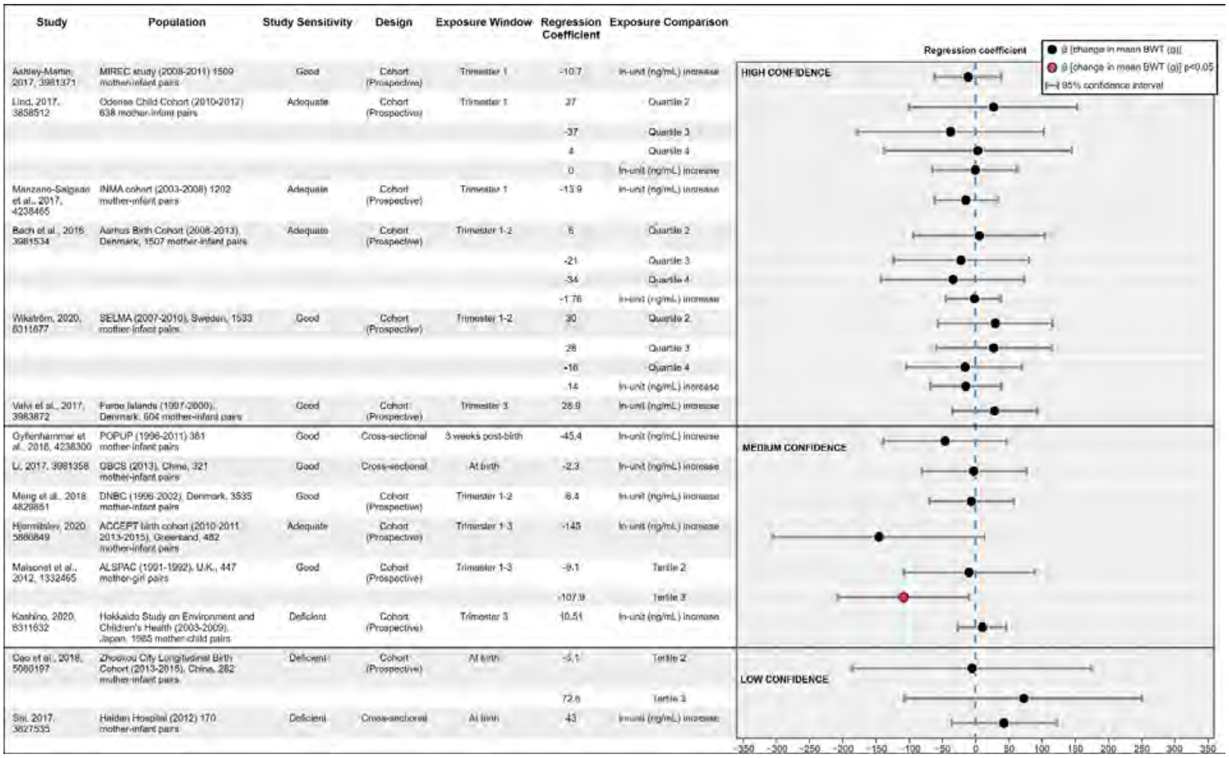


Figure 3-24. Sex-specific female infants only mean birth weight results for 14 PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: BWT= Birth Weight
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.
[c]Gyllenhammar et al. (2018) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort compared to a smaller sample size of 381 in their 2018 publication.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Birth Weight - Standardized – Background

Twelve of thirteen studies in the overall population that reported a continuous standardized birth weight scores in relation to different PFHxS measures (see Figures 3-25 and 3-26), while the Gardener et al. (2021) study not included on the forest plot examined odds of being in the lowest standardized birthweight category (vs. the top 3 birth weight z-score quartiles). Four of the 13 studies also reported sex-specific results (Eick et al., 2020; Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019), while Gardener et al. (2021) only examined interactions across sex for associations between PFHxS and standardized birth weight measures.

Among the 13 studies that examined PFHxS exposure in relation to standardized birth weight scores in the overall population, eight were *high* (Gardener et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016), three were *medium* (Gyllenhammar et al., 2018; Meng et al., 2018; Hamm et al., 2010) and two were *low* (Gross et al., 2020; Workman et al., 2019) confidence. Six studies had good (Wikström et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017) study sensitivity ratings, while five were adequate (Gardener et al., 2021; Eick et al., 2020; Xiao et al., 2019; Bach et al., 2016; Hamm et al., 2010) and two were deficient (Gross et al., 2020; Workman et al., 2019).

Birth Weight - Standardized – Study Results

Null associations between PFHxS exposure and standardized birth weight scores were reported in six studies (Wikström et al., 2020; Workman et al., 2019; Gyllenhammar et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Hamm et al., 2010) (see Figures 3-25 and 3-26). Similar to results from categorical and continuous exposures in Wikström et al. (2020) and Sagiv et al. (2018), birth weight z-score results were largely null in relation to PFHxS tertiles in the *high* confidence Eick et al. (2020) study in the overall population and across the sexes. They did report larger birth weight z-scores in the overall population for tertile 3 (β= 0.15; 95% CI: −0.12, 0.42 compared to tertile 1) that appeared to be driven primarily by results in females (β= 0.22; 95%CI: −0.18, 0.63). The *high* confidence study by Gardener et al. (2021) detected non-significant increased odds for their lowest standardized birthweight category (vs. the top three birth weight z-score quartiles) across PFHxS quartiles (Q3: OR= 1.70; 95%CI: 0.81, 3.74); Q4: OR= 1.20; 95%CI: 0.55, 2.62). They also found no statistically significant interactions for their birth weight z-score measures by sex.

Although their continuous exposure results were null per each ln-unit PFHxS increase, the *high* confidence study by Bach et al. (2016) reported a small decrease in standardized birth weight scores (β= −0.11; 95%CI: −0.25, 0.03) in PFHxS quartile 4 compared to quartile 1. Similar results were seen for both tertiles 2 and 3 only (β range: −0.12 to −0.13) in the *high* confidence Shoaff et al. (2018) study. Statistically significant results similar in magnitude were detected in the *medium* confidence Meng et al. (2018) study (β= −0.14; 95%CI: −0.22, −0.07 per each ln-unit PFHxS increase). Larger statistically significant lower birth weight z-scores results were reported in the *low* confidence study by Gross et al. (2020) for the overall population (β= −0.65; 95%CI: −0.99, −0.39), males (β= −0.60; 95%CI: −1.14, −0.06) and females (β= −0.77; 95%CI: −1.25, −0.29) for PFHxS levels greater than the mean level of dried-blood spot samples. Associations large in magnitude per each ln-unit increase were also detected in the *high* confidence study by Xiao et al. (2019) for the overall population (β= −0.74; 95% CI: −1.23, −0.26), male neonates (β= −0.62; 95% CI: −1.28, 0.06), and female neonates (β= −0.87; 95% CI: −1.50, −0.22).

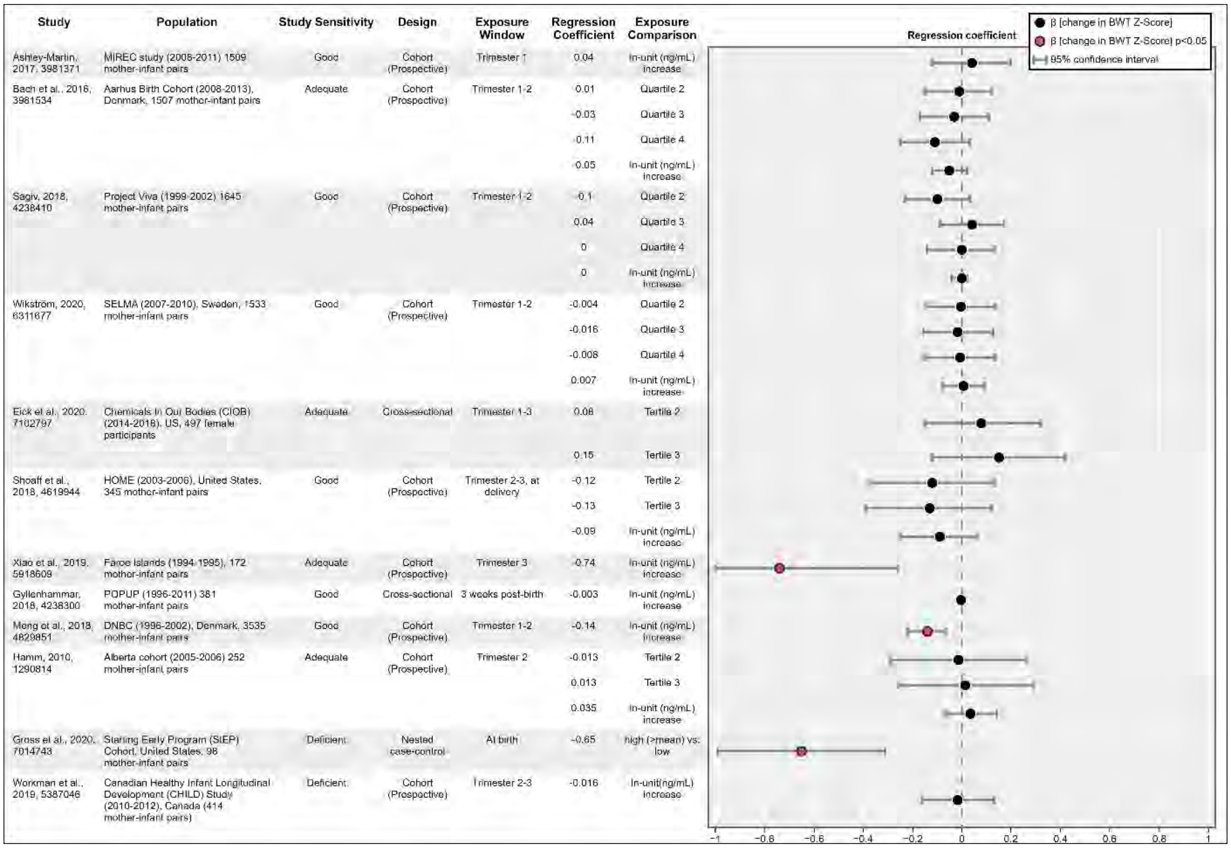


Figure 3-25. Overall population standardized birth weight results for 12 epidemiologic studies. For additional details see HAWC link.

Abbreviations: BWT= Birth Weight

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b](Xiao et al., 2019) results are truncated: the complete 95% CI ranges from -1.23 to -0.26 grams.

[c]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Study	Population	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	Good	Cohort (Prospective)	Trimester 1-2	-0.081	Quartile 2
					-0.083	Quartile 3
					0.016	Quartile 4
					-0.017	ln-unit (ng/mL) increase
Sagiv, 2018, 4238410	Project Viva (1999-2002) 1645 mother-infant pairs	Good	Cohort (Prospective)	Trimester 1-2	-0.01	ln-unit (ng/mL) increase
Eick et al., 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	Adequate	Cross-sectional	Trimester 1-3	0.14	Tertile 2
						Tertile 3
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), 172 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-0.62	ln-unit (ng/mL) increase
Gross et al., 2020, 7014743	Starting Early Program (StEP) Cohort, United States, 98 mother-infant pairs	Deficient	Nested case-control	At birth	-0.6	high (>mean) vs. low
Wikström, 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	Good	Cohort (Prospective)	Trimester 1-2	0.071	Quartile 2
					0.062	Quartile 3
					-0.043	Quartile 4
					0.031	ln-unit (ng/mL) increase
Sagiv, 2018, 4238410	Project Viva (1999-2002) 1645 mother-infant pairs	Good	Cohort (Prospective)	Trimester 1-2	0.04	ln-unit (ng/mL) increase
Eick et al., 2020, 7102797	Chemicals In Our Bodies (CIOB) (2014-2018), US, 497 female participants	Adequate	Cross-sectional	Trimester 1-3	0.06	Tertile 2
					0.22	Tertile 3
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), 172 mother-infant pairs	Adequate	Cohort (Prospective)	Trimester 3	-0.87	ln-unit (ng/mL) increase
Gross et al., 2020, 7014743	Starting Early Program (StEP) Cohort, United States, 98 mother-infant pairs	Deficient	Nested case-control	At birth	-0.77	high (>mean) vs. low

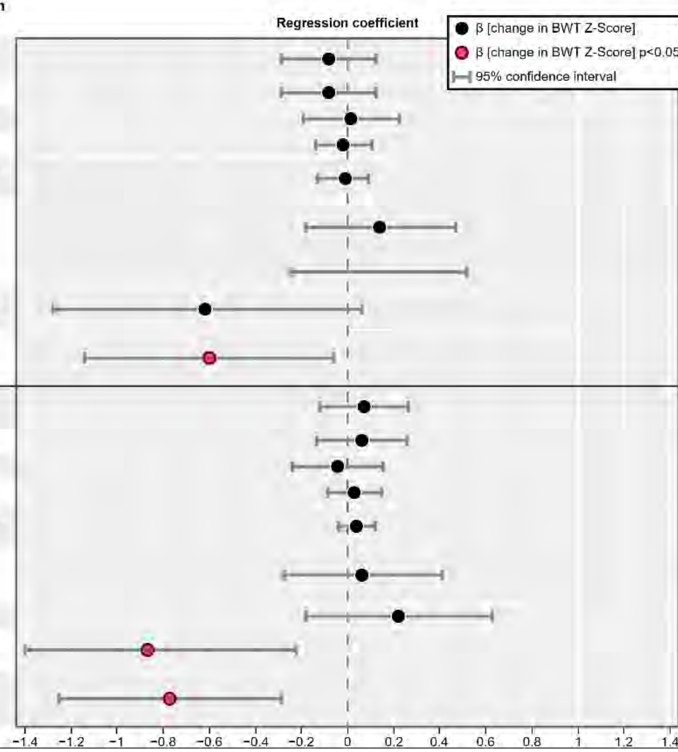


Figure 3-26. Sex stratified standardized birth weight results for 5 epidemiologic studies (boys above reference line, girls below). For additional details see HAWC link.

Abbreviations: BWT= Birth Weight
[a]Studies are sorted first by overall study confidence level, then by Exposure Window(s) examined.
[b](Xiao et al., 2019) results are truncated: the complete 95% CI ranges from -1.5 to -0.22.
[c]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

*Birth Weight – Summary of Differe*nt Measures and Analyses

Six of 13 studies showed some evidence of inverse associations between PFHxS and standardized birth weight measures in the overall population. Among the 12 studies examining continuous birth weight measures in the overall population, 3 showed some associations of at least -0.1 in relation to either categorical or continuous PFHxS exposures. Two other studies (1 high and 1 low confidence) showed stronger associations in excess of −0.74 as well as comparable results in both sexes. The high confidence study by Gardener et al. (2021) also reported non-significant odds of being in the lowest standardized birthweight category (vs. the top 3 BWT z-score quartiles) based on PFHxS quartiles 3 (OR range: 1.20 to 1.74). There was limited evidence of exposure-response relationships in support of the continuous study results expressed per a unit change. Few patterns and minimal differences were seen across sexes. Among the six studies in the overall population that showed some suggestion of inverse associations, two studies (1 high and 1 low confidence) reported large associations consistent in magnitude for both male and female neonates. Study sensitivity did also not seem to explain null study findings as four of these six studies had good ratings in this domain. There was a slight preponderance of inverse associations with four of the six studies using later biomarker samples.

Overall, 17 of the 31 epidemiological studies with mean birth weight in either/both sex or the overall population detected some deficits in relation to PFHxS exposures (see Table 3-17), although these deficits were at times limited to sex-specific findings (Marks et al., 2019a; Lind et al., 2017; Maisonet et al., 2012) and often were not statistically significant (see Figures 3-20, 3-21, 3-23, and 3-24). This included 14 (4 low and 5 each medium and high confidence) of the 27 studies in the overall population. Two different studies (out of 14) with categorical data in the overall population or either sex showed some evidence of exposure-response relationships. Overall, the magnitude of changes in those studies showing deficits ranged from −25 to −109 grams for the highest quantile (compared to the lowest quantile). Those results were consistent in magnitude with 12 studies with continuous exposure metrics data showing birth weight-related deficits with increasing exposures in the overall population (β ranging from –12 to –93 grams per each unit change in PFHxS). Seven of these ranged from −12 to −30 grams, and the remaining five ranged from −53 to −93 grams. These data were supported by an EPA meta-analysis that showed also showed a small birth weight deficit (β= −7.7 g; 95% CI: −14.8, −0.5) per each ln-unit PFHxS among all 27 studies and were consistent in magnitude (β range: −7 to −10 g) across 12 high confidence studies, 11 medium confidence studies, and the combined high and medium studies. Although deficits were largest among post-partum samples, the results among the 12 early samples studies were comparable (β= −7.3 g; 95% CI: −16.0, 1.4) to that seen in the overall population of all studies. Although deficits were largest among post-partum samples, the results among the 12 early sampled studies were comparable (β= −7.3 g; 95% CI: −16.0, 1.4) to that seen in the overall population of all 27 studies.

Limited patterns were evident in the mean birth weight findings as overall confidence, study sensitivity, exposure levels and other study design elements were not explanatory for the null or inverse associations. The mean birth weight differences in the overall population may be influenced by hemodynamic changes during pregnancy, as only ten of the fourteen were based on late biomarker sampling. Similar to that seen for standardized birth measures, the sex-specific data were more mixed in relation to sample timing as four of six studies showing birth weight deficits were based on late biomarker collection.

Birth Length – Background of Studies

Nineteen studies examined the relationship between PFHxS exposures and birth length in the overall population or across sexes; one study (Alkhalawi et al., 2016) was classified as uninformative and is not discussed here (see Figure 3-27). Two of the 10 studies reporting sex-specific findings did not report overall population results; both studies were from the ALSPAC population, including a study in boys (Marks et al., 2019a) and girls (Maisonet et al., 2012). Two studies (Xiao et al., 2019; Gyllenhammar et al., 2018) reported standardized birth length measures, while the remaining studies examined mean birth length differences in relation to PFHxS. As noted above, two studies (Bjerregaard-Olesen et al., 2019; Bach et al., 2016) from the Aarhus birth cohort are discussed when discrepancies arise or in isolation as for some sex-specific findings. They are both listed together below in the background materials just below, but only counted as one study when evaluating consistency and between-study heterogeneity patterns.

Six of the 18 included PFHxS studies examining birth length studies were classified as *high* (Luo et al., 2021; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Bach et al., 2016), and five were *medium* (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018; Maisonet et al., 2012) confidence. Seven of birth length studies were classified as *low* confidence (Gao et al., 2019; Marks et al., 2019a; Workman et al., 2019; Xu et al., 2019; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016) largely due to concerns with participant, selection, confounding, and study sensitivity. For example, seven of those studies were considered deficient for study sensitivity (Kashino et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016). Five studies were rated good (Luo et al., 2021; Marks et al., 2019a; Gyllenhammar et al., 2018; Valvi et al., 2017; Maisonet et al., 2012) and six were adequate (Chen et al., 2021; Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016).

Birth length-Overall Population Results

Nine of the 16 studies in the overall population reported shorter birth length in relation to PFHxS exposure (see Figure 3-28; Table 3-17). Five of the six *high* confidence studies observed that PFHxS exposure was associated with shorter birth length in at least one comparison set, including statistically significant changes in three high confidence studies examining mean (Buck Louis et al., 2018; Manzano-Salgado et al., 2017a) or standardized birth length measures (Xiao et al., 2019). For example, Xiao et al. (2019) reported smaller birth length z-scores in overall population (β= −0.52; 95% CI: −1.04, −0.13 each ln-unit increase). The Manzano-Salgado et al. (2017a) study reported birth length reductions consistent in magnitude across all three PFHxS quartiles (β range: −0.31 to −0.33 cm), although results were largely null for each ln-unit increase (β= −0.09; 95%CI: −0.25, 0.09). The study by Valvi et al. (2017) reported small deficits in mean birth length in the overall population (β= −0.14 cm; 95% CI: −0.35, 0.04). Based on a ln-unit PFHxS increase, null results were reported in the Bach et al. (2016) study, and their smaller subset analysis (n = 671 participants) reported in Bjerregaard-Olesen et al. (2019) (the latter data are not plotted given from same cohort). The Bach et al. (2016) study based on 1,507 participants did report decreased birth length in the third (β= −0.1 cm; 95% CI: −0.5, 0.3) and fourth (β= −0.2 cm; 95% CI: −0.5, 0.2) quartiles compared with the lowest quartile (not included on Figure Y given overlapping population). The study by Buck Louis et al. (2018) reported that PFHxS was associated with reductions in birth length (and upper thigh length; the latter data not shown) in the overall population (β= −0.22 cm; 95% CI: −0.39, −0.05 per each ln-unit increase), as well as Black (β= −0.43 cm; 95% CI: −0.71, −0.14) and Hispanic neonates (β= −0.34 cm; 95% CI: −0.70, 0.03).

Three out of four *medium* confidence studies in the overall population were null for birth length deficits in relation to PFHxS exposures. The Chen et al. (2021) study reported a small deficit (β= −0.15 cm; 95% CI: −0.42, 0.11) per each ln-unit increase and non-monotonic consistent deficits across quartiles (β range: −0.33 to −0.46 cm). Three out of five *low* confidence studies reported some suggestion of birth length deficits in relation to PFHxS. Although results were null for tertile 3 relative to tertile 1, the low confidence study by Cao et al. (2018) reported a statistically significant result (β= −0.33 cm; 95% CI: −0.68, −0.01) for tertile 2. Compared to tertile 1, the *low* confidence study by Gao et al. (2019) reported a statistically significant result (β= −0.43 cm; 95% CI: −0.78, −0.07) for tertile 2 but a smaller deficit in tertile 3 (β= −0.20 cm; 95% CI: −0.64, 0.25). Callan et al. (2016) reported an imprecise deficit of −0.20 cm (95% CI: −0.78, 0.38) per each ln-unit increase. In contrast, Xu et al. (2019) reported a large increased birth (β= 0.66 cm; 95% CI: −0.01, 1.26 per each ln-unit increase).

Overall, 9 (5 *high*, 1 *medium*, and 3 *low* confidence) out of 16 studies in the overall population provided some evidence of birth length deficits with increasing PFHxS exposure. Some of these results were not always internally consistent across different exposure expressions (continuous vs. categorical). The five studies with categorical data in the overall population did not provide any evidence of any exposure-response relationships. Although mean birth length results for continuous PFHxS exposures were smaller, two of the three studies with PFHxS quartiles showed deficits similar in magnitude (β= −0.31 to −0.46 cm). There was a consistent pattern by sample timing among those studies demonstrating birth length deficits in the overall population, as six of the nine studies were based on late biomarker sampling. No other patterns by study characteristics were evident.

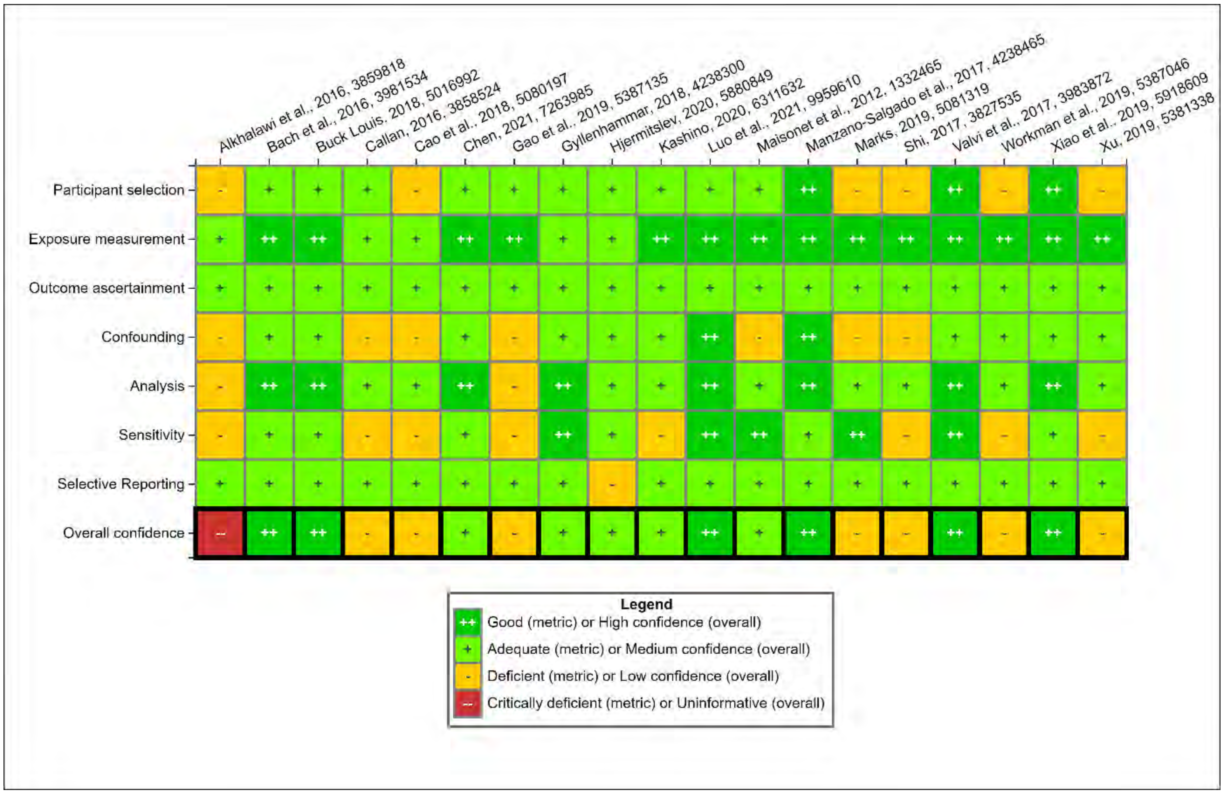


Figure 3-27. Study evaluation results for 19 epidemiological studies of birth length and PFHxS. For additional details see HAWC link.

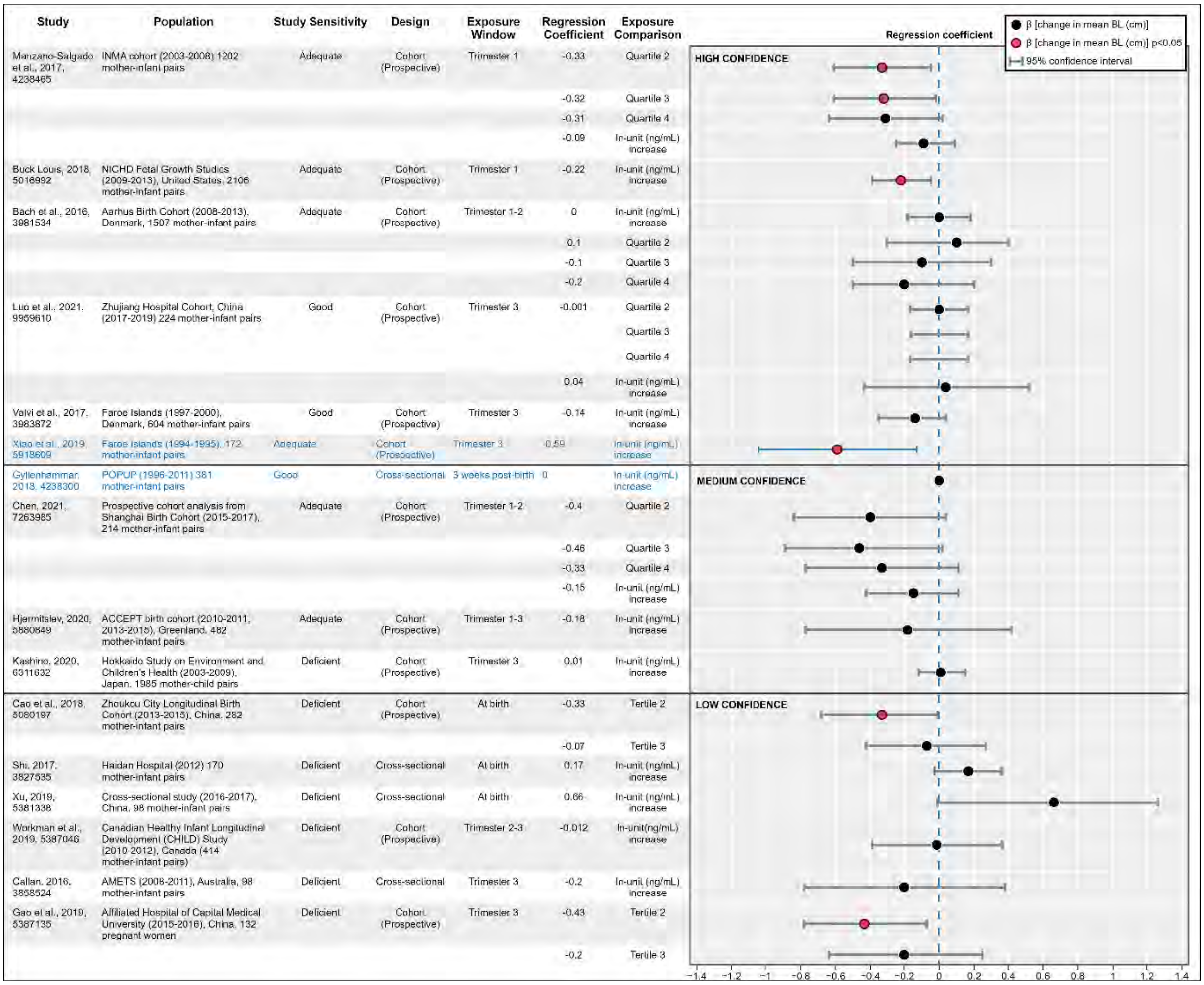


Figure 3-28. Overall population mean birth length results for 16 PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: BL= Birth Length
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b](Xiao et al., 2019) and (Gyllenhammar et al., 2018) in blue text report birth length z-score data.
[c]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Birth Length-Sex-Specific Results

Among these 11 studies with results in either boys, girls or both, some birth length deficits were detected in 7 different studies (see Figure 3-29). The *high* confidence study by Xiao et al. (2019) reported deficits in both sexes including larger and statistically significant birth length z-scores among girls (β= −0.72; 95% CI: −1.33, −0.12 each ln-unit increase). Sex-specific results were null based in both sexes based on continuous (per each ln-unit increase) data in the Manzano-Salgado et al. (2017a) and Kashino et al. (2020) studies. Four of the remaining six studies in females were null (Chen et al., 2021; Bjerregaard-Olesen et al., 2019; Cao et al., 2018; Shi et al., 2017). The *medium* confidence Maisonet et al. (2012) study of girls only reported dose-dependent statistically significant associations across exposure tertiles (β range: −0.52 to −0.82). The *medium* confidence Hjermitslev et al. (2020) study reported deficits among female neonates only (β= −0.42 cm; 95% CI: −1.07, 0.22 per each ln-unit increase).

The *medium* confidence Chen et al. (2021) study reported a small birth length deficit (β= −0.15 cm; 95% CI: −0.61, 0.31) per each ln-unit increase in boys only. The high confidence study by Valvi et al. (2017) reported deficits among male neonates only (β= −0.22 cm; 95% CI: −0.49, 0.04 per each ln-unit increase). The *low* confidence study by Cao et al. (2018) detected non-monotonic reductions in birth length across tertiles (β range: −0.18 to −0.44) in boys, while another *low* confidence study of boys only (Marks et al., 2019a) detected evidence of an exposure-response relationship across PFHxS tertiles (β range: −0.25 to −0.39). In contrast, increased birth length (β range: 0.20 to 0.40 cm per ln-unit PFHxS increase) was detected in males in three studies (Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019; Shi et al., 2017).

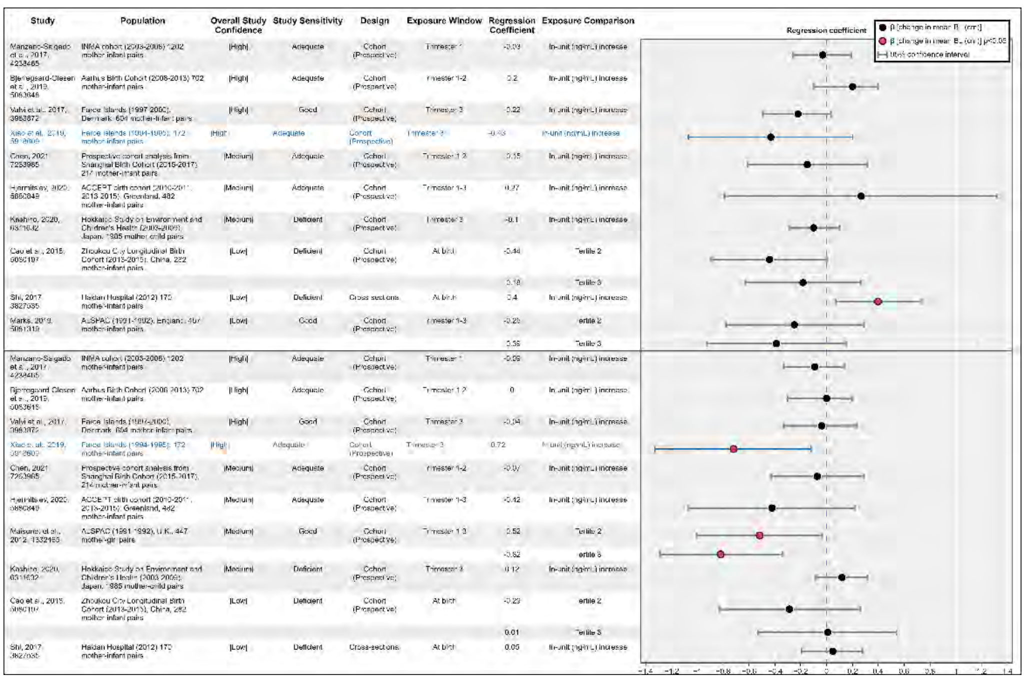


Figure 3-29. Sex stratified birth length results for 11 epidemiologic studies (boys above reference line, girls below). For additional details see HAWC link.

Abbreviations: BL= Birth Length.
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Xiao et al. (2019) in blue text reports birth length z-score data.
[c]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Summary-Birth Length-Sex-Specific

Stronger evidence of birth length deficits was observed in males (5 of 10 studies) compared to females (3 of 10 studies); however, these deficits were generally smaller in magnitude among males (β range: −0.15 to −0.39 cm) than females (β range: −0.42 to −0.82 cm). In addition to the two null studies in males, three other studies reported increased birth length in relation to PFHxS exposures. Two of the three studies with categorical data provided evidence of an inverse exposure-response relationships, albeit only in males (Marks et al., 2019a) and females (Maisonet et al., 2012) derived from the same ALPSAC study population.

Exposure levels were higher in the studies reporting birth length deficits in males, including the top four and five of the top six highest exposure measures of centrality reported. Besides this and the slightly more consistent results in males in general, no other patterns across study characteristics explained the between-study heterogeneity including the null results. For example, there was no definitive pattern of results by study confidence across the seven different studies (two *high*, three *medium,* and two *low* confidence) nor sample timing (four had early biomarker samples compared to three with late).

Summary-Birth Length

Overall, 12 out of 18 included studies provided some evidence of birth length deficits with increasing PFHxS exposure in either the overall population or either sex. Some of these results were not always internally consistent across different exposure expressions (continuous vs. categorical). Two of the seven studies with categorical data provided some evidence of any exposure-response relationships, both of these were from sex-specific studies in the same cohort. There was no pattern among the null studies based on study sensitivity or other study characteristics. Mean and median exposure levels were higher among the male studies showing deficits, but this did not appear to explain results in females or the overall population. There was not a consistent pattern by sample timing among the studies showing inverse associations in either/both sex (four of seven had early sampling) or the overall population (three of nine had early sampling). Among the 11 different studies demonstrating birth length deficits, six of them relied on early sampling suggesting limited overall potential impact of pregnancy hemodynamics.

Head Circumference at Birth – Study Background

Fourteen studies examined PFHxS in relation to head circumference measured at birth including two studies (Xiao et al., 2019; Gyllenhammar et al., 2018) reporting standardized head circumference measures (see Figure 3-30). Among the other 12 studies, 10 (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Workman et al., 2019; Xu et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Valvi et al., 2017); Bach et al. (2016); (Callan et al., 2016) of these studies reported data in the overall population. Eight studies analyzed sex-specific results include two studies (Marks et al., 2019a; Lind et al., 2017) that only reported these data.

Four studies were classified as *low* confidence (Marks et al., 2019a; Workman et al., 2019; Xu et al., 2019; Callan et al., 2016) and five each were *medium* (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018; Lind et al., 2017) and *high* (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Valvi et al., 2017); Bach et al. (2016). Seven of the 14 PFHxS studies on head circumference had adequate study sensitivity (Chen et al., 2021; Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a), while four were deficient (Kashino et al., 2020; Workman et al., 2019; Xu et al., 2019; Callan et al., 2016) and three had good study sensitivity (Marks et al., 2019a; Gyllenhammar et al., 2018; Valvi et al., 2017).

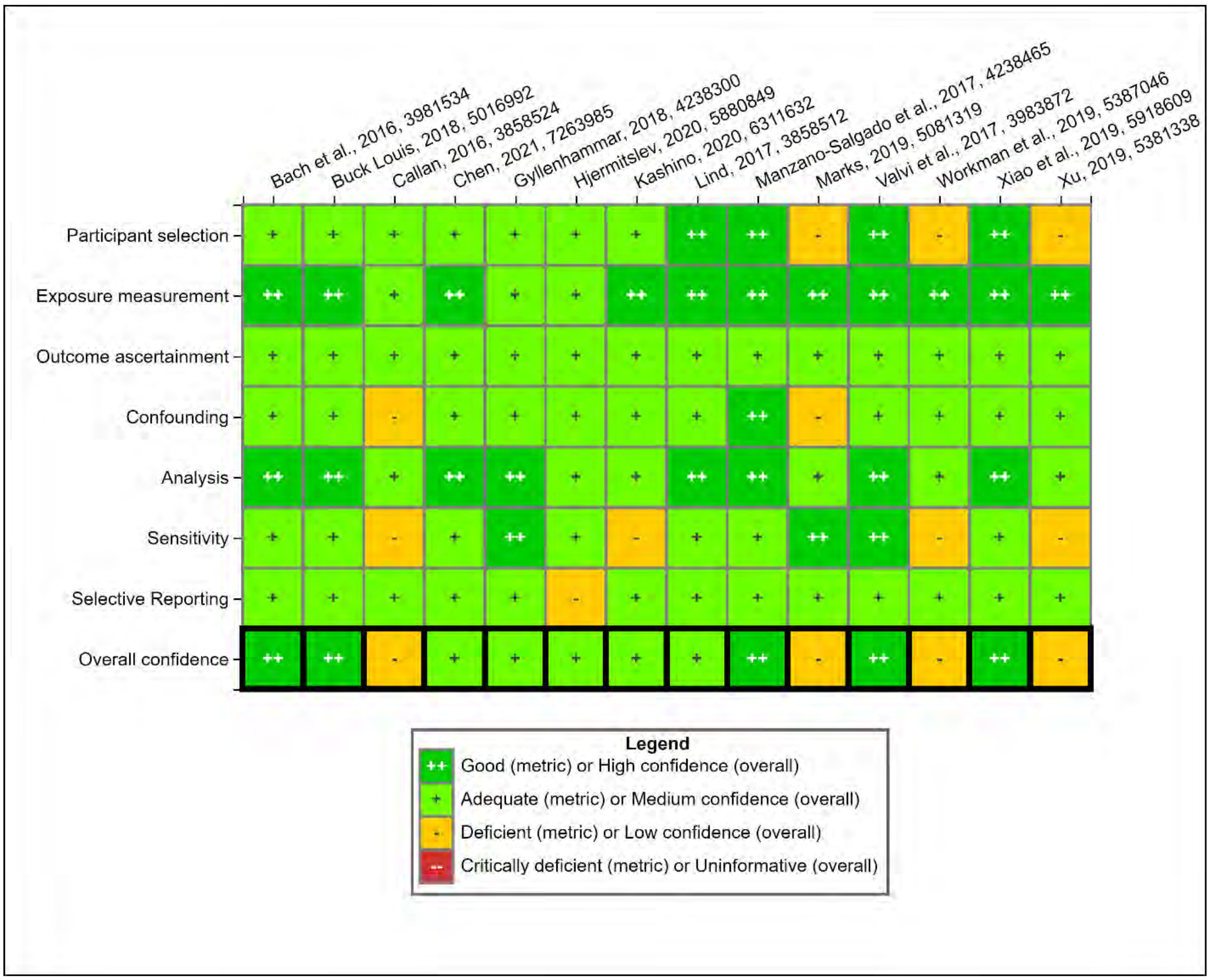


Figure 3-30. Study evaluation results for 14 epidemiological studies of head circumference and PFHxS. For additional details see HAWC link.

Head Circumference at Birth – Overall Population Results

Seven out of the 12 studies in the overall population reported some evidence of reduced mean or standardized head circumference at birth with increasing PFHxS exposures including four of five *high* confidence studies, two of four *medium* and one of three *low* confidence studies (see Figure 3-31). Three studies detected null associations (Kashino et al., 2020; Xu et al., 2019; Gyllenhammar et al., 2018). Two studies reported small increases in head circumference per each ln-unit increase including the *high* confidence Valvi et al. (2017) study (β= 0.16 cm; 95% CI: 0.01, 0.29) and the *low* confidence Workman et al. (2019) study (β= 0.12 cm; 95% CI: −0.18, 0.42).

The *high* confidence Xiao et al. (2019) study reported lower head circumference z-scores in the overall population (β= −0.52; 95% CI: −1.04, 0.00 per each PFHxS ln-unit increase). The *high* confidence study by Bach et al. (2016) detected consistent deficits across quartiles two through four (all βetas were −0.2 cm), but they reported null findings based on the continuous PFHxS measure as well as in their smaller subset in a separate publication (Bjerregaard-Olesen et al., 2019) (the latter data are not plotted given from same cohort). Similarly, the *high* confidence study by Manzano-Salgado et al. (2017a) showed some evidence of an exposure-response relationship across the PFHxS quartiles (β range: −0.08 to −0.16) but not among the continuous exposure results (β= −0.01 cm; 95% CI: −0.13, 0.10). The *high* confidence study by Buck Louis et al. (2018) reported a precise but small deficit in the overall population (β= −0.09 cm; 95%CI: −0.19, 0) and saw a statistically significant reduction in head circumference for Black (β= −0.25 cm; 95% CI: −0.41, −0.08) neonates per each ln-unit increase in PFHxS. Two *medium* confidence studies detected an imprecise head circumference difference of −0.14 cm per each ln-unit PFHxS increase including Hjermitslev et al. (2020) (95%CI: −0.52, 0.25) and Chen et al. (2021) (95%CI: −0.46, 0.19). A larger difference was detected in the *low* confidence Callan et al. (2016) study (β= −0.31 cm; 95% CI: −0.74, 0.12 per each ln-unit PFHxS increase).

Overall, 7 of 12 studies showed some evidence of associations between PFHxS and different head circumference measures in the overall population. Some of these results were not always internally consistent across different exposure expressions (continuous vs. categorical). One of two studies with categorical data showed some evidence of an exposure-response relationship across quartiles. There was no clear pattern in study characteristics among the null studies, although two of the four had deficient study sensitivity. Five of the seven studies were based on early biomarker samples, so pregnancy hemodynamics did not appear to explain the study findings.

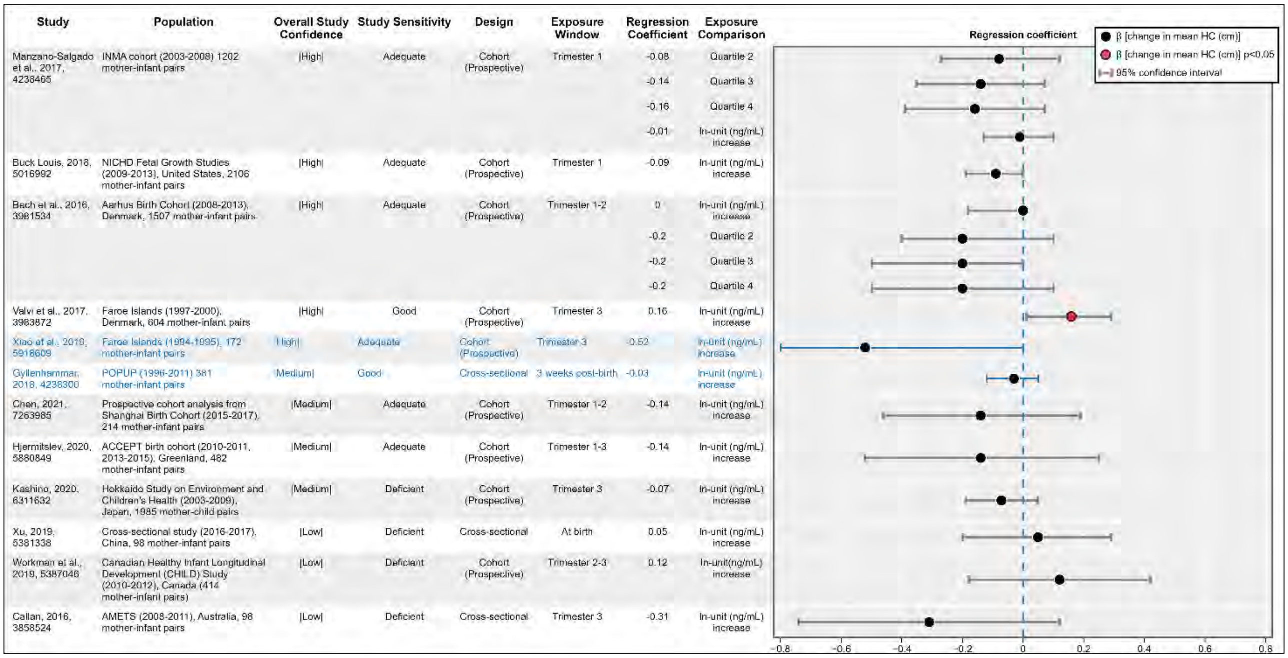


Figure 3-31. Overall population head circumference results for 12 epidemiologic studies. For additional details see HAWC link.

Abbreviations: HC= Head Circumference
[a]Studies are sorted first by overall study confidence level, then by Exposure Window(s) examined.
[b]Xiao et al. (2019) and Gyllenhammar et al. (2018) in blue text report head circumference z-score data.
[c]Xiao et al. (2019) results are truncated: the complete 95% CI ranges from -1.04 to 0.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Head circumference at birth - Sex and Race-specific Results

Eight studies examined PFHxS and head circumference differences among sexes (see Figure 3-32). Two *high* confidence studies were null in both sexes (Bjerregaard-Olesen et al., 2019; Manzano-Salgado et al., 2017a) and only one study (Xiao et al., 2019) showed inverse associations in both sexes. Four of eight studies were null in boys, and one showed larger head circumference differences with increasing PFHxS exposures. Five studies were null in girls and two studies showed inverse associations between head circumference differences and PFHxS exposures.

Three of eight studies in boys and two of seven studies in girls reported associations with PFHxS. The *high* confidence study by Xiao et al. (2019) reported smaller head circumference z-scores with larger results in female (β= −0.76; 95% CI: −0.19, 0.23 per each ln-unit increase) compared to male (β= −0.26; 95% CI: −0.46, 0.07 per each ln-unit increase) neonates. All of the other studies examined mean head circumference differences in relation to PFHxS. For example, the medium confidence study by Hjermitslev et al. (2020) showed head circumference differences among females only (β= −0.26; 95% CI: -0.73, 0.20 per each ln-unit increase). Among boys, the *medium* confidence study by Kashino et al. (2020) reported head circumference differences smaller in magnitude relation to PFHxS (β= −0.14 cm; 95%CI: −0.29, 0.02 per each ln-unit PFHxS increase), as did the *medium* confidence study by Lind et al. (2017) (β= −0.1 cm; 95%CI: −0.4, 0.2 per each ln-unit PFHxS increase). The Lind et al. (2017) study showed non-monotonic head circumference deficits across exposure categories (β range: −0.1 to −0.7 cm), including one that was statistically significant for PFHxS quartile 3 (β= −0.7 cm; 95% CI: −1.2, −0.2).

Overall, four (1 *high*; 3 *medium* confidence) of eight studies showed some evidence of associations between PFHxS and different head circumference measures among either or both sexes (including three of eight studies in boys and two of seven studies in girls). No study characteristics (i.e. study design features or study quality domains) appeared to explain between-study heterogeneity of results including sample timing, as half of the studies reporting inverse association were based on early biomarker samples.

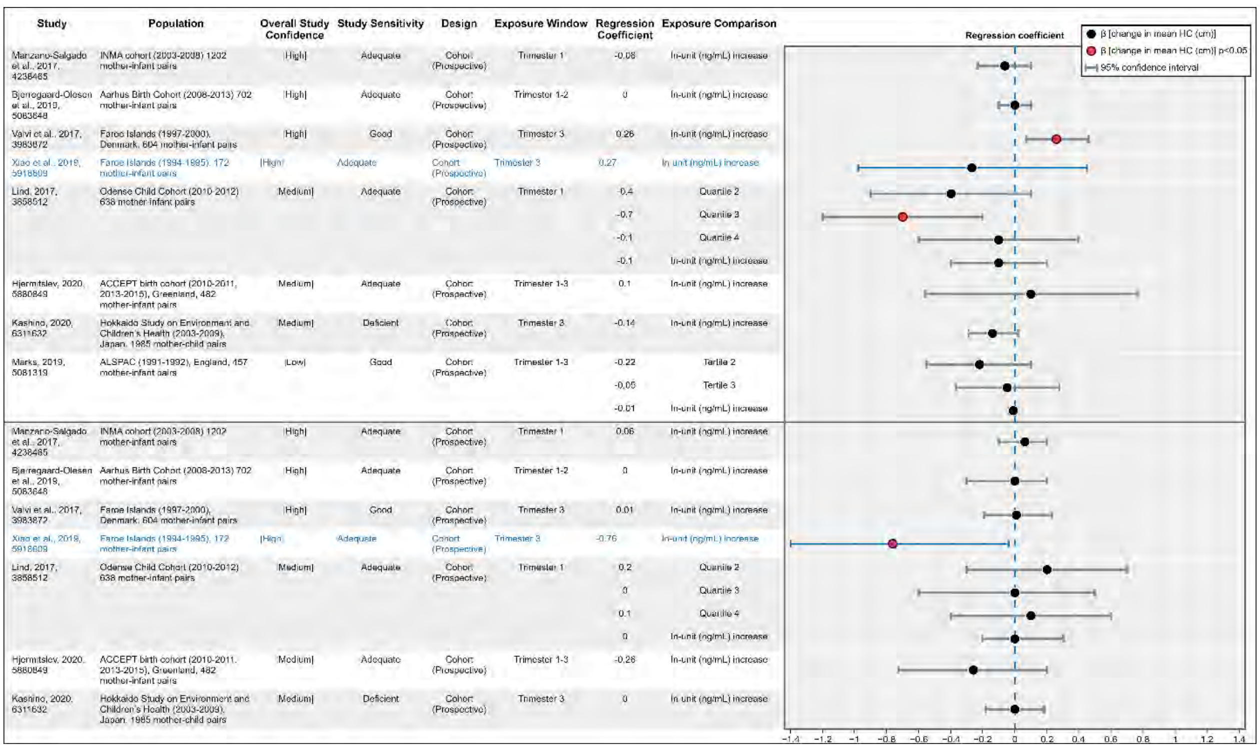


Figure 3-32. Sex stratified head circumference results for 8 epidemiologic studies (boys above reference line, girls below). For additional details see HAWC link.

Abbreviations: HC= Head Circumference
[a]Studies are sorted first by overall study confidence level, then by Exposure Window(s) examined.
[b]Xiao et al. (2019) in blue text report head circumference z-score data.
[c]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Head Circumference Summary

Overall, 8 of 14 total studies showed some head circumference deficits in either sex or in the overall population in relation to PFHxS exposures. There was fairly consistent evidence of associations in the overall population as 6 out of 12 studies (including five of the nine *high* and *medium* confidence studies) reported some evidence of deficits for at least one exposure comparison. Overall, one of the three studies with categorical data showed evidence of an exposure-response relationship in either sex or in the overall population. There was no pattern among the null studies based on study sensitivity and exposure levels/contrasts. There was not a consistent pattern by sample timing among those studies demonstrating head circumference deficits, as half other studies in both the overall population and sex-specific analyses that were based on late biomarker sampling.

Small for Gestational Age and Low Birth Weight

Seven epidemiological studies included here examined associations between PFHxS exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints) (Chang et al., 2022; Wikström et al., 2020; Xu et al., 2019; Hamm et al., 2010) or low birth weight (LBW) (Hjermitslev et al., 2020; Meng et al., 2018; Manzano-Salgado et al., 2017a) (see Figure 3-33). Two studies were *high* confidence (Wikström et al., 2020; Manzano-Salgado et al., 2017a), three were *medium* confidence (Hjermitslev et al., 2020); Meng et al. (2018); (Hamm et al., 2010) and two were *low* confidence (Chang et al., 2022; Xu et al., 2019). Two of these studies had good study sensitivity (Wikström et al., 2020; Manzano-Salgado et al., 2017a), four had adequate study sensitivity (Chang et al., 2022; Hjermitslev et al., 2020; Wikström et al., 2020; Manzano-Salgado et al., 2017a) while one was deficient (Xu et al., 2019). All seven studies reported results in the overall population, while two (Wikström et al., 2020; Manzano-Salgado et al., 2017a) provided results in both the overall population and across sexes.

Three (Wikström et al., 2020; Xu et al., 2019; Hamm et al., 2010) of four SGA studies showed some adverse associations (see Figure 3-34) in relation to PFHxS. The *medium* confidence study by Hamm et al. (2010) showed increased odds (OR=2.35; 95%CI: 0.63, 8.72) in the overall population among tertile 3 compared to tertile 1. The *low* confidence by Xu et al. (2019) reported showed an even larger statistically significant odds of SGA (OR=9.14; 95%CI: 1.15, 72.8 per each ln-unit increase). Although their overall population results were null, some of the quartile results were elevated (OR=1.76; 95%CI: 0.79, 3.90) but in a non-monotonic fashion. Their results based on a ln-unit increase were largely null for both sexes. In addition to the Wikström et al. (2020) study, two other studies in the overall population were null (Chang et al., 2022; Hjermitslev et al., 2020). The Manzano-Salgado et al. (2017a) study was null for the overall population, girls, and boys.

Two studies reported largely null results between PFHxS and LBW in the overall population (Hjermitslev et al., 2020; Manzano-Salgado et al., 2017a) as did the *medium* confidence study by Meng et al. (2018) based on their quartile comparisons. Based on the continuous exposure expressions, Meng et al. (2018) reported a larger risk (OR=1.5; 95%CI: 0.7, 2.9 per each ln-unit increase) for a very LBW (i.e., <2,260 grams) measure compared to the typical LBW definition of <2,500 grams (OR=1.3; 95%CI: 0.8, 2.1). Although term LBW results were null in girls in the Manzano-Salgado et al. (2017a) study, non-significant increases were seen amongst boys (OR=1.33; 95%CI: 0.47, 3.82 per ln-unit increases).

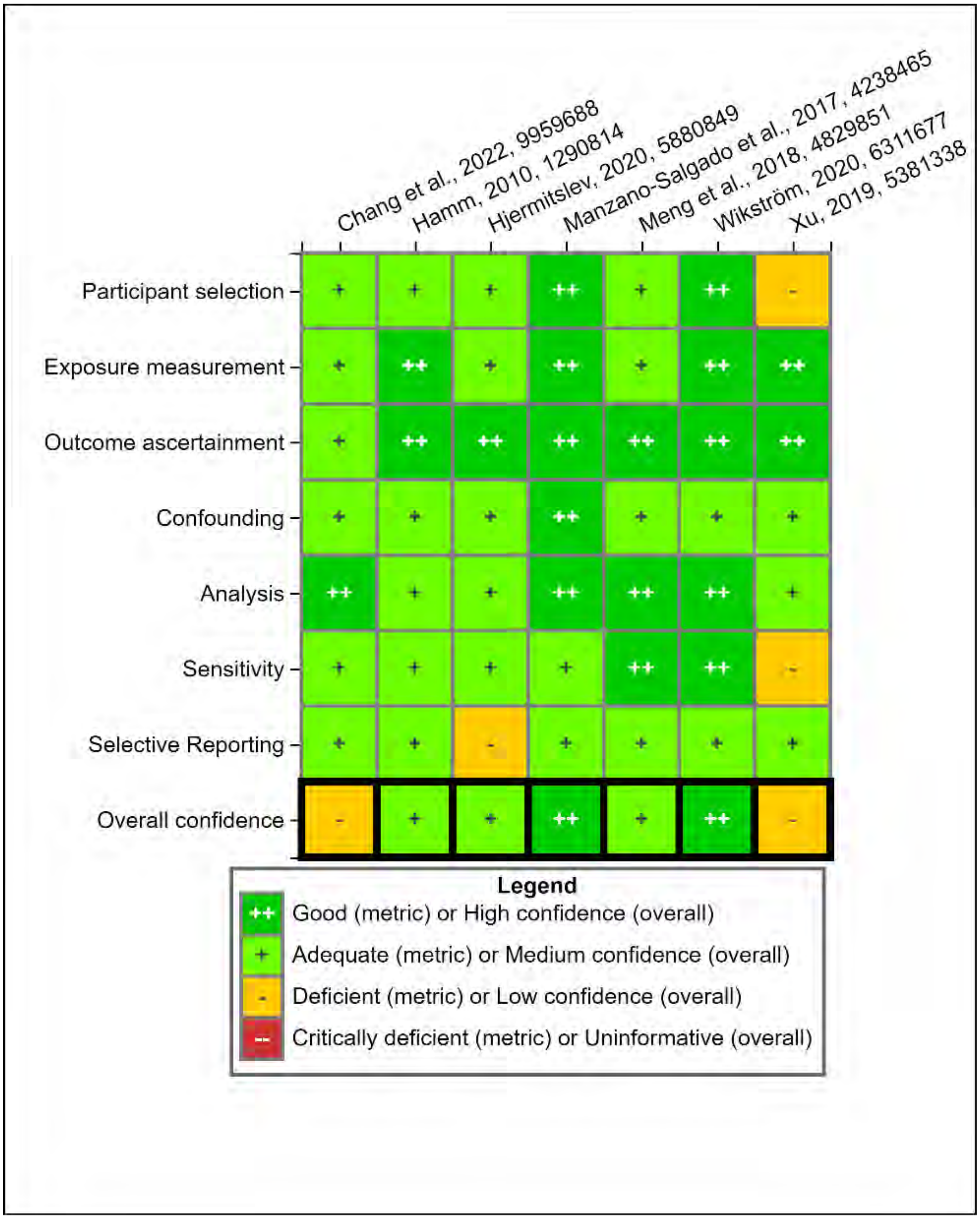


Figure 3-33. Study evaluation results for 7 epidemiological studies of small for gestational age and low birth weight and PFHxS. For additional details see HAWC link.

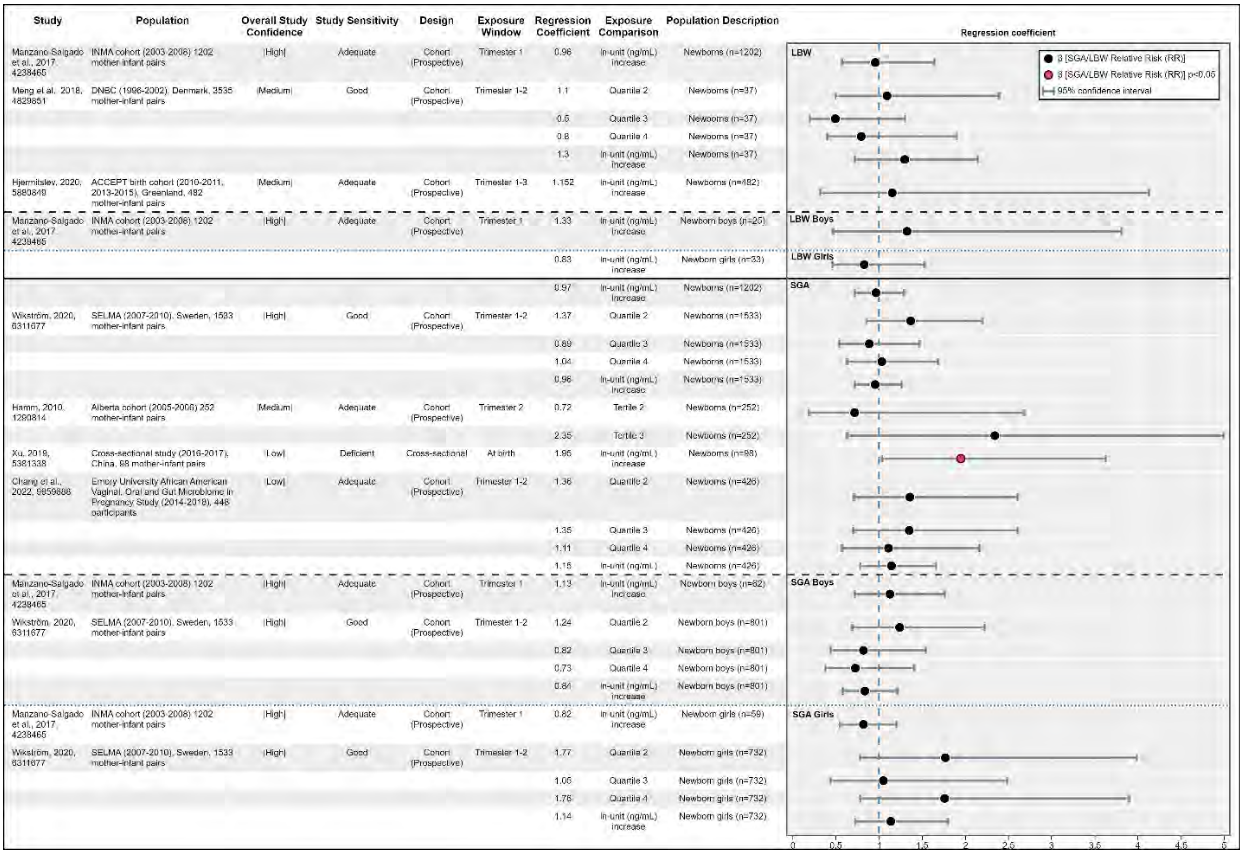


Figure 3-34. Small for gestational age and low birth weight results for 7 epidemiologic studies. For additional details see HAWC link.

Abbreviations: SGA= Small for Gestational Age; LBW= Low Birth Weight
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Low birth weight overall population data above black reference line.
[c]Overall population data above black dotted line; sex-stratified data below blue dotted line.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Small for Gestational Age/Low Birth Weight Summary

Although they were not always statistically significant, five different (Wikström et al., 2020; Xu et al., 2019; Meng et al., 2018; Manzano-Salgado et al., 2017a; Hamm et al., 2010) of the seven studies examining either SGA, LBW or very LBW showed some increased risks with increasing PFHxS exposures among the overall population or either girls or boys. The associations were quite variable (OR range: 1.3–9.1) in magnitude including some large but imprecise increased odds, but there was no evidence of exposure-response relationships based on categorical data in three separate studies. There were no patterns of results based on sample timing and other characteristics.

Fetal Growth Restriction Summary

Among the most accurate fetal growth restriction endpoints examined, there was reasonably consistent evidence for birth weight deficits across different measures and types of PFHxS exposure metrics considered. Some mean or standardized birth weight deficits were detected in 20 of the 34 included studies, including 14 out of 16 *medium* and *high* confidence studies. Inverse associations were also noted in 17 of 31 studies that examined mean birth weight associations in the overall population (5 *high*; 5 *medium* and 4 *low* confidence). Although smaller birth weight deficits were seen in the five *high* confidence studies (β= −11 to −22 g), the remaining studies reporting reductions ranged from −30 to −93 grams per each ln-unit PFHxS increase. Similarly, 9 out of 12 sex-specific analyses, including 5 out of 9 *medium* and *high* confidence studies, showed deficits in either or both male and female neonates. Results were larger based on categorical comparisons in two *low* confidence studies (β range: −108 and −109 g for highest tertiles), but also consistent among these sex-specific studies expressing results per each ln-unit increase in both *medium* (β range: −45 to −71 g) and *high* confidence studies (β range: −11 to −14 g).

The findings in the overall population were supported by meta-analysis results of a larger study subset (n = 27) presented above (and detailed in Appendix C) that showed a small deficit (β= −7.7 g; 95% CI: −14.8, −0.5 per each ln-unit increase) in analyses of the overall populations. This overall meta-analysis birth weight result (β= −7.7 g) was comparable to analyses restricted to just the *high* (β= −6.8 g) and *medium* (β= −9.6 g) confidence studies. The analysis restricted to only studies with some early pregnancy (β= −7.3 g) biomarkers was also comparable in magnitude to these results. This early pregnancy data subset would be less prone to any potential impact of bias related to pregnancy hemodynamics. As noted above, many of the individual study results lacked precision and were not statistically significant, especially the sex-stratified results. Two of the 16 *medium* and *high* confidence studies examining categorical data for the overall population or different sexes, showed evidence of exposure-response relationships, which was supported by the findings based on continuous PFHxS exposure data.

The evidence for birth length deficits was also consistent, with all four of the *high* confidence studies showing deficits with increasing PFHxS exposures. However, among the *high* confidence studies based on the overall populations, the birth length results were often imprecise and fairly small in magnitude (−0.14 to −0.43 cm). In contrast, the results for PFHxS studies of head circumference and ponderal index were largely null. Across these different endpoints there is some evidence of an association between fetal growth restriction and PFHxS exposure, but important uncertainties remain. For example, there was a pattern suggestive of potential bias in studies with biomarker samples collected after pregnancy (i.e., postpartum), given these studies showed larger deficits in birthweight. Some additional uncertainty also remains regarding whether any other PFAS co-exposures are likely to be confounders in these studies; as such, this could potentially affect study findings.

Growth restriction – postnatal growth (infancy and early childhood up to 2 years of age)

Postnatal Weight, Height, and Head Circumference – Background

Thirteen studies were identified that assessed postnatal growth in relation to PFHxS (see Figure 3-35) with each examining some measures of infant weight and/or height. Two *uninformative* studies (Jin et al., 2020a; Alkhalawi et al., 2016) are not further considered here mainly due to deficiencies or critical deficiencies in participant selection, confounding, analysis, and study sensitivity. As shown in Figure 3-37 and Table 3-18, 5 of the 11 included studies were considered *high* confidence (Gao et al., 2022; Zhang et al., 2022; Starling et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b), while three each were *medium* (Jensen et al., 2020a; Gyllenhammar et al., 2018; Maisonet et al., 2012) and *low* confidence (Gross et al., 2020; Cao et al., 2018; Lee et al., 2018). Of the 11 postnatal growth studies, study sensitivity in three were considered adequate (Gao et al., 2022; Starling et al., 2019; Manzano-Salgado et al., 2017b), while four each were good (Gyllenhammar et al., 2018; Lee et al., 2018; Shoaff et al., 2018; Maisonet et al., 2012) and deficient (Zhang et al., 2022; Gross et al., 2020; Jensen et al., 2020a; Cao et al., 2018) largely owing to small exposure contrasts.

Although there was some overlap across studies, limited serial measures during infancy as well as inconsistent age at examinations and analyses may limit some comparisons here. For example, Zhang et al. (2022) examined growth up to 12 months and Starling et al. (2019) took measurements at 5 months only. Manzano-Salgado et al. (2017b) examined growth from birth until 6 months of age. Lee et al. (2018) examined postnatal growth at 2 years, while the Cao et al. (2018) analyses were based on a mean of 19 months in participants. Gyllenhammar et al. (2018) had serial postnatal growth measures for most endpoints at 3, 6, 12 and 18 months but was limited to 36 months and beyond for BMI SDS measures. Gross et al. (2020) completed examinations at 18 months, while Maisonet et al. (2012) did so at 20 months. Jensen et al. (2020a) examined different adiposity measures at 3 and 18 months, while Gao et al. (2022) examined growth trajectory based on serial measurements at five time periods within the first 2 years (at birth, 42 days, 6 months, 12 months, and 24 months). Shoaff et al. (2018) examined postnatal growth with repeated measures at age 4 weeks to 2 years.

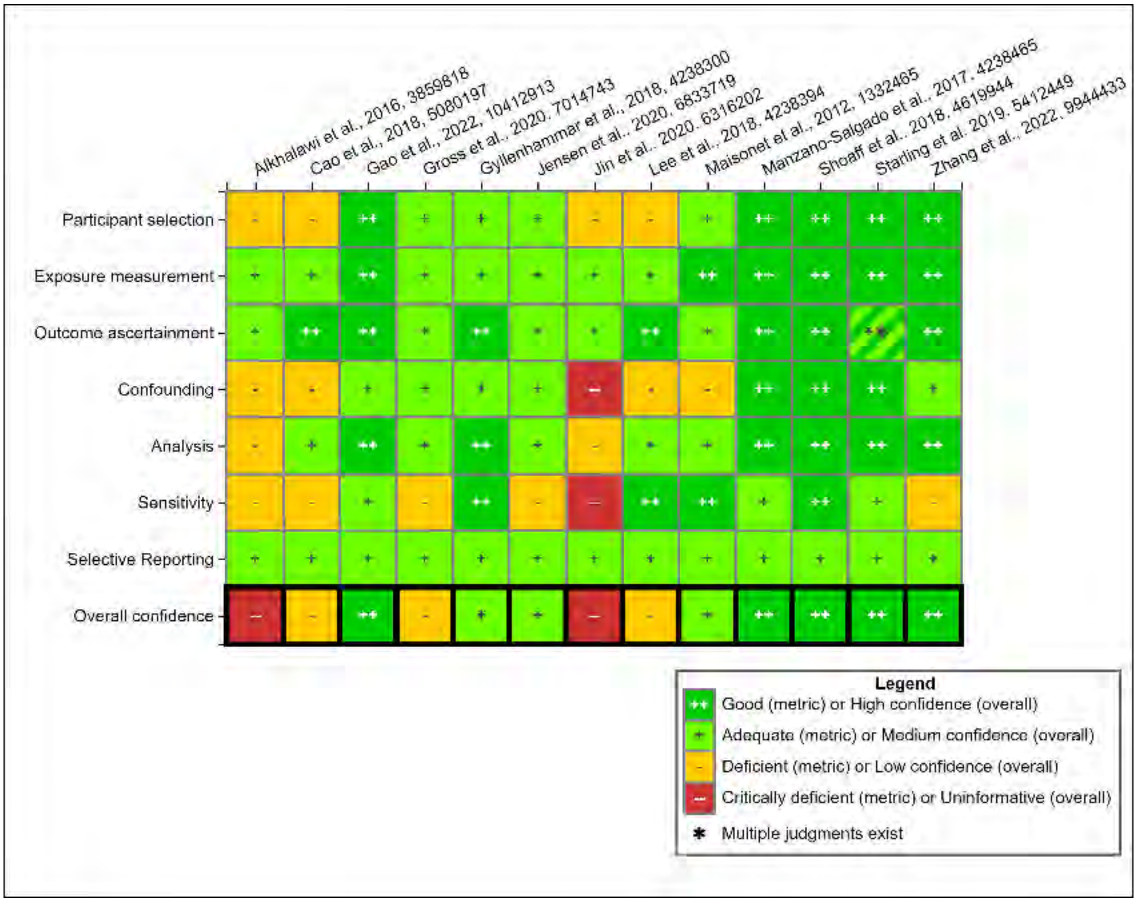


Figure 3-35. Study evaluation results for 13 epidemiological studies of postnatal growth and PFHxS. For additional details see HAWC link.

Postnatal Weight Standardized Results

In the overall population, eight postnatal studies (four high, two medium, and two low confidence) examined PFHxS in relation to either standardized (Zhang et al., 2022; Starling et al., 2019; Gyllenhammar et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) or mean weight measures (Cao et al., 2018; Lee et al., 2018; Maisonet et al., 2012) (see Figure 3-36). Three of five studies with standardized postnatal weight measures reported some inverse associations with PFHxS exposures, while the medium confidence Gyllenhammar et al. (2018) study of standard deviation scores (SDS) for weight measured at 3 to 18 months was null. Results in the high confidence study by Zhang et al. (2022) were largely null for standardized weight measures in the overall population and both sexes, with the only association seen for increased weight among tertile 2 exposures among girls examined up to 12 months (β= 0.15; 95% CI: 0.05, 0.25).

The results in the high confidence study by Starling et al. (2019) for the overall population and both sexes were largely null for both weight-for-age and weight-for-length z-scores, although they reported a statistically significant lower weight-for-age z-score at 5 months of age (β = −0.17; 95%CI: −0.33, −0.01 per each ln-unit increase) among girls. The authors did show an exposure-response relationship for weight-for-age z-scores among girls across PFHxS tertiles (T2: β = −0.24; 95%CI: −0.54, 0.05; T3 β = −0.38; 95%CI: −0.69, −0.08), but the opposite was seen for boys (T2: β = 0.31; 95%CI: −0.01, 0.62; T3: β = 0.26; 95%CI: −0.09, 0.61). Results were smaller in magnitude but fairly comparable for weight-for-length z-scores albeit in a non-monotonic fashion for girls (β range: −0.20 to −0.23).

Compared with tertile 1, the *high* confidence study by Shoaff et al. (2018) detected small nonstatistically significant deficits in z-scores for several outcomes including weight-for-age and weight-for-length for PFHxS tertile 3 (β range: −0.15 to −0.16). They also reported non-significant results per each ln-unit increase for both weight-for-age (β = −0.12; 95%CI: −0.29. 0.06) and weight-for-length (β = −0.12; 95%CI: −0.26. 0.01) z-scores. Although they were also not statistically significant, small weight z-score changes from birth to 6 months of age were also reported in the Infancia y Medio Ambiente (INMA) birth cohort (β = −0.09; 95% CI: −0.22, 0.03 per each ln-unit increase) from the other *high* confidence Manzano-Salgado et al. (2017b) study. These data seemed largely driven by the findings in girls (β = −0.13; 95% CI: −0.29, 0.03).

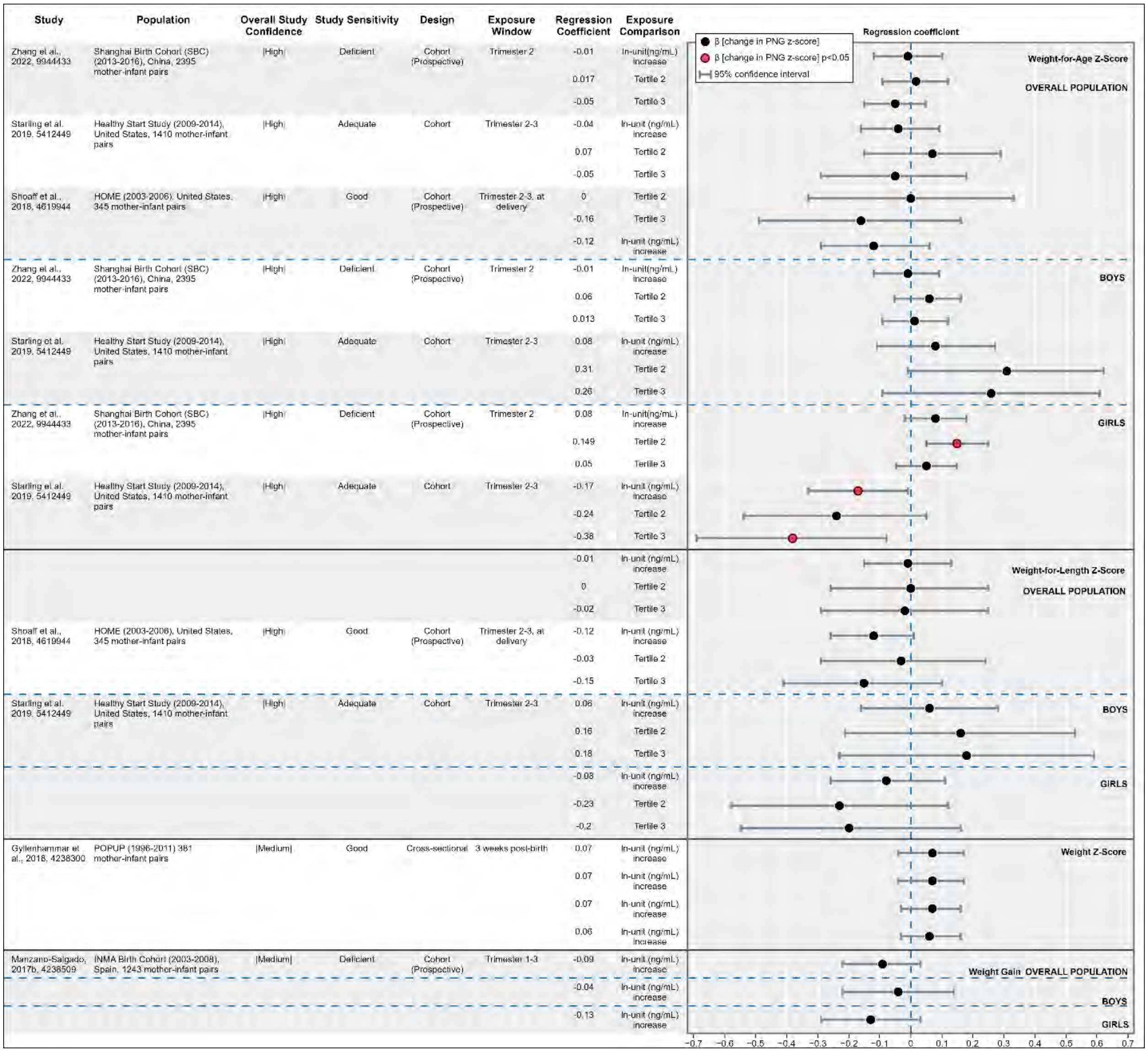


Figure 3-36. Standardized postnatal weight results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: (Gyllenhammar et al., 2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom); (Starling et al., 2019) at 5 months; (Zhang et al., 2022) between 42 days and 12 months; (Shoaff et al., 2018) at 4 weeks, 1 year, and 2 years; (Manzano-Salgado et al., 2017b) at 6 months.
[c]Solid black lines divide the figure into four categories. Listed from top to bottom they are as follows: Weight-for-Age Z-Score, Weight-for-Length Z-Score, Weight Z-Score, and Weight Gain Z-Score
[d]Within each category, overall population is located above the first blue dashed lines, boys are between the two blue dashed lines, and girls are below the second blue dashed line.
[e]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal Weight_Mean - Results

Three studies examined associations between PFHxS exposures and mean postnatal weight measures (Cao et al., 2018; Lee et al., 2018; Maisonet et al., 2012) (see Figures 3-37). The low confidence study by Lee et al. (2018) detected associations infant weight at age 2 (β= −200 g; 95% CI: −420, 20) per each ln-unit increase and monotonically across PFHxS quartiles (β range: -160 to -360 grams). For example, a large difference was detected for quartile 4 (≥1.81 ng/mL) (β= −360 g; 95% CI: −740, 20) compared with quartile 1 (<0.77 ng/mL). They detected weight change associations from birth to age 2 per each ln-unit increase (β= −170 g; 95% CI: −330, 160) but was considerably smaller among quartile 4 exposures (β= −60 g; 95%CI: −400, 270). The Cao et al. (2018) study was null for all comparisons, but they did report an imprecise postnatal (mean = 19 months) weight difference for tertile 2 (β= −145 g; 95% CI: −584, 294) in the overall population. Tertile 2 results were imprecise and in opposite directions for boys (β= −387 g; 95% CI: −916, 143) and girls (β= 155 g; 95% CI: −605, 915), while there was some suggestion of reduced weight in tertile 3 among girls (β= −101 g; 95% CI: −811, 608). The medium confidence study of girls from the ALSPAC study (Maisonet et al., 2012) were largely null and inconsistent across tertile (β range: -32 to 63 g) over the first 20 months of life.

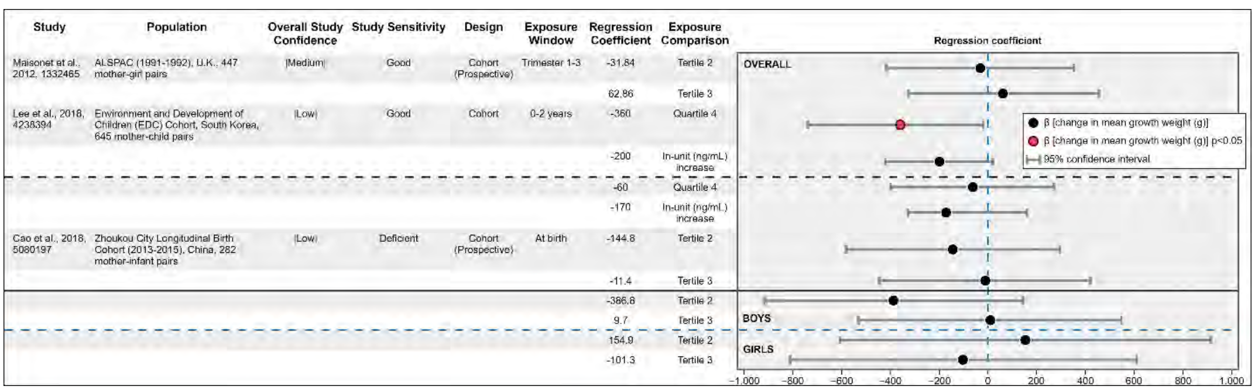


Figure 3-37. Mean postnatal weight results for PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: CI = Confidence Interval
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]For Lee et al. (2018) above the dashed line is PNG at two years, while below the dashed line is PNG change from birth to two years.
[c]Data for overall population is found above the reference line; sex-stratified data is found below the reference line.
[d]For Cao et al. (2018), sex-specific data is found below the reference line. Above the blue dashed line is data for boys; below the blue dashed line is data for girls.
[e]While a monotonic exposure-response relationship was seen for PFHxS quartiles in relation to weight at 2 years in Lee, the 95%CIs were not estimable and only quartile 4 is plotted here.
[f]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal Weight Summary

Five of eight studies in total showed some evidence of associations in the overall population or other sex for either mean or standardized infant weight measures. This included one *high* confidence study (Shoaff et al., 2018) showing associations for both weight for age and weight for length measures in the overall population and both *low* confidence studies. There was a preponderance of inverse associations between PFHxS and infant weight among girls only (based on three of four, including two of three weight standardized studies and one mean weight study). No patterns across the few studies with associations were evident.

Postnatal Height Standardized Results

In the overall population, five postnatal studies (two high, one medium, and two low confidence) examined PFHxS in relation to either standardized (Zhang et al., 2022; Gyllenhammar et al., 2018; Shoaff et al., 2018) or mean height measures (Cao et al., 2018; Lee et al., 2018) (see Figures 3-38). Five studies in total examined postnatal height measures in relation to PFHxS including three that examined standardized postnatal height (Zhang et al., 2022; Gyllenhammar et al., 2018; Shoaff et al., 2018). None of these studies showed any evidence of an association between PHFxS in relation to standardized infant height measures. The medium confidence by Gyllenhammar et al. (2018) was null for standardized height measures in the overall population. The high confidence study by Zhang et al. (2022) were null for standardized height measures in the overall population and both sexes. The high confidence study by Shoaff et al. (2018) was largely null for length-for-age z-score for continuous ($\beta = -0.07$; 95% CI: −0.27, 0.14) for each ln-unit increase and categorical PFHxS exposures (T3 ($\beta = -0.13$; 95%CI: −0.52, 0.27)).

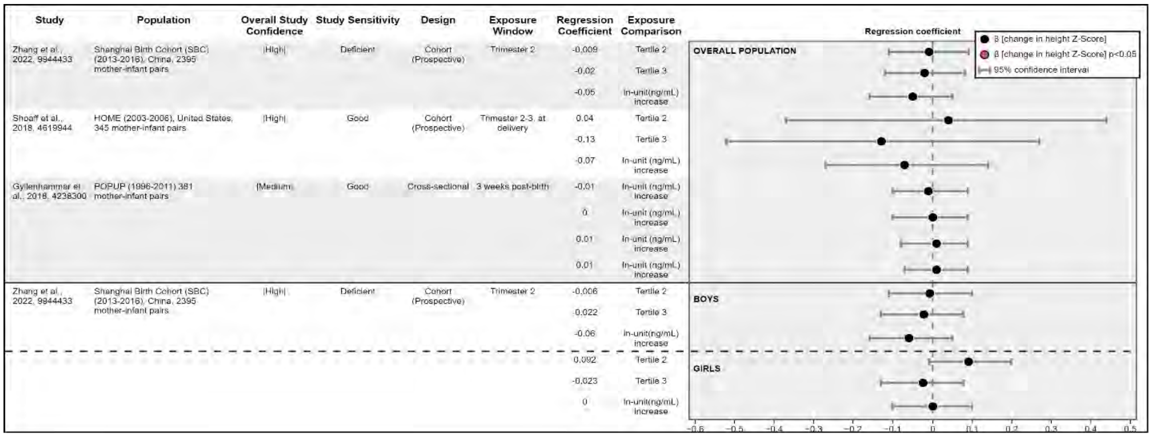


Figure 3-38. Standardized postnatal height results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: Gyllenhammar et al. (2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom); Zhang et al. (2022) between 42 days and 12 months; Shoaff et al. (2018) between 4 weeks and 2 years.
[c]Zhang et al. (2022) and Shoaff et al. (2018) examined length-for-age z-score.
[d]Overall, population is above the solid black line, while sex-stratified data is below. Within sex-stratified data, boys are above the dashed line, girls below.
[e]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal Height Mean Results

Two studies (Cao et al., 2018; Lee et al., 2018) examined associations between PFHxS exposures and mean postnatal height measures (see Figures 3-39). The low confidence study by Lee et al. (2018) reported statistically significant decreased mean height (β= −0.84 cm; 95% CI: −1.26, −0.42 per each ln-unit increase) at age 2 as well as reductions in height (β= −0.89 cm; 95% CI: −1.45, −0.33 per each ln-unit increase) from birth to age 2. They also detected exposure-response relationships and statistically significant infant height reductions in quartiles 3 and 4 for both weight at 2 years (Q4 β= −1.34 cm; 95% CI: −2.09, −0.60; Q3 β= −0.82 cm; 95% CI: −1.57, −0.07) and weight change from birth to 2 year (Q4 β= −1.63 cm; 95% CI: −2.62, −0.64; Q3 β= −1.20 cm; 95% CI: −2.10, −0.30). The low confidence study by Cao et al. (2018) reported non-monotonic increased postnatal length in the overall population (β range: 0.95 to 1.42 cm across tertiles). Similar results were seen for girls (β range: 1.32 to 2.01 cm across tertiles) but were null for boys (β range: 0.30 to 0.32 cm across tertiles).

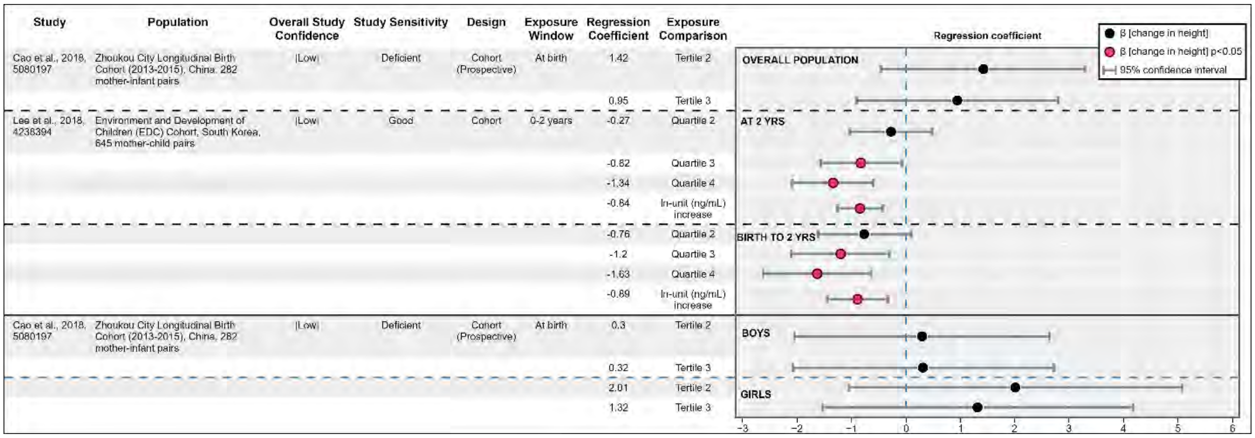


Figure 3-39. Mean postnatal height results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b]Above the solid black line is overall population data, while below is sex-stratified. Within the sex-stratified data, above the dashed blue line is boys, below is girls.

[c]For Lee et al. (2018) data, above the black dashed line is data referring to at two years, below the line is data referring to change from birth to 2 years.

[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Rapid Weight Gain

Four high confidence studies (Gao et al., 2022; Starling et al., 2019; Shoaff et al., 2018); Manzano-Salgado et al. (2017b) examined different rapid weight gain measures in relation to PFHxS (see Figures 3-40 and 3-41). In the Health Outcomes and Measures of the Environment (HOME) study, Shoaff et al. (2018) examined rapid growth based on weight z-scores in relation to PFHxS in the overall population. In the Healthy Start study, Starling et al. (2019) examined different rapid weight gain measures in relation to PFHxS for the overall population and both sexes. In the Shanghai Birth Cohort, Gao et al. (2022) examined various measures of growth trajectories in the overall population and across sex for various postnatal growth measures. In the INMA Birth Cohort Study, Manzano-Salgado et al. (2017b) examined rapid growth from birth to six months.

Two of the four studies showed some increased odds of rapid growth measures with increasing PFHxS exposures, although results were not always internally consistent. Shoaff et al. (2018) reported null associations for odds of weight z-score differences across tertiles (e.g., tertile 3 OR=0.95, 95%CI: 0.65, 1.40). The study by Manzano-Salgado et al. (2017b) was also null for rapid growth (OR=0.87; 95%CI: 0.72, 1.04). The study by Starling et al. (2019) reported an OR of 1.49 (95%CI: 1.02, 2.18) for rapid weight gain per each ln-unit increase based on the weight-for-age z-score data but was null for weight-for-length z-score (OR=0.95; 95%CI: 0.63, 1.44).

In the Gao et al. (2022) study, most relative risks were null based on standardized weight for age and weight for length measures in the overall population and both sexes. Compared to the moderate-stable referent, Gao et al. (2022) reported elevated odds for the low-rising *weight-for-age z-score* (*WAZ*) trajectory (OR=1.92; 95% CI: 1.19, 3.08 per each ln-unit PFHxS increase) in the overall population. This seemed driven by results in males (OR=2.96; 95%CI: 1.51, 5.82 per each ln-unit PFHxS increase) given that females showed null associations. Using a weighted quantile sum mixture approach, they reported a statistically significant inverse association (OR=1.53; 95%CI: 1.13, 2.06 per each ln-unit PFAS Sum increase) for WAZ among low-rising participants (vs. moderate-stable) with PFHxS having the highest weight among the PFAS mixture constituents.

Among males only, Gao et al. (2022) reported increased odds for weight-for-length z-score (WLZ) trajectory in low-rising (OR=2.43; 95% CI: 1.00, 5.87 per each ln-unit PFHxS increase) and low-stable participants (OR=2.04; 95% CI: 0.70, 6.02 per each ln-unit PFHxS increase). Compared to the moderate-stable referent, Gao et al. (2022) reported elevated odds in females only for the moderate-falling (OR=1.85; 95% CI: 0.97, 3.47 per each ln-unit PFHxS increase) and high-rising length-for-age z-score (LAZ) trajectories (OR=1.61; 95% CI: 0.41, 6.38 per each ln-unit PFHxS increase). The odds of LAZ for high-rising participants from the overall population was null in the single pollutant model but was elevated for the PFAS mixture metric based on a weighted quantile sum approach (OR=1.59; 95% CI: 0.90, 2.82 per each ln-unit PFHxS increase), with PFDA having the highest weight among the PFAS mixtures.

Although most were not statistically significant, Gao et al. (2022) reported inverse associations in the single PFAS models for *head-circumference-for-age* z-score for high-rising, moderate-rising, low-rising, and low-stable vs. moderate-stable participants (OR range: 0.46 to 0.71 per each ln-unit PFHxS increase). They also reported a statistically significant inverse association (OR=0.37; 95%CI: 0.18, 0.72) for low-rising vs. moderate-stable groups based on a PFAS mixture metric (per each ln-unit increase) using a weighted quantile sum approach.

Rapid Weight Gain Summary

Overall, two of four studies showed increased odds of rapid growth in relation to PFHxS exposures. Although results were a bit mixed across different growth trajectory measures, there was only evidence of inverse associations between PFHxS and rapid growth as measured by head circumference z-scores in the Gao et al. (2022) study. In contrast, most of the associations they detected using weight for age, weight for length and length for age z-scores showed increased risk of rapid growth per each ln-unit PFHxS increase. These associations were most evident among the weight and height measures among the participants with a low baseline growth trajectory followed by a rapid increased trend afterward (i.e., low-rising group). These data were supported by another study (Starling et al., 2019) that reported a statistically significant OR (1.49; 95%CI: 1.02, 2.18 per each ln-unit increase) for rapid weight gain based on weight-for-age z-score data only. Both of these studies are consistent with a hypothesis that rapid weight growth in childhood that may have followed intrauterine growth retardation from PFHxS exposures. These individuals may be at most risk for metabolic syndrome, as evidenced by changes in obesity and other health effects later in life.

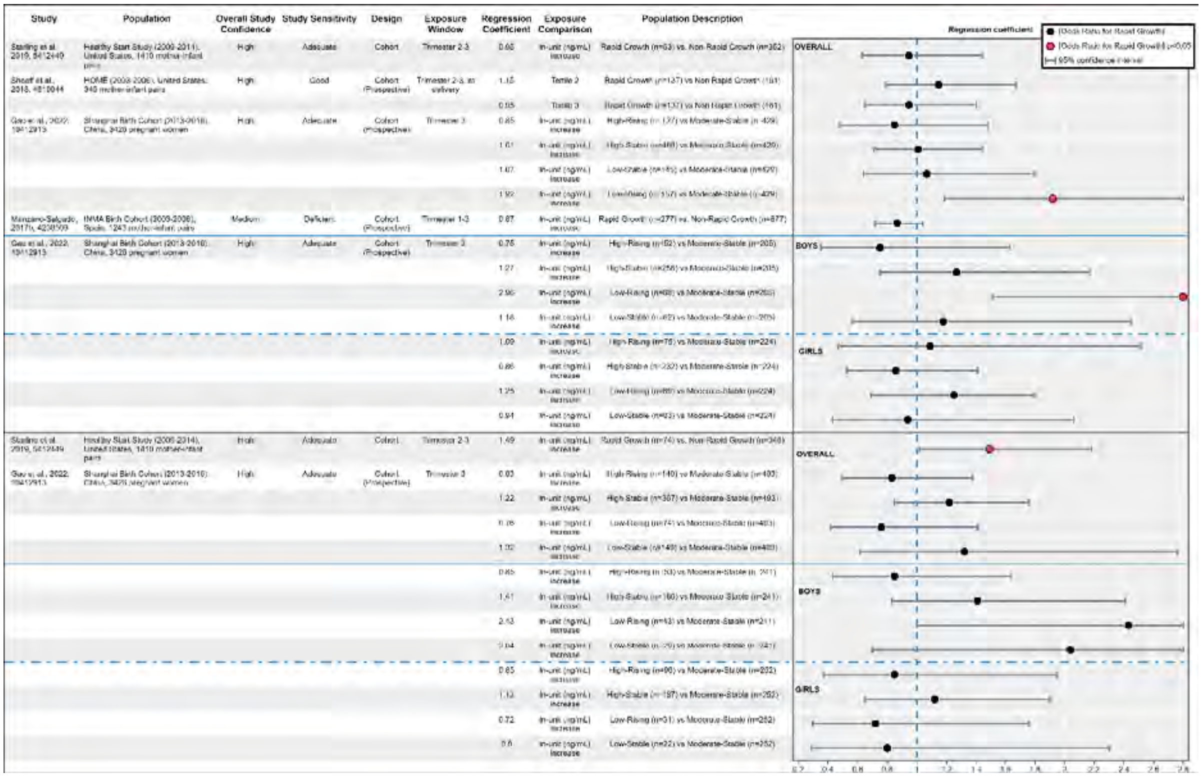


Figure 3-40. Postnatal rapid growth (weight-for-age and weight-for-length z-score) results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: Starling et al. (2019) at 5 months, Gao et al. (2022) modeled data (collected at 42 days, 6 months, 12 months, and 24 months).
[c]Weight-for-Age Z-Score data above the black reference line; weight-for-length below.
[d]Overall population data above the blue line; Sex-stratified data below.
[e]Sex-Stratified data: male infants above the blue dash-dotted line; females below.
[f]Quantile 2 in Starling et al. (2019) represents dichotomized exposure at median (quantile 1 referent: LOD-0.1 ng/mL; quantile 2: 0.2–3.5 ng/mL).
[g]The following Gao et al. (2022) results have been truncated: 1.92 [1.19–3.08], 2.96 [1.51–5.82], 2.43 [1–5.87], and 2.04 [0.7–6.02].
[h]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

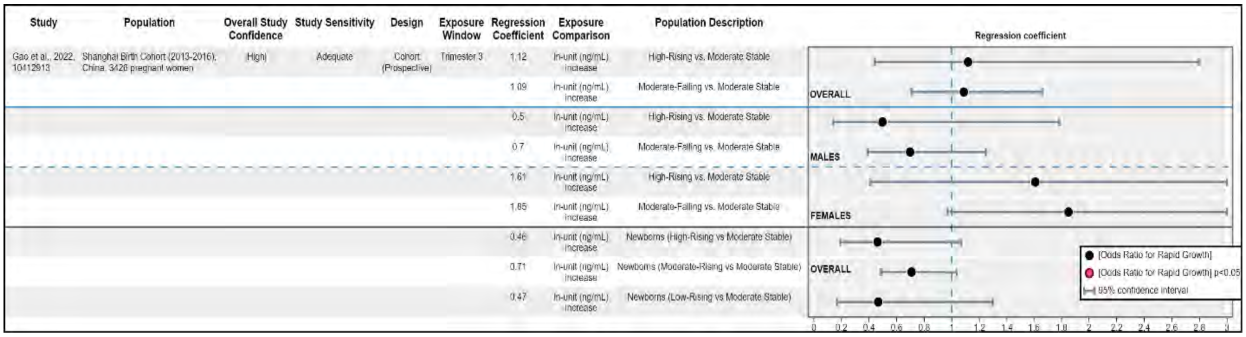


Figure 3-41. Postnatal rapid growth (length-for-age and head circumference z-score) results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: Gao et al. (2022) modeled data (collected at 42 days, 6 months, 12 months, and 24 months).
[c]Length-for-Age Z-Score data above the black reference line; Head Circumference Z-Score below.
[d]Sex stratified Length-for-Age Z-Score data below blue solid line; males above blue dotted line; females below.
[e]Overall population data above the blue line; Sex-stratified data below.
[f]Female confidence intervals have been truncated; the data points are 1.61 [0.41–6.38] and 1.85 [0.97–3.47].
[g]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal Head Circumference

Three studies examined postnatal head circumference in relation to PFHxS (Zhang et al., 2022; Cao et al., 2018; Gyllenhammar et al., 2018) (see Figure 3-42). Null results were detected in the high confidence study by Zhang et al. (2022) for head circumference-for-age Z score per each ln-unit PFHxS increase (β = −0.08; 95%CI: −0.19, 0.02). The medium confidence study by Gyllenhammar et al. (2018) showed monotonic head circumference-for-age Z increases as children aged from 3 to 18 months (β range: 0.05 to 0.12). The low confidence study by Cao et al. (2018) reported non-monotonic increased postnatal head circumference in the overall population (β range: 0.90 to 1.33 cm across tertiles). These results were comparable across boys (β range: 0.97 to 1.27 cm across tertiles) and girls (β range: 0.78 to 1.34 across tertiles). Overall, two of three studies showed some evidence of increased postnatal head circumference in relation to PFHxS exposures.

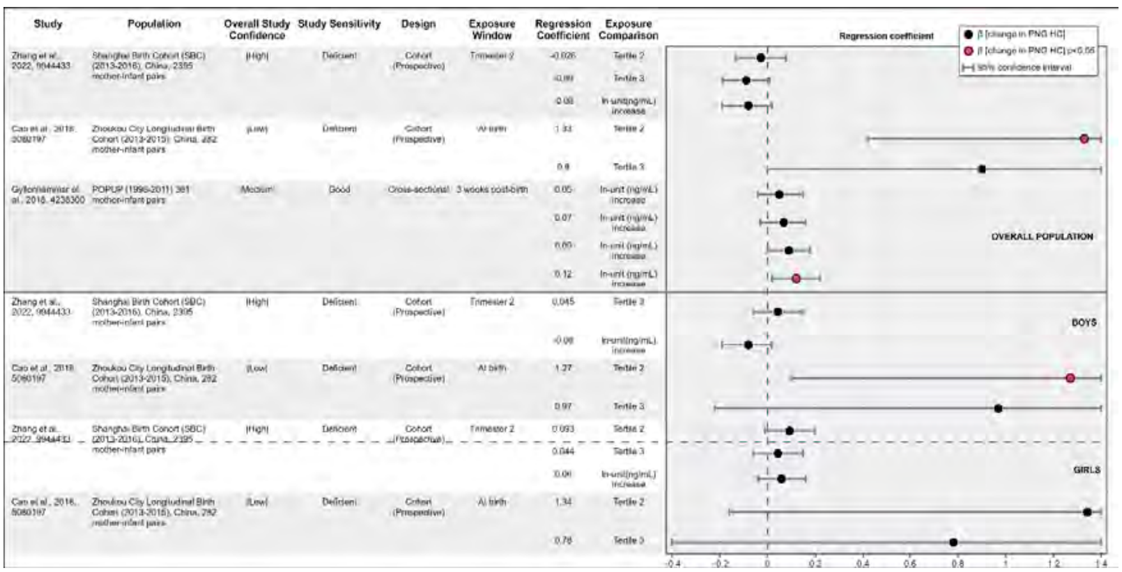


Figure 3-42. Postnatal head circumference results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Age at Outcome Measurement: Gyllenhammar et al. (2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom); (Zhang et al., 2022) between 42 days and 12 months; Cao et al. (2018) at a mean of 19 months.
[c]Zhang et al. (2022) reports head circumference-for-age Z-Score, Gyllenhammar et al. (2018) report head circumference Z-Score, and Cao reported odds ratios.
[d]Overall population is above the solid black line, while sex-stratified data is below. Within sex-stratified data, boys are above the dashed blue line, girls below.
[e]Cao et al. (2018) upper and lower bounds have been truncated. For overall population, the Tertile 2 bounds are [0.42, 2.26] and the Tertile 3 bounds are [0, 1.81]. For boys, the Tertile 2 bounds are [0.1, 2.43] and the Tertile 3 bounds are [-0.22, 2.16]. For girls, the Tertile 2 bounds are [-0.16, 2.84] and the Tertile 3 bounds are [-0.62, 2.18].
[f]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal Adiposity/Body Mass Index/Ponderal Index/Weight Status

Five studies (Zhang et al., 2022; Gross et al., 2020; Jensen et al., 2020a; Starling et al., 2019; Shoaff et al., 2018) enabled examination of different measures of infant adiposity such as body mass index (BMI), overweight status, and ponderal index (see Figure 3-43). Three of the five studies were null (Zhang et al., 2022; Jensen et al., 2020a; Starling et al., 2019) for associations in the overall population, while the remaining two showed decreased measures of adiposity in relation to PFHxS. For example, the low confidence study by Gross et al. (2020) showed an inverse but non-significant association between overweight status at 18 months (OR=0.75 g; 95% CI: 0.30 to 1.85) and dried blood spot PFHxS levels above the mean (compared to below the mean) with similar relative risks among boys (OR=0.74; 95%CI: 0.17, 3.24) and girls (OR=0.68; 95%CI: 0.15. 3.12). The high confidence study by Shoaff et al. (2018) exposure-response relationship detected for PFHxS and BMI z score across tertile 2: (β= −0.12; 95%CI: −0.37, 0.13) and tertile 3 (β= −0.22; 95%CI: −0.47, 0.03) and per each ln-unit increase (β= −0.12; 95%CI: −0.26, 0.01).

The results were a bit more mixed when examined by sex, with two of three sex-specific studies showing some suggestion of increased adiposity among boys only. For example, the *medium* confidence by Jensen et al. (2020a) reported null associations at age 3 and 18 months for standardized (i.e., SDS) postnatal waist circumference, body mass index, and ponderal index measures in their overall population. Although they did not detect statistically significant interactions by sex for any endpoints evaluated, slight non-significant increases in boys BMI (β= 0.13; 95%CI: −0.34, 0.60 per each ln-unit increase) and Ponderal Index (β= 0.34; 95%CI: −0.14, 0.82 per each ln-unit increase) SDS scores were noted. The high confidence study by (Starling et al., 2019) was null for infant adiposity per each ln-unit PFHxS increase among the overall population (β= 0.01 fat mass increase %; 95%CI: −0.67, 0.68). Results were divergent for males (β= 0.54 fat mass increase %; 95%CI: −0.51, 1.58 per each ln-unit increase) versus females (β= −0.42 fat mass increase %; 95%CI: −1.31, 0.47 per each ln-unit increase). Similar results were seen in their tertile analyses with more adiposity in males (β range: 0.89 to 1.90% fat mass increase) and females (β= −0.85 to −1.11% fat mass increase). The high confidence study by (Zhang et al., 2022) reported null associations for PFHxS and BMI-for-age z-scores (β= −0.01; 95%CI −0.12, 0.09 per each ln-unit increase) in the overall population, males (β= -0.01; 95%CI −0.12, 0.09 per each ln-unit increase) and females (β= 0.10; 95%CI: −0.01, 0.20 per each ln-unit increase).

Postnatal Adiposity Summary

Overall, none of the five studies in the overall population reported increased adiposity with increasing PFHxS exposures up to age 2 years. However, two of three studies in boys did show some suggestion of increased adiposity in relation to PFHxS exposures. None of the three studies in girls reported increased adiposity.

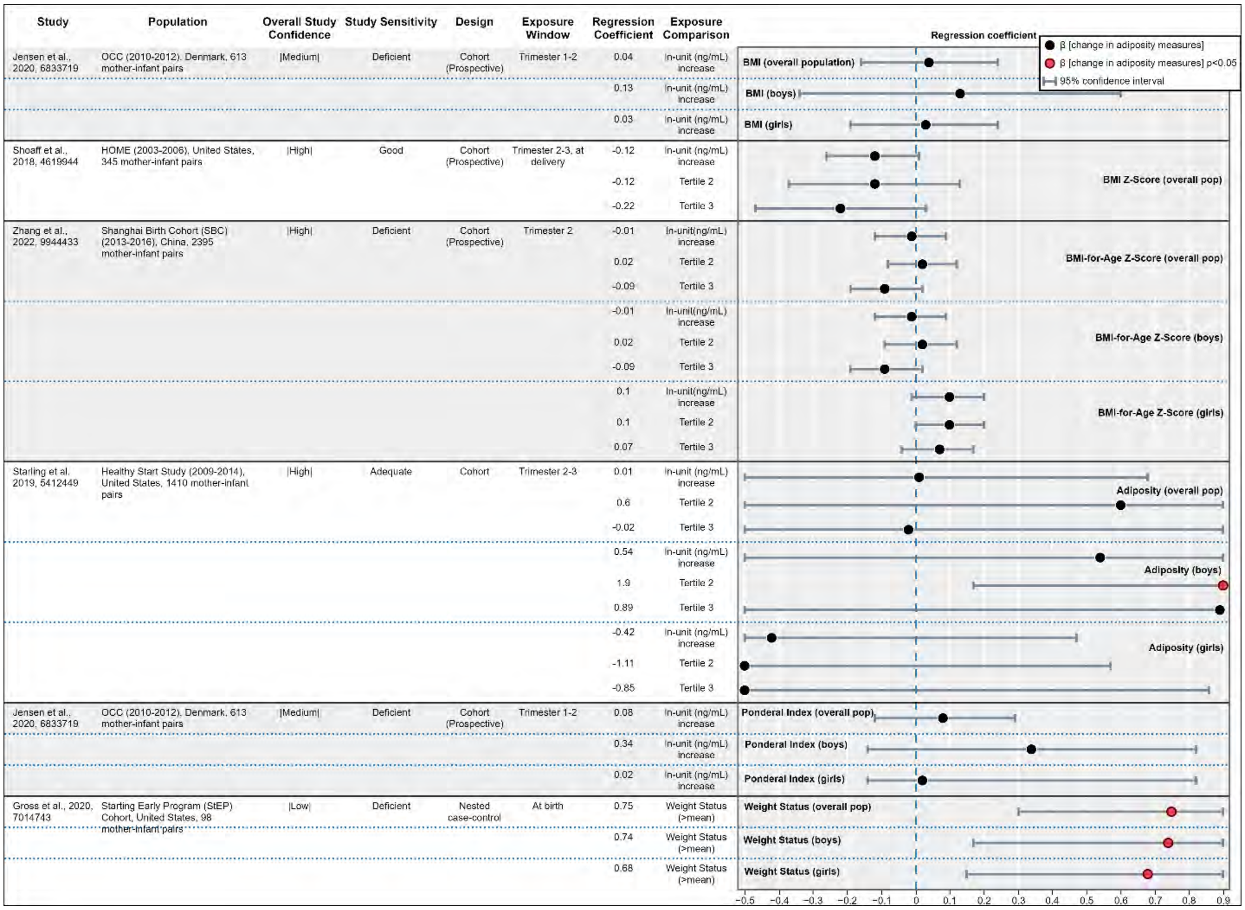


Figure 3-43. Postnatal body mass index, adiposity, and ponderal index and weight status results for PFHxS epidemiological studies. For additional details see HAWC link.

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]Measurement types are separated by the solid black reference lines and are as follows (in descending order): BMI, BMI Z-Score, BMI-for-Age Z-Score, Adiposity, Ponderal Index, and Weight Status.
[c]Within each category, above the first dotted blue line are values for overall population, between the two dotted lines are values for boys, and below the second dotted line are values for girls.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Postnatal growth summary

Overall, there were mixed results within and across the 13 available postnatal PFHxS studies of postnatal growth with the most consistent evidence for postnatal weight. Five of eight studies in total showed some evidence of associations with mean or standardized infant weight measures including three high confidence studies in the overall population and three of four studies in girls. No other patterns were evident. Only one low confidence study out of five total studies showed any evidence of smaller height based on either with mean or standardized height measure in the overall population or either sex. None of three available studies showed some evidence of decreased postnatal head circumference in relation to PFHxS exposures. In contrast, two of them showed increased postnatal head circumference. Similarly, none of the five studies in the overall population reported increased adiposity in relation to PFHxS as two studies showed decreased measures of adiposity. The results for rapid growth measures were a bit mixed but two of four studies showed increased odds of rapid growth in relation to PFHxS.

Although few studies examined exposure-response relationships based on categorical data in the overall population or across sexes, three different studies did show dose-dependence for some measures such as infant weight (one of six studies), height (one of four studies) and adiposity (one of three studies). No study characteristics were obvious explanatory factors for between-study heterogeneity. Few patterns by sex were evident outside a preponderance of inverse associations between PFHxS and infant weight among girls. There was also evidence in two of three studies in boys of increased adiposity. However, limited exposure contrasts and statistical power may have hampered the ability to detect associations small in magnitude especially among the sexes. In summary, the evidence was mixed across various postnatal measures and different examination windows, with only minimal evidence of exposure-response relationships to support the continuous exposure scaled results. One challenge in evaluating consistency across heterogeneous studies includes disparate periods of follow-up and assessment (e.g., childhood age at examination).

Table 3-18. Summary of 11 epidemiologic studies of PFHxS exposure and post-natal growth measured

Author	Study location, years	Sample size	Median exposure (range) in ng/mL	Weight	Height	HC	Adiposity	Rapid growth
High Confidence Studies								
Gao et al. (2022)	China, 2013–2016	1,350	0.54 (0.21, 3.75)					↑ Overall
Manzano-Salgado et al. (2017b)	Spain, 2003–2008	1,154	0.58 (0.05, 11.01)	- Overall/ Girls ∅ Boys				∅ Overall
Shoaff et al. (2018)	OH, USA, 2003–2006	345	1.5 (0.1, 32.5)	- Overall	- Overall		- Overall[a]	∅ Overall
Starling et al. (2019)	CO, USA, 2009–2014	415	0.7 (0.2, 2.8)[b]	- Overall/Girls[a] + Boys[a]			∅ Overall + Boys - Girls	↑ Overall
Zhang et al. (2022)	China, 2013–2016	2,395	0.53 (0, 25.4)	∅ Overall/Boys + Girls			∅ Overall/ Boys/Girls	
Medium Confidence Studies								

Author	Study location, years	Sample size	Median exposure (range) in ng/mL	Weight	Height	HC	Adiposity	Rapid growth
Gyllenhammar et al. (2018)	Sweden, 1996–2001	381	2.4 (0.32, 26.0)	∅ Overall				
Maisonet et al. (2012)	United Kingdom, 1991-–992	422	1.6 (0.2, 54.8)	– Girls				
Low Confidence Studies								
Cao et al. (2018)	China, 2013–2015	337	0.09 0.03, 0.31[c]	∅ Overall/Boys + Girls	+ Overall/ Girls ∅ Boys	+ Overall/ Girls/Boys		
Gross et al. (2020)	USA, 2014	98	0.108 (N/A)[d]				↓ Overall/ Girls/Boys	
Jensen et al. (2020a)	Denmark, 2010–2012	589	0.30 (0.08, 0.66)[b]				∅ Overall/Girls + Boys	
Lee et al. (2018)	S. Korea, 2012–2013	361	1.19 (0.22, 1.69)	– Overall	– Overall*[a]			

Abbreviations: N/A: not available

*Denotes statistical significance at $p < 0.05$; Æ represents a null association; + represents a positive association; - represents a negative association; - represents increased odds ratio; ¯ represents decreased odds ratio

Note: "Adverse effects" are indicated by both increased ORs (-) for dichotomous outcomes and negative associations (-) for the other outcomes.

/ Denotes multiple groups with the same direction of associations.

[a]Exposure-response relationship detected based on categorical data.

[b]No range provided but 5th-95th percentiles included.

[c]No range provided but 10th-90th percentiles included.

[d]Dried Blood spot PFHxS sample collected within 48 hours of birth.

Anogenital distance

Four *medium* confidence studies examined the associations between PFHxS and AGD in infants (see Figure 3-44). Reduced AGD is associated with clinically relevant outcomes in males, including cryptorchidism, hypospadias, and lower semen quality and testosterone levels (Thankamony et al., 2016), but adversity of reduced AGD is less established in females. Three studies examined boys and girls (Christensen et al., 2021; Arbuckle et al., 2020; Lind et al., 2017), while one included boys only (Tian et al., 2019b). All four studies were birth cohorts in Denmark (Lind et al., 2017), Faroe Islands (Christensen et al., 2021) (cross-sectional analysis within cohort sample), Canada (Arbuckle et al., 2020), and China (Tian et al., 2019b). In Arbuckle et al. (2020) and Tian et al. (2019b), AGD was measured shortly after birth (median 3.5 days). Christensen et al. (2021) measured AGD at two weeks after the expected term date. Tian et al. (2019b) additionally measured AGD at 6 and 12 months, and Lind et al. (2017) measured at 3 months. With greater variability in timing of measurements, there is additional potential for misclassification with these measures, but age at time of measurement was included in the statistical models in all studies.

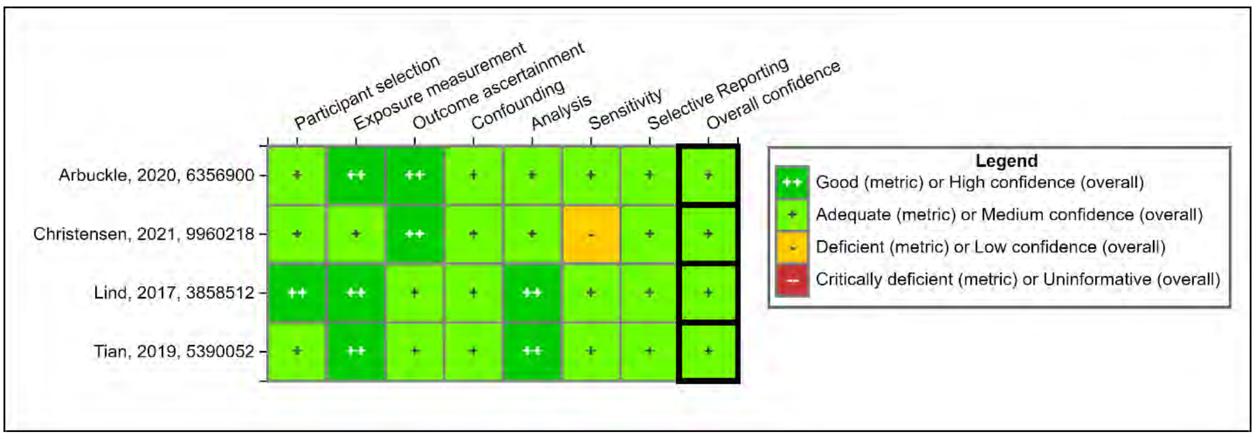


Figure 3-44. Summary of study evaluation for epidemiology studies of anogenital distance. For additional details see HAWC link.

In Lind et al. (2017), there was a statistically significant inverse association (i.e., shorter AGD with higher exposure) with ASD among boys. The other three studies did not report decreased AGD, despite greater exposure contrasts (see Table 3-19). In girls, there was an inverse association with PFHxS for ACD Lind et al. (2017). This was statistically significant with PFHxS analyzed as continuous, although there was not a monotonic decrease across quartiles. A consistent but smaller and non-significant association was also observed in the third and fourth quartiles for AFD. This association is coherent with the decrease in testosterone observed in some studies (described below in the Reproductive Effects section). However, in the other two studies (Christensen et al., 2021); Arbuckle et al. (2020), there was no decrease in either AGD measure with higher PFHxS exposure.

AGD is a marker of androgen exposure, and thus an inverse in AGD would be expected to correspond with a decrease in testosterone. This was not observed in the two studies of testosterone in male neonates, but an inverse association was observed in a study of female neonates (see Male and Female Reproductive Effects). The lack of coherence for males does not reduce confidence in the AGD findings due to low confidence in the reproductive hormone studies. However, the inconsistency across studies results in considerable uncertainty for an association with AGD.

Table 3-19. Associations between PFHxS and anogenital distance in *medium* confidence epidemiology studies

Boys					
Reference	**Population**	**Median exposure (IQR) (ng/mL)**	**Effect estimate**	**ASD**	**APD**
Christensen et al. (2021)	Cross-sectional analysis within birth cohort in the Faroe Islands; 232 boys at 2 wks post term	Serum 0.2 (0.1–0.3)	β (95% CI) for ln-unit increase	0.2 (-0.3, 0.7)	NR
Lind et al. (2017)	Birth cohort in Denmark; 299 boys at 3 months	Serum 0.3 (0.2–0.4)	β (95% CI) for ln-unit increase	−1.2 (−2.3, −0.2)	−0.6 (−1.8, 0.5)
			Quartiles vs. Q1	Q2: 0.6 (−1.3, 2.4) Q3: −0.3 (−2.1, 1.6) Q4: −0.8 (−2.7, 1.2)	Q2: 2.6 (0.5, 4.6) Q3: 0.9 (−1.0, 2.9) Q4: 0.1 (−2.0, 2.3)
(Arbuckle et al., 2020)	Birth cohort in Canada; 198 boys at birth	Plasma 1.1 (0.7–1.7)	β (95% CI) for unit increase	0.22 (−0.54, 0.98)	0.24 (−0.52, 1.01)
			Quartiles vs. Q1	Q2: −0.08 (−1.99, 1.83) Q3: 0.13 (−1.80, 2.06) Q4: 0.57 (−1.33, 2.46)	Q2: −0.91 (−2.74, 0.91) Q3: 0.64 (−1.23, 2.51) Q4: 0.57 (−1.30, 2.44)
Tian et al. (2019b)	Birth cohort in China; 439 boys at birth	Plasma 2.8 (2.2–3.6)	β (95% CI) for ln- unit increase	Birth: −0.19 (−0.97, 0.58) 6 mos: 0.69 (−1.86, 3.23) 12 mos: 2.21 (−0.47, 4.89)	Birth: 0.35 (−0.55, 1.26) 6 mos: 0.04 (−2.53, 2.61) 12 mos: 0.60 (−2.62, 3.83)
Girls					
Reference	**Population**	**Median exposure (IQR) (ng/mL)**	**Effect estimate**	**ACD**	**AFD**
Christensen et al. (2021)	Cross-sectional analysis within birth cohort in the Faroe Islands; 231 girls at 2 wks post term	Serum 0.2 (0.1–0.3)	β (95% CI) for ln-unit increase	NR	-0.1 (-0.4, 0.3)
Lind et al. (2017)	Birth cohort in Denmark; 212 girls at 3 mos	Serum 0.3 (0.2–0.4)	β (95% CI) for ln-unit increase	−0.9 (−1.9, 0.0)	−0.3 (−1.1, 0.4)
			Quartiles vs. Q1	Q2: −1.6 (−3.4, 0.2) Q3: −2.3 (−4.1, −0.5) Q4: −1.6 (−3.4, 0.2)	Q2: 0.2 (−1.2, 1.6) Q3: −0.8 (−2.2, 0.6) Q4: −0.5 (−1.6, 0.9)

Arbuckle et al. (2020)	Birth cohort in Canada; 205 girls at birth	Plasma 1.1 (0.7–1.7)	β (95% CI) for unit increase	0.3 (−0.47, 1.07)	0.14 (−0.79, 1.07)
			Quartiles vs. Q1	Q2: 1.01 (−0.56, 2.59) Q3: 0.31 (−1.40, 2.02) Q4: 0.92 (−0.94, 2.79)	Q2: 1.23 (−0.66, 3.13) Q3: −0.51 (−2.56, 1.54) Q4: 0.52 (−1.71, 2.75)

Abbreviations: ASD: AGD measured from anus to the posterior base of the scrotum; APD: AGD measured from the center of the anus to the cephalad insertion of the penile; ACD: AGD measured from the from the center of the anus to the top of the clitoris; AFD: AGD measured from the top of the center of the anus to the posterior fourchette; mos: months

Gestation duration

As shown in Figure 3-47, 19 informative epidemiological studies assessed PFHxS in relation to changes in gestational duration measures. All 19 studies examined gestational age, with 10 of these providing analyses of both preterm delivery and gestational age. Fourteen of the 19 gestational duration studies were nested case-control studies or prospective cohort studies (Yang et al., 2022a; Gardener et al., 2021; Hjermitslev et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Buck Louis et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016; Maisonet et al., 2012; Hamm et al., 2010), and five were cross-sectional (Bangma et al., 2020; Eick et al., 2020; Xu et al., 2019; Gyllenhammar et al., 2018; Li et al., 2017b). The 19 epidemiological studies examined here had maternal exposure biomarkers collected either during trimesters one (Buck Louis et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a), two (Huo et al., 2020; Hamm et al., 2010), three (Gardener et al., 2021; Gao et al., 2019) across multiple trimesters (Eick et al., 2020; Hjermitslev et al., 2020; Workman et al., 2019; Meng et al., 2018; Sagiv et al., 2018; Bach et al., 2016; Maisonet et al., 2012), or had post-partum maternal or infant samples (Yang et al., 2022a; Bangma et al., 2020; Xu et al., 2019; Gyllenhammar et al., 2018; Li et al., 2017b).

Nine studies each were classified as having late (defined as trimester 2 exclusive onward) and early sampling biomarker sampling (defined as having at least some trimester 1 maternal sampling). Four of the five-cross-sectional studies/analyses had late biomarker sampling. Among the 14 cohort or nested case-control studies, eight studies had early biomarker sampling (Hjermitslev et al., 2020; Buck Louis et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016; Maisonet et al., 2012), while six were classified as late (Yang et al., 2022a; Gardener et al., 2021; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Hamm et al., 2010). For examination of consistency and between-study heterogeneity, the type of statistical analyses in addition to the type of study design was evaluated. As part of this evaluation, cross-sectional analyses are considered for any study that used maternal serum/plasma, umbilical cord or placental post-partum PFHxS measures in relation to gestational duration even if the data were derived from prospective cohort or nested case-control studies (e.g., (Yang et al., 2022a)).

Preterm Birth

Two (Huo et al., 2020; Manzano-Salgado et al., 2017a) of the ten preterm birth (typically defined as <37 gestational weeks) studies reported sex-specific findings in addition to overall population results (see Figure 3-45 and Table 3-20). Ten studies examined PFHxS and preterm birth including six *high* (Gardener et al., 2021; Eick et al., 2020; Huo et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016) and four *medium* confidence (Yang et al., 2022a; Hjermitslev et al., 2020; Meng et al., 2018; Hamm et al., 2010) studies. Two studies had good study sensitivity (Meng et al., 2018; Sagiv et al., 2018), six had adequate study sensitivity (Gardener et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Manzano-Salgado et al., 2017a; Bach et al., 2016; Hamm et al., 2010) and two were rated as deficient (Yang et al., 2022a; Huo et al., 2020).

Six of the ten studies showed no increased odds for preterm birth in relation to PFHxS (Yang et al., 2022a; Eick et al., 2020; Hjermitslev et al., 2020; Manzano-Salgado et al., 2017a; Bach et al., 2016; Hamm et al., 2010) with two reporting decreased risks (see Figure 3-46). The *medium* confidence study by Hamm et al. (2010) found a statistically significant decreased exposure-response relationship between preterm birth and the upper two PFHxS exposure tertiles (OR range: 0.31 to 0.59). An inverse association (OR = 0.59; 95%CI: 0.33, 1.06) was also detected in girls in the largely null Manzano-Salgado et al. (2017a) study.

Six studies were null for based on the overall population. The other four *high* and *medium* confidence studies reported some increased ORs but were not always internally consistent in direction of the effect estimates reported for different PFHxS exposure comparisons. The *high* confidence Sagiv et al. (2018) study reported largely null results based on continuous PFHxS exposures but showed some associations based on their categorical analysis that were not dose-dependent. For example, they reported an increased OR of preterm birth for PFHxS quartile 3 (OR=1.8; 95%CI: 1.1, 3.1 for 2.5–3.7 ng/mL) and 4 (OR = 1.3; 95%CI: 0.7, 2.2 for 3.8–74.5 ng/mL) compared with quartile one. Similarly, the *medium* confidence study by Meng et al. (2018) reported no associations for the various definitions of preterm birth examined for PFHxS quartile 4 or per a ln-unit increase. They did detect an increased OR of preterm birth for the second (OR=2.3; 95%CI: 1.1, 4.6) and third (OR=1.5; 95%CI: 0.7, 3.2) PFHxS quartiles compared with the first quartile. However, small sample sizes limited the interpretation of these categorical data. The categorical analysis in the *high* confidence Gardener et al. (2021) also found no dose-dependence but showed a non-significant two-fold increased risk of preterm birth in quartile 2 (OR = 2.11; 95%CI: 0.76, 5.81) relative to quartile 1.

In the *high* confidence study by Huo et al. (2020), associations between PFHxS and different preterm birth measures (including overall and different sub-types) were just above the null value based on continuous or categorical exposures for the overall population. However, an association was seen for clinically indicated preterm births for each ln-unit increase (OR = 1.58; 95%CI: 0.82, 3.05) and for tertile 3 (OR=1.43; 95%CI: 0.66, 3.08). A small non-significant increased risk was also seen for overall preterm birth (OR=1.33; 95%CI: 0.77, 2.27 per each ln-unit) in girls only, with larger statistically significant associations noted among girls only for the clinically indicated preterm birth subtype (OR = 2.56; 95%CI: 1.18, 5.53).

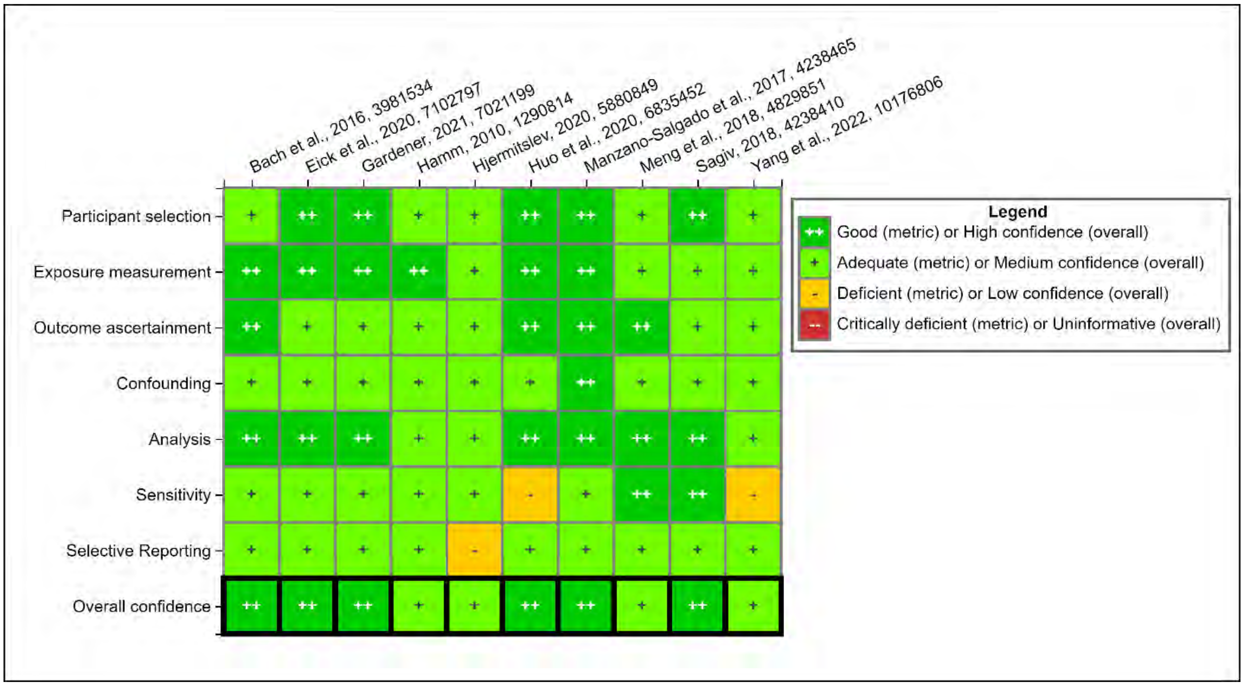


Figure 3-45. Summary of study evaluation for 10 epidemiology studies of preterm birth. For additional details see HAWC link.

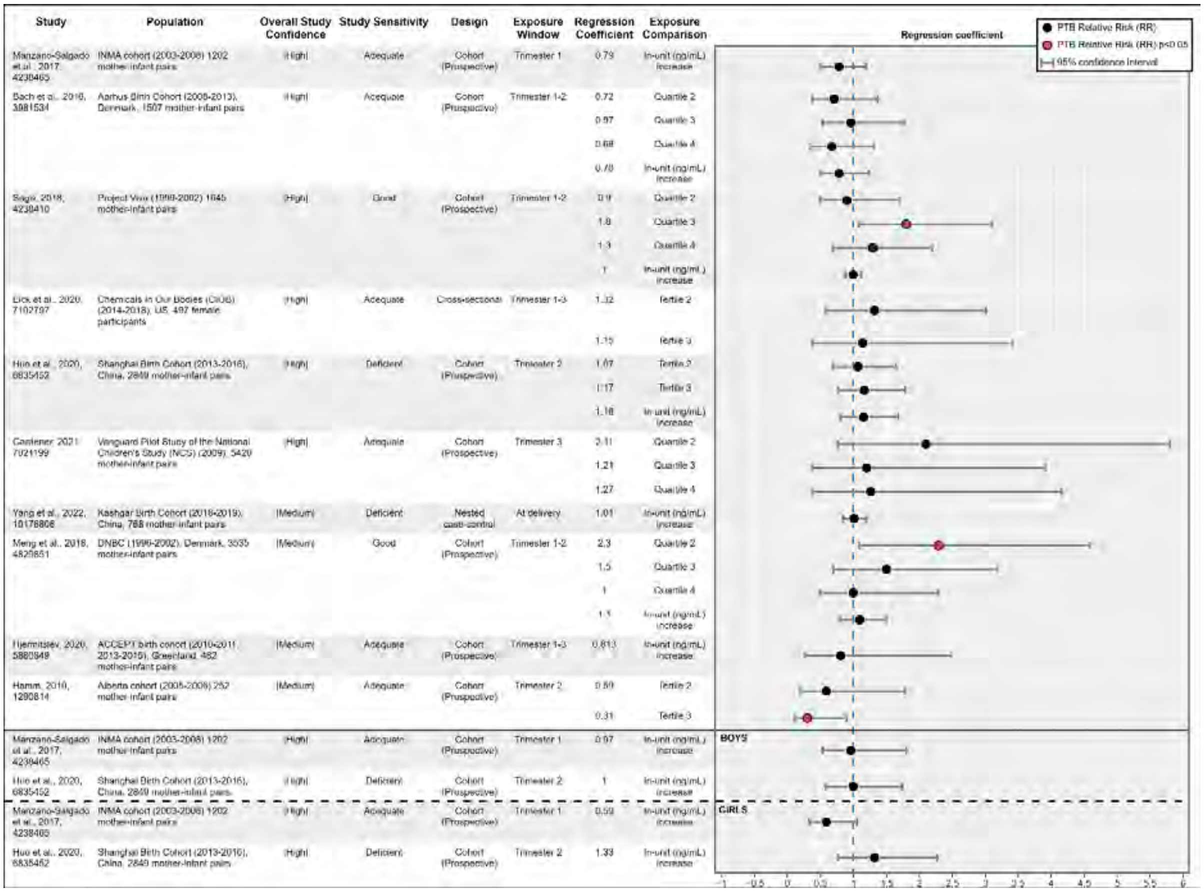


Figure 3-46. Preterm birth results for 10 PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: PTB= Preterm Birth

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b]Sex specific data below solid black line; newborn boys above dotted line, newborn girls below.

[c]For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (e.g., Yang et al. (2022a)).

[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Gestational age-overall population results

Seventeen of the 19 epidemiological studies examined mean gestational age data in the overall population, with the other two only reporting sex-specific findings (Lind et al., 2017; Maisonet et al., 2012) for PFHxS and gestational age relationships. Four studies reporting both sex-specific and overall population results (Hjermitslev et al., 2020; Meng et al., 2018; Li et al., 2017b; Manzano-Salgado et al., 2017a). Among the 19 studies with gestational age measures, eight were *high* confidence (Gardener et al., 2021; Eick et al., 2020; Huo et al., 2020; Buck Louis et al., 2018; Sagiv et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016), five were *medium* (Yang et al., 2022a; Hjermitslev et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018; Maisonet et al., 2012), and six were *low* confidence studies (Bangma et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019; Li et al., 2017b; Hamm et al., 2010) (see Figure 3-47). Five (Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Li et al., 2017b; Maisonet et al., 2012) of the 19 studies received a good rating in the study sensitivity domain, while eight (Gardener et al., 2021; Eick et al., 2020; Hjermitslev et al., 2020; Buck Louis et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a; Bach et al., 2016; Hamm et al., 2010) were considered adequate and six were deficient (Yang et al., 2022a; Bangma et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019).

Six (Bangma et al., 2020; Huo et al., 2020; Workman et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Bach et al., 2016) of the 17 studies in the overall population reported no associations between gestational age and PFHxS exposures, while four reported an increased gestational age with increasing PFHxS exposures (Eick et al., 2020; Xu et al., 2019; Li et al., 2017b; Hamm et al., 2010) (see Table 3-20 or Figure 3-48). For example, the *low* confidence study by Xu et al. (2019) reported a very large increase in gestational age (β= 3.38 weeks; 95%CI: −0.80, 7.55) per ln-unit increase in PFHxS. The Buck Louis et al. (2018) study was largely null in the overall population and reported some small non-significant differences for black (β= −0.14 weeks; 95%CI: −0.34, 0.05 for each ln-unit increase) and Asian (β= −0.09 weeks; 95%CI: −0.40, 0.21 for each ln-unit increase) neonates.

Seven studies reported some gestational age reductions in relation to PFHxS in the overall population. Although their continuous PFHxS exposure results were null, the *high* confidence study by Sagiv et al. (2018) showed small non-significant decreases for quartiles 3 and 4 albeit not in a non-monotonic fashion. Although their overall population results were null, based on each ln-unit increase, the *high* confidence study by Manzano-Salgado et al. (2017a) did show a small decrease in gestational age for quartile 4 (β= −0.16 weeks; 95%CI: −0.43, 0.1). The *medium* confidence study by Hjermitslev et al. (2020) reported a relatively large gestational age reduction (β= −0.32 weeks; 95%CI: −0.72, 0.08 per each ln-unit increase). The *medium* confidence study by Yang et al. (2022a) showed larger gestational age reductions among term births (β= −0.64; 95%CI: −1.64, 0.36) compared to preterm births (β= −0.20 weeks; 95%CI: −3.32, 2.93) per each ln-unit increase in Total PFHxS exposures. The *medium* confidence Meng et al. (2018) study reported a decrease based on continuous exposure (β= −0.29 weeks; 95%CI: −1.15, 0.58 per each ln-unit PFHxS increase) and small non-monotonic decreases across quartiles (β range: −0.06 to −0.17 weeks). The *low* confidence study by Gao et al. (2019) reported a non-monotonic decreased gestational age in relation to PFHxS tertiles 2 (β= −0.37 weeks; 95%CI: -0.82, 0.09) and 3 (β= −0.22 weeks; 95%CI: −0.71, 0.27). Although there was no evidence of an exposure-response relationship, the *high* confidence study by Gardener et al. (2021) reported that participants in the three upper PFHxS quartiles had smaller gestational ages (β range: −0.18 to −0.75) relative to quartile 1.

Although they were not always internally consistent across exposure metrics, seven (3 *high*, 3 *medium*, and 1 *low* confidence) of 17 studies in the overall population showed some gestational age reductions in relation to PFHxS exposures. Few study characteristics appeared to be related to patterns across the study results. For example, four of the seven studies showing inverse associations were based on early biomarker sampling. Study sensitivity in the six (three *high*, one *medium*, and one *low* confidence) may explain some of the null findings as half of the studies had deficient ratings (one good, two adequate, and three deficient).

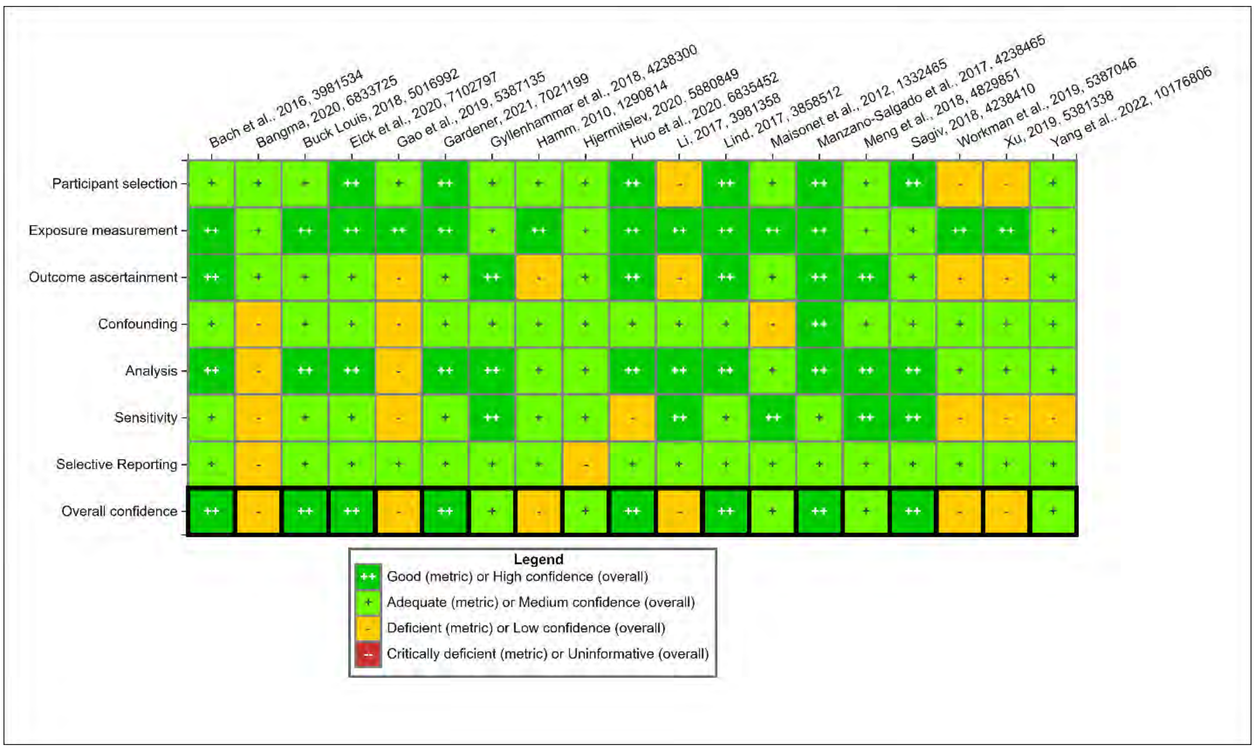


Figure 3-47. Study evaluation results for 19 epidemiological studies of gestational age and PFHxS. For additional details see HAWC link.

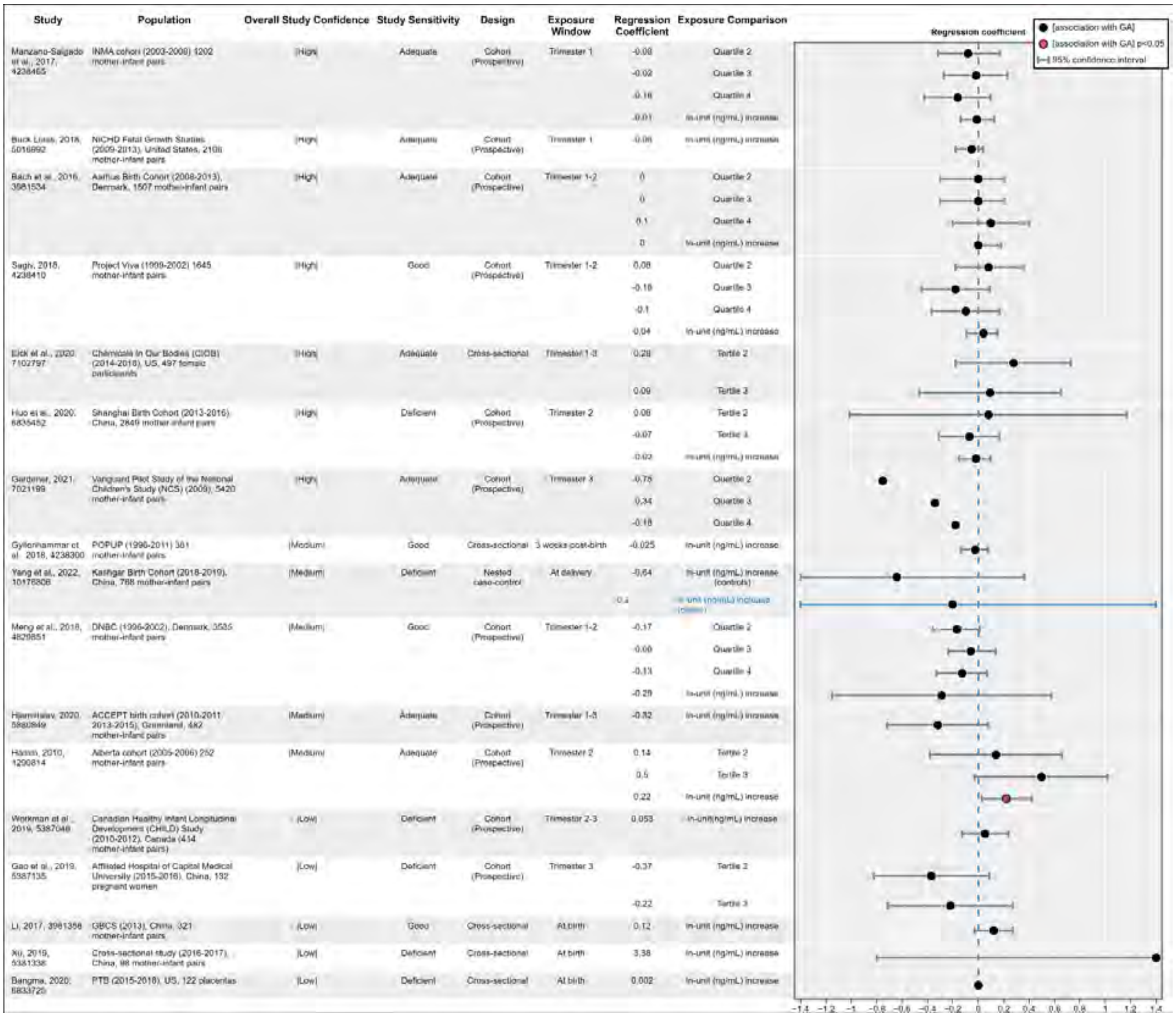


Figure 3-48. Overall population gestational age results for 17 PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: GA= Gestational Age

[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b]The (Yang et al., 2022a) -0.64 per IQR Increase value is reported in the term birth population; the -0.2 per IQR increase value is in the preterm birth population.

[c]Gardener gestational age differences estimated from digitization of their Figure 4; 95%CIs were not estimable.

[d]For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (e.g., Yang et al. (2022a)).

[e]Yang et al. (2022a) preterm results are truncated: the complete 95% CI ranges from -3.32 to 2.93.

[e]Yang et al. (2022b) term results are truncated; the complete 95% CI ranges from -1.64 to 0.36.

[f]Xu et al. (2019) results are truncated: the complete 95% CI ranges from -0.8 to 7.55.

[g]Unlike other studies that relied on maternal or cord serum or plasma (in ng/mL), Bangma et al. (2020) used placental exposure measures (in ng/g).

[h]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Gestational Age - Sex-specific Results

Eight (four *high*, three *medium,* and one *low* confidence) epidemiological studies examined mean gestational age in relation to PFHxS in either or both sexes including one that evaluated data in girls only (Maisonet et al., 2012) (see Figure 3-49). None of the seven studies in boys showed decreased gestational age with increasing PFHxS, with six studies showing null associations (Eick et al., 2020; Hjermitslev et al., 2020; Meng et al., 2018; Sagiv et al., 2018; Lind et al., 2017; Manzano-Salgado et al., 2017a). The *low* confidence study by Li et al. (2017b) reported a small increased gestational age per each ln-unit PFHxS increase (β= 0.20 weeks; 95%CI: −0.02, 0.42) among boys.

Five (Eick et al., 2020; Meng et al., 2018; Sagiv et al., 2018; Li et al., 2017b; Manzano-Salgado et al., 2017a) of the eight studies in girls reported null associations between PFHxS and mean gestational age, while another study (Eick et al., 2020) reported non-significant increased gestational age across tertiles (β range: 0.18 to 0.33). Three studies in girls showed some gestational age reductions including some that were moderately large in magnitude. The *high* confidence study by Lind et al. (2017) showed some suggestion of an exposure-response relationship for mean gestational age across the upper three PFHxS quartiles (β range: −0.33 to −0.86 weeks) including a large association (β= −0.86 weeks; 95%CI: −1.34, −0.29) in quartile 4 (0.4–7.3 ng/mL) versus quartile 1 (0.2–0.29 ng/mL). The medium confidence study by Hjermitslev et al. (2020) also reported a large gestational age reduction (β= −0.57 weeks; 95%CI: −1.04, −0.10 per each ln-unit increase). In their study population of female infants only, the *medium* confidence study by Maisonet et al. (2012) reported nonstatistically significant decreases in gestational age with some suggestion of an exposure-response relationship. They reported reduced gestational age in the second (β= −0.15 weeks; 95%CI: −0.52, 0.22 for 1.3–2.0 ng/mL) and third PFHxS tertiles (β= −0.24 weeks; 95% CI: −0.62, 0.14 for 2.0–54.8 ng/mL) compared with the lowest tertile (<1.3 ng/mL).

Overall, three (one *high* and two *medium* confidence) studies out of eight studies in girls only showed reduced gestational age in relation to PFHxS exposures. Although they were not always monotonic, both of the studies with categorical data showed some evidence of exposure-response relationships which lends support to the findings based on continuous exposure metrics. There was no evidence of inverse associations among boys, although half of the studies had deficient study sensitivity. Few other study characteristics appeared to be related to patterns across the study results; however, all three of the studies showing inverse associations in females were based on early biomarker sampling.

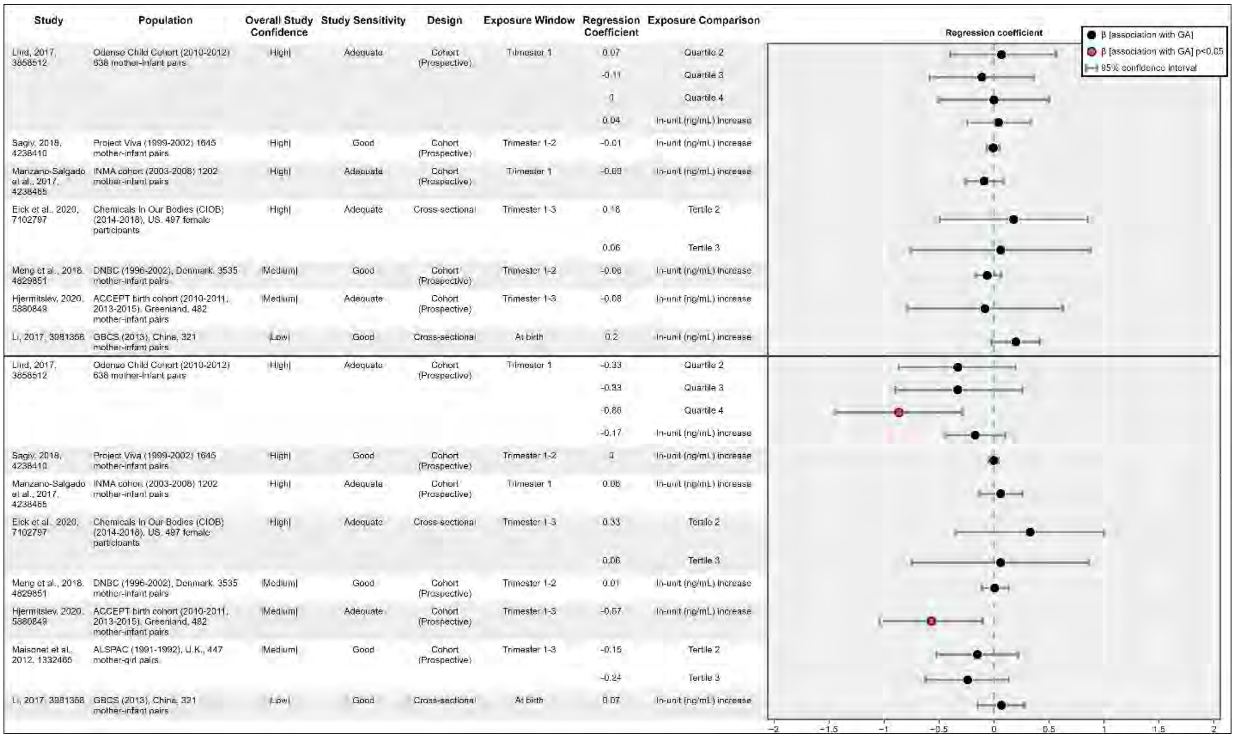


Figure 3-49. Sex stratified gestational age results for 8 PFHxS epidemiological studies. For additional details see HAWC link.

Abbreviations: GA= Gestational Age
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b]For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (e.g., Yang et al. (2022a)).
[c]Lind et al. (2017) results are truncated: the complete 95% CI ranges from –3.1 to 0.7.
[d]For evaluation of patterns of results, EPA considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses.

Gestational duration summary

There was mixed evidence within and between studies examining adverse associations between PFHxS exposure with any gestational duration measures (preterm birth or gestational age). Out of 19 total studies, 8 different ones showed gestational duration associations with PFHxS. Four of 10 studies showed some increased odds preterm birth and PFHxS exposures in the overall population or either/both of the sexes, although these were not always internally consistent. Seven of 17 studies in the overall population reported mean gestational age deficits in relation to PFHxS, while 3 of 8 studies with sex-specific data only reported inverse associations in girls. In addition to the null studies, a few studies also reported increased gestational age related to PFHxS exposures. Gestational age can be prone to some measurement error which may reduce the ability of some studies to detect statistically significant results for this endpoint. The preterm birth binary endpoint may also be less impacted by this measurement error given the broad classification of pre-term versus term births.

Table 3-20. Summary of 19 epidemiological studies of PFHxS exposure and gestational duration measures

Author	Study location/ years	N	PFHxS median (ng/mL) exposure	Overall confidence descriptor	Study sensitivity domain	PTB	GA
Bach et al. (2016)	Denmark 2008–2013	1,507	0.5	*High*	Adequate	∅ All	∅ All
Buck Louis et al. (2018)	USA, 2009–2013	2,106	0.71	*High*	Adequate		∅ All
Eick et al. (2020)	USA, 2014–2018	506	0.33	*High*	Adequate	∅ All	+ All ∅ Boys/Girls
Gardener et al. (2021)	USA, 2009–2013	354	0.5	*High*	Adequate	↑ All	– All
Huo et al. (2020)	China, 2013–2016	2,849	0.54	*High*	Deficient	∅ All/Boys ↑ Girls	∅ All
Lind et al. (2017)	Denmark, 2010–2012	636	0.3	*High*	Adequate		– Girls ∅ Boys
Manzano-Salgado et al. (2017a)	Spain, 2003–2008	1,202	0.58	*High*	Adequate	∅ All/Boys ↓ Girls	– All ∅ Boys/Girls
Sagiv et al. (2018)	USA, 1999–2002	1,645	2.4	*High*	Good	↑ All	– All ∅ Boys/Girls
Gyllenhammar et al. (2018); 2017[a]	Sweden, 1996–2001	381	2.4	*Medium*	Good		∅ All
Hjermitslev et al. (2020)	Greenland, 2010–2015	266	0.51	*Medium*	Adequate	∅ All	– All/Girls ∅Boys
Maisonet et al. (2012)	United Kingdom, 1991–1992	444	1.6	*Medium*	Good		– Girls[b]
Meng et al. (2018)	Denmark 1996–2002	2,132	~1	*Medium*	Good	↑ All	∅ All/Boys/Girls
Hamm et al. (2010)	Canada, 2005–2006	252	2.1	*Medium/ Low*[c]	Adequate	↓ All[b]	+ All
(Yang et al., 2022a)	China, 2018–2019	768	0.049–0.058[d]	*Medium*	Deficient	∅ All	– All[d]
Bangma et al. (2020)	USA, 2015–2018	122	0.067[e]	*Low*	Deficient		∅ All
Gao et al. (2019)	China, 2015–2016	132	0.24	*Low*	Deficient		– All

Author	Study location/ years	N	PFHxS median (ng/mL) exposure	Overall confidence descriptor	Study sensitivity domain	PTB	GA
Li et al. (2017b)	China, 2013	321	3.87	*Low*	Good		+ All/Boys ∅ Girls
Workman et al. (2019)	Canada, 2010–2011	414	0.44	*Low*	Deficient		∅ All
Xu et al. (2019)	China, 2016–2017	98	0.61 (0.30-1.94)[f]	*Low*	Deficient		+ Overall

Abbreviations: PTB = Preterm Birth; GA = Gestational Age.

*Denotes statistical significance at $p < 0.05$; ∅ : represents a null association; + : represents a positive association; – : represents a negative association; ↑: represents an increased odds ratio; ↓ : represents a decreased odds ratio; / implies that multiple groups shared the same classification.

Note: “Adverse effects” are indicated by both increased odds ratios () for dichotomous outcomes and negative associations (–) for the other outcomes.

[a]Gyllenhammar I (2017) and Gyllenhammar et al. (2018) results are included here (both analyzed the POPUP cohort).

[b]Exposure-response relationship detected based on categorical data.

[c]Hamm et al. (2010) was *medium* confidence for PTB and *low* confidence for GA.

[d]Median range across cases and controls.

[e]Exposure measured in placenta (ng/g).

[f]5th–95th percentiles.

Fetal Loss/Spontaneous Abortion

Five studies reported on the relationship between PFHxS exposure and spontaneous abortion (see Figure 3-50). A cohort of pregnant women enrolled at 8–16 weeks gestation (Jensen et al., 2015) was considered *low* confidence primarily due to loss to follow-up and the high risk of incomplete case ascertainment (i.e., not including women with losses that occurred prior to study enrollment, which may bias the results towards or even past the null if there is a true association between PFHxS exposure and spontaneous abortion (Radke et al., 2019)). Liew et al. (2020) is a case-control study that identified cases via medical registry and also has the potential to miss early losses. However, this study was not downgraded to *low* confidence as loss to follow-up was not a concern. Three additional studies were considered *medium* confidence, two case-control studies of first trimester miscarriage (Mi et al., 2022; Wikström et al., 2021) and a cohort of women undergoing their first *in vitro* fertilization-embryo transfer treatment cycle (Wang et al., 2021a). Notably, Mi et al. (2022) measured sodium perfluoro-1-hexanesulfonate, a related salt, rather than PFHxS.

Jensen et al. (2015) reported an increased OR (1.53; 95% CI: 0.99, 2.38) for spontaneous abortion for each ln-unit increase in exposure despite study sensitivity limitations. While this study is *low* confidence, the bias is unlikely to be away from the null (as described above), and thus the limitations are unlikely to explain the observed positive association. However, the other four studies, all *medium* confidence, reported no association between PFHxS exposure and early spontaneous abortion. It is possible that there is only an association with second trimester spontaneous abortion, but the evidence is currently not adequate to make this determination and there is considerable uncertainty due to inconsistency across studies.

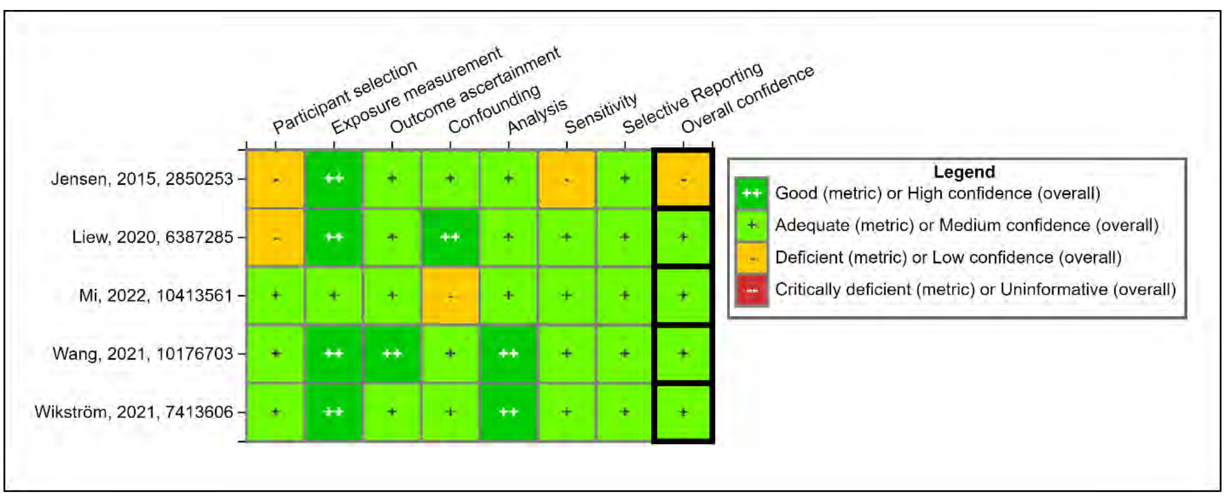


Figure 3-50. Study evaluation results for nine epidemiological studies of fetal loss and PFHxS. For additional details see HAWC link.

Birth Defects

Two studies examined birth defects in relation to PFHxS exposures (see Figure 3-51). The *medium* confidence congenital heart defect study by Ou et al. (2021) reported null associations risks for PFHxS ≥0.153 ng/mL (vs. <0.153 ng/mL) for septal defects (OR=1.07; 95%CI: 0.52, 2.22), and total heart defects (OR=1.03; 95%CI: 0.65, 1.64), although a non-significant inverse risk was seen for conotruncal defects (OR=0.64; 95%CI: 0.28, 1.49). Relative to tertile 1, the *low* confidence Cao et al. (2018) study showed evidence of monotonic associations between all birth defects and PFHxS tertiles 2 (OR=2.24; 95%CI: 1.05, 5.27) and 3 (OR=2.54; 95%CI: 1.06, 6.13). There is considerable uncertainty in interpreting results for broad all birth defect groupings which decreases study sensitivity given the etiological heterogeneity across different birth defects.

Overall, there was limited evidence of associations between PFHxS and birth defect based on the two available epidemiological studies. Despite an exposure-response relationships in one *low* confidence study based on an all (i.e., total) birth defect grouping, there is currently insufficient data for any specific birth defects to draw further conclusions given the limitations noted above.

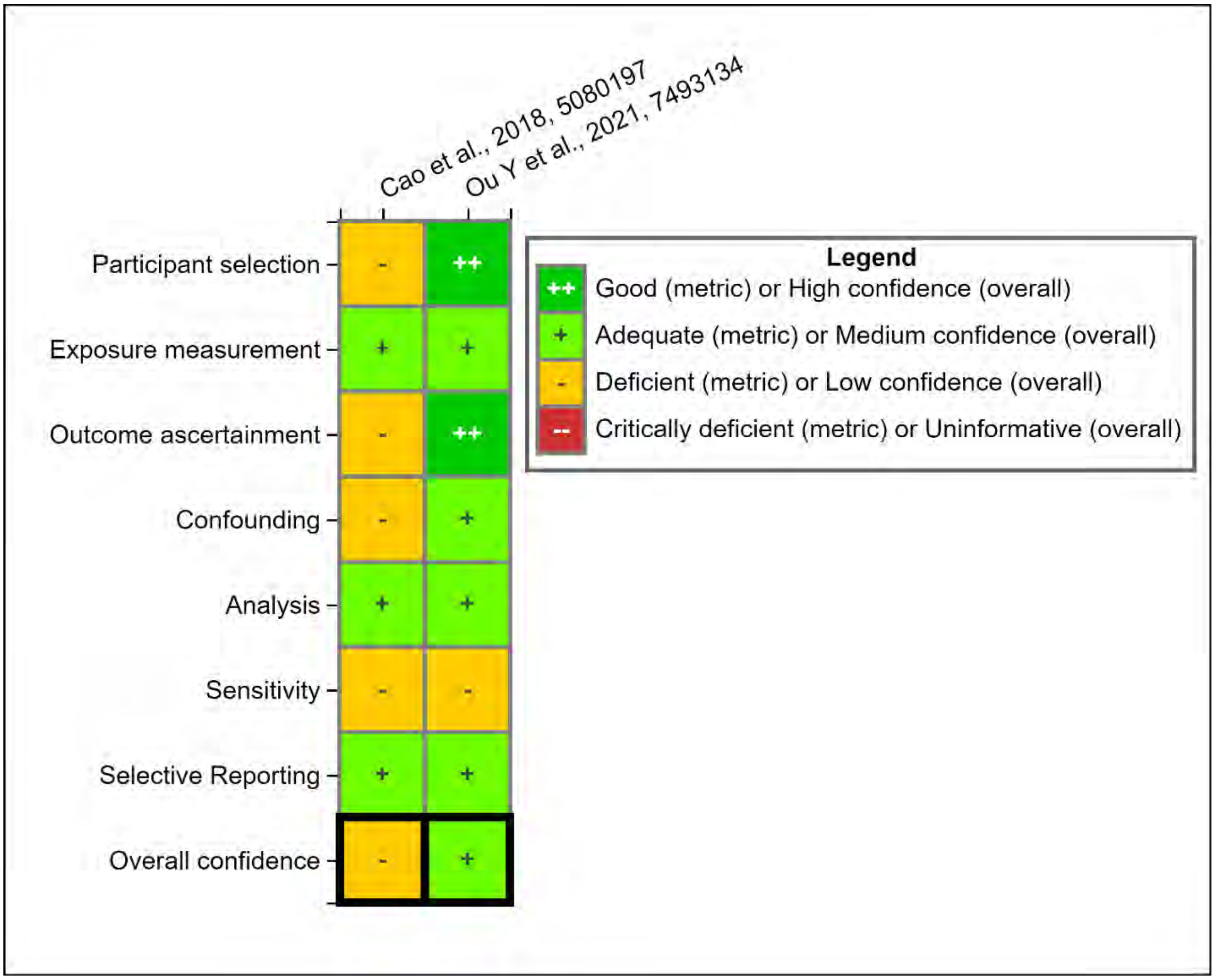


Figure 3-51. Summary of study evaluation for 2 epidemiology studies of birth defects. For additional details see HAWC link.

Animal Studies

Five of the available toxicology studies evaluated PFHxS-induced effects in developing animals. Three studies exposed Wistar rats (Butenhoff et al., 2009; 3M, 2003) or CD-1 mice (Chang et al., 2018) to PFHxS for 14 days before mating, and during mating, gestation, and lactation while Marques et al. (2021) treated CD-1 mice with PFHxS from GD1 to PND20; one study exposed Wistar rats from GD7 to PND22 (Ramhøj et al., 2018); and a separate study using Wistar rats treated animals from GD7 to GD22 and from PND1 to PND22. These studies administered PFHxS (doses ranging from 0.03 to 45 mg/kg-day) via gavage and evaluated maternal toxicity and fetal survival, growth, and morphological development. The Butenhoff et al. (2009), 3M (2003) and Chang et al. (2018) studies were evaluated as high confidence, while the Ramhøj et al. (2018), Marques et al. (2021), and Tetzlaff et al. (2021) studies were evaluated as medium confidence (see Figure 3-52). Concerns in the Ramhøj et al. (2018), Tetzlaff et al. (2021), and Marques et al. (2021) studies were noted for allocation, and the reporting of the number of animals per exposure group.

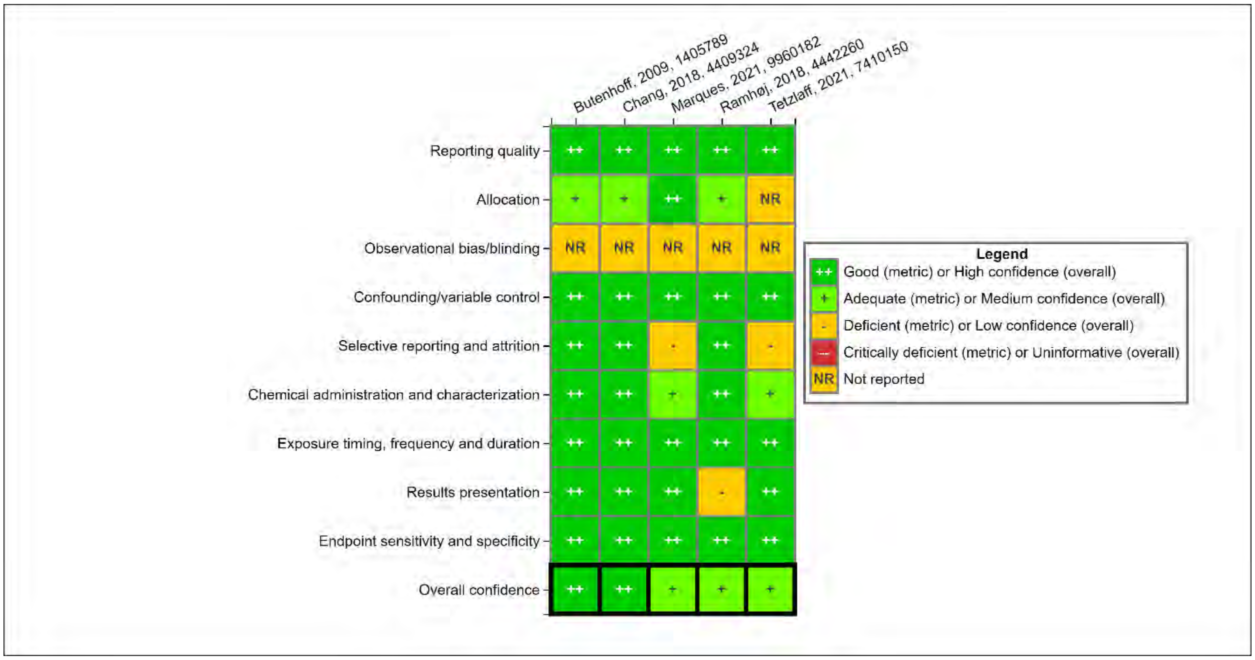


Figure 3-52. Developmental animal study evaluation heatmap. For additional details see HAWC link.

Maternal health

The health of the dams was assessed in all available studies except Tetzlaff et al. (2021) (see Figure 3-53). Butenhoff et al. (2009); 3M (2003) reported that Sprague Dawley rats administered PFHxS displayed decreased maternal body weight (6% to 8% relative to controls) during the lactation period: on PNDs 4, 6–8, 11, and 13 at the lowest dose (0.3 mg/kg-day); on PNDs 7 and 8 at 3 mg/kg-day; and on PNDs 4, 6–9, 11, 13, and 14 at the highest dose (10 mg/kg-day). However, these decrements are considered minimal, the animals recovered from these effects at weaning (PND 22), and studies in CD-1 mice (Marques et al., 2021; Chang et al., 2018) or Wistar rats (Ramhøj et al., 2018) did not report significant PFHxS-induced effects on maternal body weight during gestation or lactation. Maternal food consumption was also not affected in exposed rats or mice (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). Additional outcomes evaluated in F0 females included kidney and liver weights, reproductive organ weights and histopathology, and maternal serum thyroxine levels, which are discussed in those respective sections (see Sections 3.2.3, 3.2.4, and 3.2.10). Briefly, significant treatment-related increases were observed for mean liver weight and the incidence of histopathological findings at 3 mg/kg-day in CD-1 mice (Chang et al., 2018), and significant treatment- and dose-related decreases were observed in serum thyroxine levels in Wistar rats (Ramhøj et al., 2018); see hepatic and thyroid effect sections (see Sections 3.2.5 and 3.2.1, respectively) for more detail.

Fetal viability

Endpoints related to fetal and postnatal viability were measured in the Butenhoff et al. (2009), Chang et al. (2018), Marques et al. (2021), and Ramhøj et al. (2018) studies. Post-implantation loss, perinatal loss, number of live pups, litter size, and number of stillborn pups were not affected by PFHxS exposure in Sprague Dawley or Wistar rats (Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003), and Marques et al. (2021) reported no PFHxS-induced effects on live births per litter in CD-1 mice. However, a similar study in CD-1 mice reported that exposure to PFHxS at 1 and 3 mg/kg-day decreased the related measures of live litter size (by 14% and 12%, respectively) and the number of pups born per litter (by 12% and 11%, respectively) (Chang et al., 2018). An explanation for the lack of dose-dependence of these observations is unavailable. Decreased litter size is considered an indirect indication of pre-implantation loss and resorptions (IPCS, 2006), but the Chang et al. (2018) study did not measure either of these two outcomes. This mouse study also evaluated the number of pups born-to-implant ratio and pup survival and reported no treatment-related effects (Chang et al., 2018). The finding of reduced litter size and live pups per litter in mice but not in rats exposed to higher PFHxS levels is not explainable by differences in pharmacokinetics, study design, or study evaluation considerations. Furthermore, the toxicological significance of these effects observed in mice is not clear as these responses did not appear to be dose dependent; other measured developmental outcomes were not altered in the Chang et al. (2018) study.

Fetal growth

F1 animal growth was evaluated in all available animal developmental studies. PFHxS exposure did not affect pup body weights in male or female Sprague Dawley and Wistar rats, or in CD-1 mice (Marques et al., 2021; Tetzlaff et al., 2021; Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003). Furthermore, no significant treatment-related effects were observed on sex ratio in Sprague Dawley and Wistar rats (Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003), or in CD-1 mice (Chang et al., 2018) suggesting PFHxS exposure did not specifically affect male or female animals.

Morphological development

Gross pathological examination of F1 pups revealed no significant exposure-related developmental effects in exposed Sprague Dawley and Wistar rats, or CD-1 mice (Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003).

Small but significant alterations in F1 AGD at birth were observed in CD-1 mice and Wistar rats (Chang et al., 2018; Ramhøj et al., 2018). Chang et al. (2018) reported that adjusted (i.e., relative to cube root body weight) PND1 AGD was increased by 3% to 5% in male CD-1 mice at doses ranging from 0.3 to 3 mg/kg-day; and in female PND1 mice, adjusted AGD was decreased by 5% only at the mid-dose (1 mg/kg-day). AGD is used as a phenotypical marker of androgen levels/production during the masculinization programming window (Foster and Gray, 2013)[9]. Other phenotypical markers of androgen disruption were not altered in the available studies. On PND13 male nipple retention (another marker indicative of hormonal alterations (Foster and Gray, 2013)) was not altered by PFHxS treatment in CD-1 mice, and puberty onset was not affected in either CD-1 mice or Wistar rats (Chang et al., 2018; Ramhøj et al., 2018). Additionally, male, and female reproductive organ weights in F1 CD-1 mice (at PND36) and Wistar rats (males at PND16, females at PND17 or 22) were not affected by PFHxS treatment (Chang et al., 2018; Ramhøj et al., 2018).

The biological significance of the small and directionally inconsistent changes in androgen dependent AGD measures in animal and human studies is unclear. Taken together, the available evidence does not support an effect on reproductive organ development by PFHxS exposure in these animal studies.

[9] In rodent models and in humans AGD is longer in males when compared to females (Dean and Sharpe, 2013). Decreases in AGD are associated with androgen disruption during the masculinization programming window (Dean and Sharpe, 2013; Foster and Gray, 2013), whereas increased AGD in females could be indicative or increased androgen levels or activation of the androgen receptor (Foster and Gray, 2013). Exposure to chemicals known to impair androgen synthesis or antagonize the androgen receptor have been shown to result in decreased AGD as well as effects on other indicators of hormone disruption (e.g., increased nipple retention) or adverse effects in the reproductive system (e.g., testicular atrophy, epididymal malformations, testicular size, hypospadias, reduced size of the testis and accessory reproductive glands) (Dent et al., 2015).

Figure 3-53. PFHxS-induced developmental effects. Figure displays the *high* and *medium* confidence studies included in the analysis. For additional details see HAWC link. Details on study confidence may be found in Figure 3-30. Note: while some of the decreases in maternal body weight were statistically significant, these small changes are of unclear biological significance and not necessarily adverse.

Evidence Integration

The currently available **evidence suggests** but is not sufficient to infer that PFHxS might cause developmental effects in humans given sufficient exposure conditions[10]. This judgment is based on *slight* human evidence, specifically the fairly consistent, but notably uncertain, evidence of decreased birth weight and some coherent changes in other growth parameters from studies of exposed humans in which PFHxS was measured pre-conception or either during or shortly after pregnancy (see Table 3-21~~18~~). As discussed earlier (see Appendix C for more details), with the exception of post-partum samples, fairly consistent small (but often statistically significant) birth weight deficits were detected in EPA's meta-analysis of epidemiological studies including those based on early sample timing. Overall, although there are data that suggest changes in fetal growth are related to PFHxS exposures, additional evidence (e.g., more epidemiological study of PFHxS exposure on birth weight with earlier biomarker sampling that helps to reduce uncertainties in the current evidence base) would be needed to draw a stronger judgment.

Although not entirely consistent, the epidemiological evidence includes a large fetal growth restriction database with some of the most accurate endpoints available (e.g., birth weight is generally measured with little error). The available epidemiologic studies showing birthweight-related differences for continuous exposure data (β range: −12 to −145 grams per each ln-unit increase) and categorical (β range: −25 to −101 grams for the highest quantile compared to the lowest quantile) showed results comparable in magnitude and provided some support of a biologic gradient, albeit the categorical data to a lesser degree given lack of monotonicity across quantiles. For example, many studies based on continuous exposure data (per each increasing unit change in PFHxS) showed fairly comparable birth weight-related deficits ranges in either boys or girls (β range: −25 to −145 g) or in the overall population (β range: −12 to −93 g). There also was some evidence of exposure-response relationships based on categorical data in 3 of 16 epidemiological studies, although these were predominately driven by sex-specific findings.

Taken together, some birth weight deficits of varying magnitude were detected in 17 of 31 studies included in the main developmental synthesis, including 14 of 27 (and 10 of 21 *medium*/*high* confidence) studies that examined associations in the overall population and 8 of 14 that reported mean birth weight deficits in either male or female neonates or both. Based on EPA's meta-analysis, similar birth weight deficits per ln-unit PFHxS increase were seen across all 27 studies (β= −7.7 g; 95%CI: −14.8, −0.5 per each ln-unit increase), 23 *medium* and *high* confidence studies (β= −8.0 g; 95% CI: −15.2, −0.7), or for the 12 *high* confidence studies (β= −6.8 g; 95% CI: −16.3, 2.8). No gradient was seen across confidence levels or by biomarker sample timing. Although limited by a small sample size and considerable variation in results across studies, some deficits were detected for five post-partum sampled studies (β= −28.3 g; 95% CI: −69.3, 12.7) using umbilical cord samples or maternal samples after birth; this may be reflective of bias due to

[10] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

pregnancy hemodynamic changes. In contrast, 12 studies based on earlier pregnancy sampling periods (e.g., any first trimester sampling) showed deficits (β= −7.3 g; 95% CI: −16.0, 1.4) similar in magnitude to the overall pooled estimate of all 27 studies and those restricted to *medium* and *high* confidence. Given that these patterns are not consistent with what EPA has seen for other PFAS such as PFNA (Wright et al., 2023) and what others have reported for PFOA and PFOS Dzierlenga et al. (2020); Steenland et al. (2018), it remains unclear whether any differences noted between late pregnancy and postpartum samples is unique to PFHxS.

Examining birth weight differences in human populations is challenging, and it can be difficult to differentiate pathological deficits versus natural biological variation in distributions within study populations. The magnitude of birth weight deficits across categorical and continuous exposures in the individual studies, for example, ranged from −12 to −145 grams, depending on the exposure contrasts being compared. The meta-analysis of the 27 studies that EPA conducted showed a small but statistically significant decrease in mean birth weight (β= −7.7 g; 95% CI: −14.8, −0.5) per ln-unit increase in PFHxS. This overall result was similar when studies were restricted to just the 12 *high* (β= −6.8 g; 95% CI: −16.3, 2.8) confidence studies or the 19 combined *medium* and *high* confidence studies (β= −7.1 g; 95% CI: −15.2, 1.0). The public health significance of small changes in birth weight noted here in this meta-analysis may not be immediately evident. On a population level, even small changes, if causally related, can increase the number of infants at higher risk for other co-morbidities and mortality especially during the first year of life. And, therefore, small decrements may have a large public health impact if these shift the birth weight distribution to include more infants in the low-birth-weight category. Additionally, decreased birth weight has been associated with long-term adverse health outcomes such as cardiovascular disease and diabetes (Osmond and Barker, 2000). Thus, this magnitude of decrease is considered to be of concern.

Providing some evidence for changes coherent with the observed birth weight decreases, decreases, 5 of 7 small for gestational age and low birth weight studies showed increased risk in relation to PFHxS exposures. Additional evidence was seen in 12 of 18 (including 9 of 16 in the overall population) birth length studies that showed associations of smaller birth length with increasing PFHxS exposures, including 5 of 6 available *high* confidence studies. These results were fairly small in magnitude. In addition, there was some support for these findings from coherent effects related to postnatal weight measures (as 5 of 8 studies showed inverse associations), albeit the other postnatal growth endpoints were null or mixed.

In addition to the uncertainty related to potential bias from pregnancy hemodynamics in developmental epidemiological studies, a common area of concern when interpreting epidemiological findings on individual PFAS is the potential for confounding by PFAS co-exposures. As noted for other endpoints in general, despite extensive and advanced statistical modeling attempts, it can be difficult at times to completely isolate an independent effect for each individual PFAS when real-world exposures involve a myriad of sources. Although there were some moderate to strong positive correlations between PFHxS and some other PFAS, there were no consistent patterns in magnitude of effects detected in models that adjusted for other PFAS (see detailed write-up in Appendix C). Thus, while confounding by other PFAS remains a general source of uncertainty in epidemiological studies, the lack of a consistent patterns across the available studies here does not provide strong evidence of this possibility.

The available evidence on PFHxS-induced developmental effects in animal toxicity studies is considered *indeterminate*. The available animal studies do not provide evidence coherent with the epidemiological observations of effects on fetal growth (i.e., rodent offspring body weights were generally unaffected). Similarly, PFHxS exposure during early developmental stages did not impact the incidence of developmental malformations or alter reproductive organ development. One *high* confidence study reported a significant decrease in litter size and numbers of pups per litter in CD-1 mice that was not dose-dependent (Chang et al., 2018) (note: a single, *low* confidence epidemiological study evaluating an outcome related to fetal survival showed a marginally statistically significant increased odds of fetal loss with increasing PFHxS exposure). However, (Chang et al., 2018) also reported that the number of pups born-to-implant ratio was unaffected, and two separate *high* and *medium* confidence studies in rats reported no significant treatment-related effects on fetal survival endpoints at the same or higher PFHxS levels (Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003). Chemical-induced reduction in litter size can provide an indirect indication of pre-implantation loss (IPCS, 2006); however, this was not evaluated in any of the available gestational PFHxS exposure studies in animals, highlighting a significant data gap.

Several epidemiological and animal toxicity studies report alterations in AGD. However, the biological significance of the small and directionally inconsistent changes as well as lack of consistency with other markers of androgen-dependent phenotypical outcomes and developmental measures adds uncertainty to the available evidence. Overall, the available studies do not support an effect on reproductive organ development by PFHxS exposure.

Overall, the available **evidence suggests** but is not sufficient to infer that PFHxS exposure may have the potential to cause developmental toxicity in humans given sufficient exposure conditions[11]. A stronger evidence integration judgment was not drawn due to some important sources of uncertainty in the epidemiological literature (most notably, uncertainty due to potential bias by pregnancy hemodynamics) that appear to reflect complex patterns of biological influence that are not completely understood. Nonetheless, the consistent and coherent epidemiological findings on fetal growth restriction warrant further examination to disentangle these uncertainties and improve understanding of whether and to what extent PFHxS exposure during these sensitive lifestages might contribute to growth restriction in children.

[11] Given the uncertainty in this judgement and the available evidence, this assessment does not derive a toxicity value that might better define the “sufficient exposure conditions” for developing this outcome (see Section 5 discussion).

Table 3-21. Evidence profile table for PFHxS related developmental effects

Evidence stream summary and interpretation					**Evidence integration summary judgment**
Evidence from studies of exposed humans (see Development Human Section)					⊕⊙⊙ **Evidence suggests, but is not sufficient to infer**
Evidence from human studies-fetal growth restriction					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Fetal growth (Mean birth weight /z scores/small for gestation age/low birth weight) 9 *high*, 7 *medium*, and 5 *low* confidence studies	• Consistent findings of some inverse associations in 20 of 34 (including 14 of 26 high or medium confidence) studies • Inverse associations in 17 of 31 mean birth weight studies and 14 (5 high; 5 *medium*; 4 low) of 27 in overall population across all study confidence levels • Although they varied across confidence	• Imprecision of some birth weight deficits • Concern for potential confounding by co-exposures to highly correlated PFAS • Exposure-dependence limited, including monotonic relationships, in only 3 of 14 different birth weight studies with categorical data in overall population or either sex; lends limited support to studies based	• 20 of 34 overall birth weight studies (including 14 of 26 medium or high confidence) studies showed inverse associations in the overall population, or among boys or girls • Meta-analysis conducted by US EPA showed a small but statistically significant birth weight deficit (7.7 g; 95% CI: −14.8, -0.5) per each ln-unit PFHxS increase; results were	⊕⊙⊙ *Slight* Based primarily on consistent evidence for birth weight reductions and coherent findings for other fetal and postnatal weight endpoints, but strength was reduced due to concern for confounding and limited evidence of dose-dependence across most studies with categorical data.	*Primary basis*: Consistent human evidence of decreased birth weight and coherent findings across multiple other fetal and early-life measures of growth. Median PFHxS values spanned from 0.09 to 10.36 ng/mL across the birth weight meta-analysis studies. *Human relevance*: N/A (based on human evidence) *Cross-stream coherence*: N/A (animal evidence indeterminate) *Susceptible populations and lifestages*: Pregnancy and early life

Evidence stream summary and interpretation					Evidence integration summary judgment
	levels, some reported mean birth weight deficits (up to −145 g) and relative risks were fairly large in magnitude • Statistically significant meta-analysis results for mean birth weight from continuous exposure metrics (-7.7 g; 95%CI: -14.8, -0.5 per each ln-unit increase); this was comparable to high (-6.8 g) and medium (-9.6 g) confidence studies • Overall meta-analysis birth weight results (-7.7 g) comparable to early pregnancy (-7.3 g) studies; suggests results not likely due to	on continuous exposure metrics	comparable in magnitude across early sampled studies and high and medium confidence studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
	pregnancy hemodynamics • Evidence among 6 of 13 standardized birth weight studies primarily seen in high (4 of 8 high and medium (1 of 3) confidence studies • 5 of 7 studies examining either small for gestational age, low birth weight or very low birth weight showed some increased risks with increasing PFHxS exposures among the overall population or either girls or boys (quite variable in magnitude, OR range: 1.3–9.1)				

Evidence stream summary and interpretation					Evidence integration summary judgment
Fetal growth restriction (birth length) 6 *high, 5 medium*, and 7 *low* confidence studies	• Consistent findings of some inverse associations in 9 of the 16 studies in the overall population (5 high, 1 medium, and 3 low confidence)	• None of the 5 studies with categorical data showed dose-dependent associations in the overall population although 2 of 3 sex-specific analyses did (both from same birth cohort). • Concern for potential confounding by co-exposures to highly correlated PFAS • Some concern for potential bias due to sample timing (pregnancy hemodynamics) as 6 of 9 studies with inverse associations were based on later biomarker sampling; although this did not bear out in	• 9 of 16 studies reported adverse effects, including all 5 of 6 high and 1 of 5 medium confidence studies		

Evidence stream summary and interpretation					**Evidence integration summary judgment**
		the sex-specific analyses.			
Fetal growth restriction (head circumference) 5 high, 5 medium, and 4 low confidence studies	• 8 of 14 studies in total showed inverse associations, including 7 of 12 studies in the overall population (4 of 5 high; 2 of 4 medium and 1 of 3 low confidence) • Exposure-dependence in 1 of 2 studies with categorical data • Limited concern over pregnancy hemodynamics as 5 of 7 studies with inverse associations in the overall population were based on early biomarker sampling	• Concern for potential confounding by co-exposures to highly correlated PFAS	• 8 of 14 studies (5 high; 2 medium and 1 low confidence) reported adverse associations, including 4 of 5 high confidence studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
Anogenital distance (AGD) 4 medium confidence studies	• No factors noted	• No factors noted	• Inverse association between PFHxS exposure and AGD in 1 of 4 *medium* confidence studies in boys and in 1 of 3 studies in girls		
Evidence from human studies postnatal growth					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Postnatal growth-Weight measures: 5 high, 3 medium, and 3 low confidence studies	• Consistent findings of inverse associations across 5 of the 8 studies of infant weight with more evidence among girls • Mixed results were seen among four studies of rapid growth (2 of 4 studies). • Limited to no evidence of	• Inconsistent periods of follow-up and assessment (e.g., childhood age at examination) precludes more direct comparison across studies. • Concern for potential confounding by co-exposures to highly correlated PFAS	• 5 of 8 studies showed some evidence of postnatal weight reductions which showed some coherence with birth weight deficits. • The other endpoints were mixed or provided limited or no evidence of associations.		

Evidence stream summary and interpretation					Evidence integration summary judgment
	associations for postnatal height (1 of 5 studies), head circumference (0 of 3 studies) in overall population or either sex. • No evidence of associations with adiposity (0 of 5 studies) in the overall population, but 2 of 3 studies did report this for boys.				
Evidence from human studies-gestational duration					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
<u>Preterm birth</u> 6 *high* and 4 *medium* confidence studies	• All 10 published studies were *high* or *medium* confidence	• Unexplained inconsistency • Concern for potential confounding by co-exposures to highly correlated PFAS	• 4 of 10 studies showed some evidence of adverse associations		

Evidence stream summary and interpretation					Evidence integration summary judgment
<u>Gestational age</u> 8 *high,* 5 *medium,* and 6 *low* confidence studies	• 4 of the 7 studies were based on early biomarker sampling; suggesting that pregnancy hemodynamics may have less of an impact in this subset. • There was a preponderance of associations among girls with 2 of 3 of the studies with categorical data showing some exposure-response relationship.	• Unexplained inconsistency • One-half of the studies in boys were deficient in study sensitivity • Concern for potential confounding by co-exposures to highly correlated PFAS	• 8 of 19 studies in total as well as 7 (3 *high*, 3 *medium*, and 1 *low* confidence) of 17 studies in the overall population showed some gestational age reductions • 5 of the 8 sex-specific studies reported associations in girls, while none of the studies in the boys did.		
<u>Spontaneous abortion</u> 4 *medium* and 1 *low* confidence study	• No factors noted	• *Low* confidence study reporting an effect	• 1 *low* confidence reported a positive association despite bias towards null, but 4 *medium* confidence studies reported no associations.		

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from in vivo animal studies (see Developmental Animal Section)					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Maternal health, fetal viability, fetal growth, morphological development 2 *high* confidence studies: • GD0 – PND22 • GD7 – PND22 1 *high* confidence study: • GD0 – PND22	• *High* confidence studies	• Unclear biological significance of small maternal weight changes • Lack of expected dose-dependence for litter size decrease in 1 study	• Decreased litter size in 1 of 3 studies • No notable PFHxS-induced effects on maternal health, fetal viability, fetal growth, and gestation duration. • Studies did not evaluate pre-implantation loss	⊙⊙⊙ *Indeterminate*	

3.2.4. Hepatic Effects

Human Studies

Thirteen epidemiology studies (reported in 14 publications) report on the relationship between PFHxS exposure and liver effects, primarily serum liver enzymes. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Alkaline phosphatase (ALP), bilirubin, and γ-glutamyltransferase (GGT) are also routinely used to evaluate potential hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Elevation of liver serum biomarkers is frequently an indication of liver injury, although they are not as specific as functional tests, which are currently not available for PFHxS.

Of 13 available epidemiology studies, 10 were classified as *medium* confidence, 2 as *low* confidence, and 1 was considered *uninformative* (see Figure 3-54). Jiang et al. (2014) was considered *uninformative* due to critical deficiency in the confounding domain as well as a lack of information on participant selection (deficient) and was excluded from further analysis. The majority of the available studies were cross-sectional studies in adults, four of which (Omoike et al., 2020; Jain and Ducatman, 2019c; Gleason et al., 2015; Lin et al., 2010) were analyses of different NHANES study populations (1999–2004, 2007–2010, 2011–2014, 2005–2012 respectively). The inclusion criteria in these NHANES studies varied across analyses (e.g., Gleason et al. (2015) included adolescents as well as adults, fasting was required in Lin et al. (2010), individuals who were carriers of hepatitis B or C virus were not excluded in Jain and Ducatman (2019c)). Because of the overlapping population in Omoike et al. (2020) with the previous studies, this paper was not considered a separate study. The other cross-sectional studies were in populations in Canada (Cakmak et al., 2022), China (Liu et al., 2022a). In addition, there was a cohort of elderly adults (Salihovic et al., 2018) and a birth cohort with follow-up into childhood (Mora et al., 2018). In children and adolescents, in addition to the NHANES 2007–2010 analysis in Gleason et al. (2015) that included adolescents but did not provide stratified estimates, Attanasio (2019b) examined NHANES data from 2013 to 2016 in adolescents. A multicenter birth cohort examined liver enzymes in childhood and was considered *medium* confidence (Stratakis et al., 2020). There were also two *low* confidence studies of children. Khalil et al. (2018) was a pilot cross-sectional study of 48 obese children, and there was concern for potential for selection bias and confounding. Jin et al. (2020b) was a cross-sectional study of children who had nonalcoholic fatty liver disease and analyzed the odds of severe disease (non-alcoholic steatohepatitis) with PFHxS exposure. This was the only study that did not examine liver function tests, but there were concerns for confounding due to lack of adjustment for socioeconomic status and inclusion of BMI, which may lie on the causal pathway. Across the studies of liver function, liver enzymes were analyzed appropriately in serum. Analysis of PFHxS in serum or plasma samples was also appropriate in all studies.

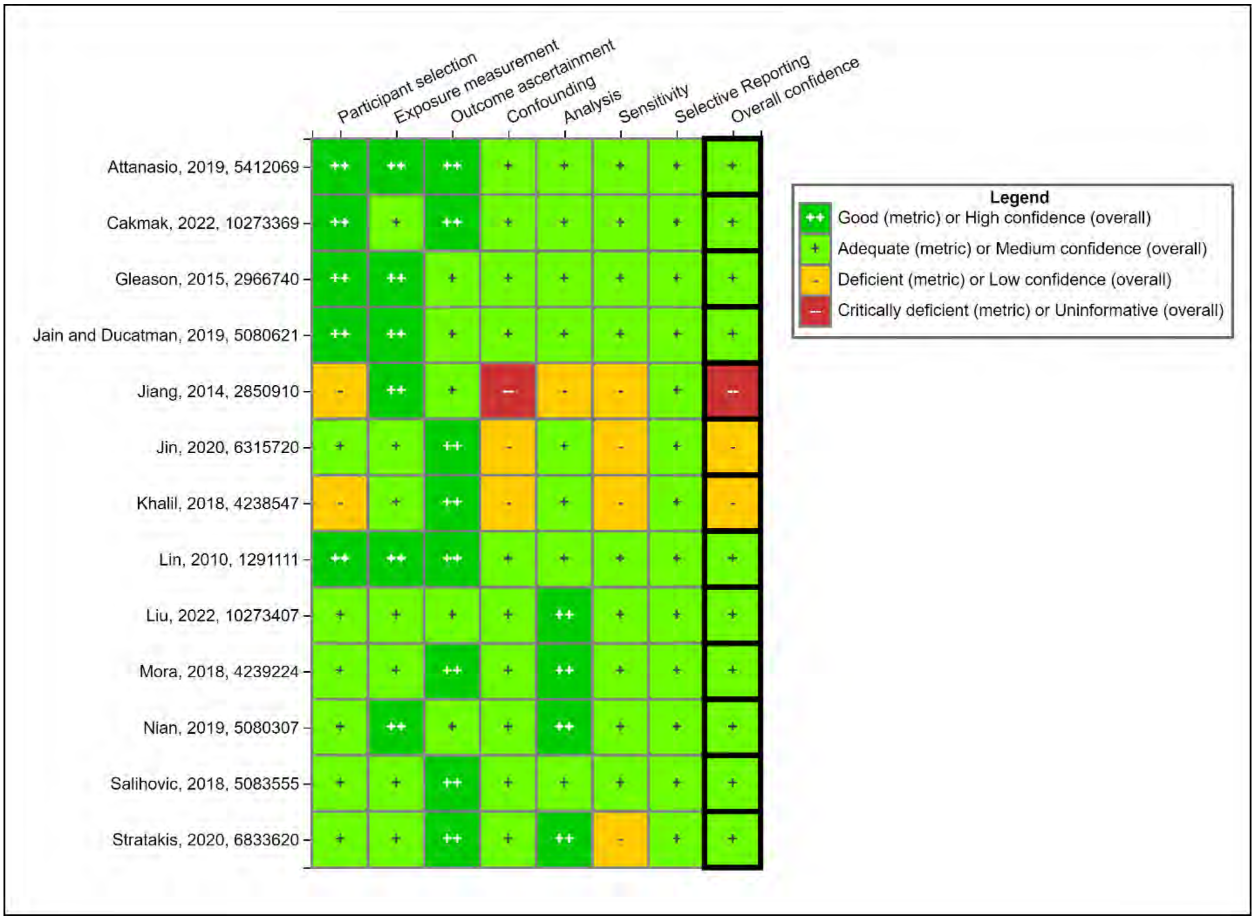


Figure 3-54. Hepatic effects human study evaluation heatmap. For additional details see HAWC link. Multiple publications of the same study: **Attanasio (2019b)** also includes **Attanasio (2019a)**

The results for the ten *medium* confidence studies are presented in Table 3-19. Five studies reported small, but statistically significant, positive associations between serum ALT and PFHxS exposure (Cakmak et al., 2022; Liu et al., 2022a; Jain and Ducatman, 2019c; Salihovic et al., 2018; Gleason et al., 2015), although in Jain and Ducatman (2019c), this was observed only in obese participants. Lin et al. (2010) and Nian et al. (2019) also reported positive associations, but with imprecise estimates. A study in children reported a nonsignificant inverse association for ALT (Mora et al., 2018).

For other enzymes, the direction of association varied across studies Gleason et al. (2015) and (Liu et al., 2022a) also reported significant positive associations with AST, ALP, and total bilirubin, while Salihovic et al. (2018) reported a significant positive association with ALP but an inverse association with total bilirubin. Other studies reported non-statistically significant associations in both directions for different enzymes (Cakmak et al., 2022; Nian et al., 2019). In adolescents, Attanasio (2019b) reported positive associations with total bilirubin but no clear associations with other enzymes analyzed continuously. There were positive associations ($p > 0.05$)

in girls with elevated ALT, AST, and GGT (dichotomous based on upper reference limits). The other *medium* confidence study in children (Stratakis et al., 2020) did not report results for individual liver enzymes but defined liver injury risk as having any liver enzyme concentration above the 90th percentile for the study population. They found no association between liver injury risk and PFHxS exposure. The *low* confidence study (Khalil et al., 2018) also reported no association between PFHxS and liver enzymes. In children with nonalcoholic fatty liver disease, higher PFHxS exposure was associated with the presence of nonalcoholic steatohepatitis (OR [95% CI]: 4.18 [1.64, 10.7] per IQR increase). Positive associations were also observed with grade of steatosis ($p > 0.05$), lobular inflammation, portal inflammation, ballooning ($p > 0.05$), and liver fibrosis (Jin et al., 2020b).

Given the consistency of direction of association for ALT across most of the studies in adults, there is some indication that PFHxS exposure may be associated with hepatic effects. However, there is still some uncertainty due to the small or imprecise nature of some ALT increases as well as the inconsistency of results for other liver enzymes. The single available *low* confidence epidemiology study of liver histology (Jin et al., 2020b) indicates an association between PFHxS exposure and disease severity (i.e., nonalcoholic steatohepatitis), but these findings should be interpreted with caution due to the potential for confounding and the nongeneralizable study population. Additional studies of functional hepatic endpoints (e.g., liver disease) are not available, so it is not possible to evaluate whether the small changes in liver enzymes observed in these studies translate to clinical hepatic injury.

Table 3-22. Associations between PFHxS and liver enzymes in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
Adults								
Nian et al. (2019)	Cross-sectional (2015–2016); China; 1,605 adults	0.7 (0.01–2.7)	% change (95% CI) for ln-unit change	0.2 (−0.8,1.2)	0.1 (−0.5,0.8)	−0.1 (−0.6,0.5)	0.4 (−0.6,1.4)	−0.3 (−1.0,0.5)
Liu et al. (2022a)	Cross-sectional (2018–2019); China; 1,303 adults	0.9 (0.5–1.4)	% difference (95% CI) vs. 25th percentile	50th: 7.69 (5.62, 9.80)* 75th: 12.15 (7.66, 16.83)* 95th: 16.90 (7.86, 26.70)*	50th: 3.43 (2.11, 4.78)* 75th: 6.16 (3.32, 9.07)* 95th: 9.66 (3.95, 15.68)*	50th: 0.90 (-0.22, 2.03) 75th: 0.88 (-1.46, 3.27) 95th: 0.44 (-4.10, 5.19)	50th: 5.65 (3.22, 8.14)* 75th: 9.01 (3.81, 14.47)* 95th: 12.65 (2.30, 24.04)	50th: 3.05 (1.57, 4.55)* 75th: 6.44 (3.25, 9.72)* 95th: 11.40 (4.92, 18.28)*
Jain and Ducatman (2019c)	NHANES cross-sectional (2011–2014), U.S.; 2,883 adults	1.4	β (*p*-value) for log-unit change	Nonobese 0.005 (0.8) Obese 0.05 (<0.01)*	Nonobese 0.007 (0.6) Obese 0.01 (0.4)	Nonobese -0.005 (0.7) Obese 0.006 (0.6)	Nonobese 0.008 (0.7) Obese 0.03 (0.1)	Nonobese 0.002 (0.9) Obese 0.04 (0.07)
Lin et al. (2010)	NHANES cross-sectional (1999–2004), U.S.; 2,216 adults	mean (SE) 1.7 (1.0) (women)	β (SE) for log-unit increase	0.2 (0.5), $p = 0.7$	NR	NR	0.0 (0.02), $p = 0.9$	0.4 (0.2), $p = 0.06$

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
Gleason et al. (2015)	NHANES cross-sectional (2007–2010), U.S.; 4,333 adults (12+ yrs)	1.8 (1.0–3.1)	β (95% CI) for ln-unit increase	0.02 (0.01,0.03)*	0.02 (0.01,0.03)*	0.02 (0.01,0.04)*	0.01 (−0.01,0.03)	0.03 (0.01,0.05)*
Cakmak et al. (2022)	Cross-sectional (2007–2017); Canada; 4,952 adults	Cycle 1: 2.2; Cycle 2: 1.7; Cycle: 1.0	% change (95% CI) for GM change	1.7 (0.2, 3.3)*	-0.3 (-1.6, 0.9)	-1.2 (-3.7, 1.3)	3.6 (-0.7, 8.0)	-0.8 (-4.8, 3.5)
Salihovic et al. (2018)	Cohort (2001–2014); Sweden; 1,002 elderly adults	2.1 (1.6–3.4)	β (*p*-value) for ln-unit change	0.02 (0.0,0.03)*	NR	0.06 (0.02,0.09)*	0.03 (−0.01,0.07)	−1.0 (−1.3,−0.7)*
Children and adolescents								
Mora et al. (2018)	Project Viva birth cohort (1999–2002), U.S.; 682 children (7–8 yrs)	prenatal 2.4 (1.6–3.8)	β (95% CI) for IQR increase	-0.1 (−0.4,0.2)	NR	NR	NR	NR
		child 1.9 (1.2–3.4)		0.0 (−0.2,0.2)	NR	NR	NR	NR

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	ALT	AST	ALP	GGT	Total bilirubin
Attanasio (2019b)	NHANES cross-sectional (2013–2016); 354 males and 305 females (12–19 yrs)	GM (SE) male 1.3 (0.09) female 0.9 (0.06)	β (95% CI) for quartiles vs. Q1	boys Q2: −0.07 (−0.15, 0.01) Q3: −0.09 (−0.20, 0.02) Q4: −0.02 (−0.12, 0.08) girls Q2: −0.01 (−0.14, 0.12) Q3: 0.05 (−0.05, 0.16) Q4: 0.03 (−0.10, 0.16)	boys Q2: −0.04 (−0.10, 0.03) Q3: −0.03 (−0.09, 0.04) Q4: 0.00 (−0.09, 0.09) girls Q2: 0.00 (−0.10, 0.10) Q3: 0.07 (−0.01, 0.15) Q4: 0.03 (−0.08, 0.14)	NR	boys Q2: −0.09 (−0.21, 0.03) Q3: −0.03 (−0.15, 0.09) Q4: 0.02 (−0.12, 0.15) girls Q2: 0.10 (−0.01, 0.20) Q3: 0.10 (−0.01, 0.20) Q4: 0.08 (−0.02, 0.18)	boys Q2: 0.11 (0.03, 0.20) Q3: 0.07 (−0.01, 0.15) Q4: 0.16 (0.07, 0.26) *p*-trend: 0.01 girls Q2: 0.08 (−0.02, 0.18) Q3: 0.19 (0.08, 0.30) Q4: 0.25 (0.11, 0.40) *p*-trend < 0.01*

*$p < 0.05$.
NR: not reported.

Animal Studies

The toxicity database for PFHxS-induced liver effects in experimental animals consists of two short-term exposure studies using SD rats (NTP, 2018a; 3M, 2000a); two subchronic exposure study using APOE*3-Leiden.CETP mice[12] (Bijland et al., 2011) or C57BL/6 mice (He et al., 2022); one chronic exposure study using C57BL/6J mice (Pfohl et al., 2020) and four multigeneration studies using Wistar (Ramhøj et al., 2018) or Sprague Dawley rats (Butenhoff et al., 2009; 3M, 2003), or CD-1 mice (Marques et al., 2021; Chang et al., 2018). All studies exposed animals orally via either gavage (Chang et al., 2018; NTP, 2018a; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003, 2000a) or the diet (Bijland et al., 2011). Outcomes evaluated and reported in these studies include histopathological effects, serum biomarkers of liver damage and lipid metabolism, and changes in absolute and relative liver weights.

Organ weight

Four *high* confidence studies five *medium* confidence studies evaluated PFHxS-induced effects on liver weight (see Figure 3-55). In both rats and mice, short-term and subchronic exposure led to increased absolute and relative liver weights[13] (NTP, 2018a; Bijland et al., 2011; 3M, 2000a) (see Figure 3-56). However, a chronic exposure study using male C57BL/6J mice reported no significant effect on liver weight after exposure to 0.15 mg/kg-day for 29 weeks (Pfohl et al., 2020). Two short-term (28-day) exposure studies using SD rats reported that exposure to PFHxS increased liver weight by 8% to 54% at doses ranging from 1.25 to 10 mg/kg-day (NTP, 2018a; 3M, 2000a). Although NTP (2018a) observed increased relative and absolute liver weights in both male and female animals, 3M (2000a) only observed exposure-related changes in male rats. A separate subchronic exposure study using APOE*3-Leiden.CETP mice also observed increased absolute liver weight (108%) in animals orally exposed to 6 mg/kg-day PFHxS for 42 days (Bijland et al., 2011).

Four multigenerational toxicity studies evaluated PFHxS-induced effects on liver weights in F0 and/or F1 animals (Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003). In F0 generation male SD rats, exposure to 3 or 10 mg/kg-day PFHxS increased absolute and relative liver weight by 20% to 67% when compared with controls, but no effects were observed in F0 females (Butenhoff et al., 2009; 3M, 2003). Two similar studies using CD-1 mice also measured liver weights, but reported different effects: (Chang et al., 2018) reported increased absolute and relative liver weight (23% to 70%) in F0 generation (male and female) animals, but (Marques et al., 2021) observed no exposure-related changes in F0 female liver weights. Both (Chang et al., 2018) and (Marques et al., 2021) exposed pregnant animals to similar doses of PFHxS, however (Chang et al., 2018) treated animals for 42 days before mating, through gestation and lactation whereas

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[12] APOE*3-Leiden.CETP mice is a genetically modified animal model which emulates human lipoprotein profiles and is used to investigate cholesterol metabolism and cardiovascular disease (Veseli et al., 2017).
[13] Alterations in liver weight are considered indicative of exposure-related responses such as enzyme induction and hepatocellular hypertrophy (Thoolen et al., 2010; Sellers et al., 2007).

([Marques et al., 2021]) exposed F0 female animals from GD1 to PND20. In F1 generation animals, significant PFHxS-induced increases in liver weight were observed in male CD-1 mice (10% increase in relative liver weight at 3 mg/kg-day) after exposure during gestation, lactation, and post-weaning (until postnatal day 36) ([Chang et al., 2018]). However, in F1 male and female SD rats sampled on PND22 and Wistar rats sampled on PNDs 16–17, there were no significant exposure-related changes in relative or absolute liver weights ([Ramhøj et al., 2020]; [Ramhøj et al., 2018]; [Butenhoff et al., 2009]; [3M, 2003]). In F1 male and female CD-1 mice exposure to a high fat diet plus PFHxS resulted in decreased relative, but not absolute, liver weight on PND21. These effects were not apparent on PND90. Overall, the majority of the available studies report fairly consistent increases in liver weight across lifestages following PFHxS exposure.

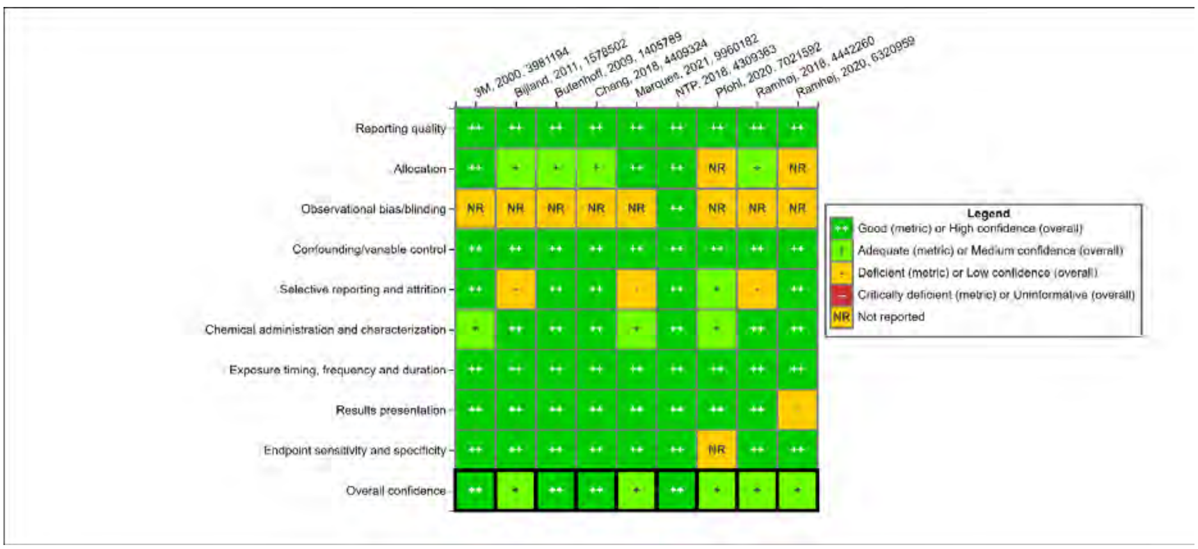

Figure 3-55. PFHxS liver weight animal study evaluation heatmap. For additional details see [HAWC] link.

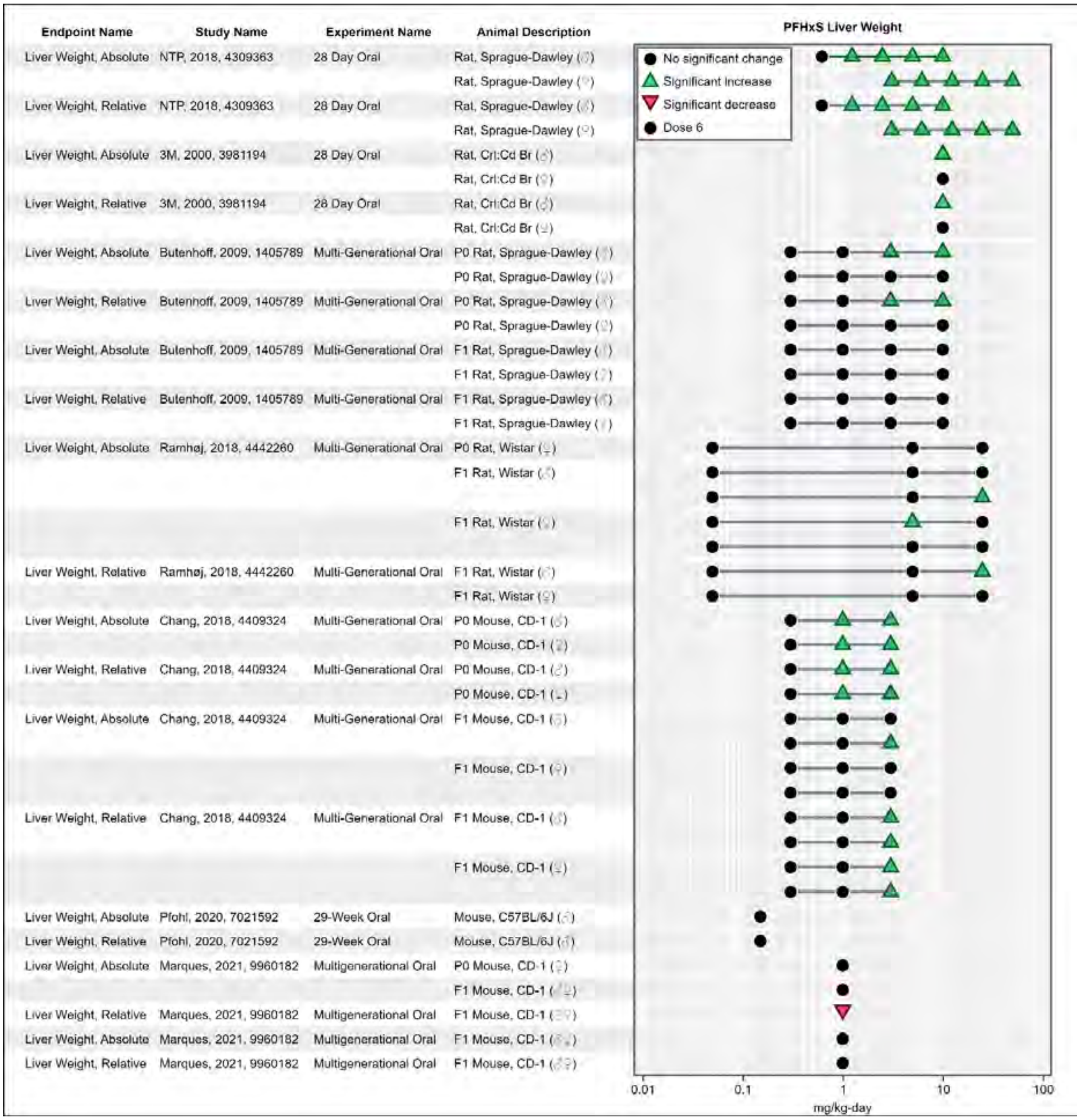

Figure 3-56. Liver weight responses from animal studies. Figure displays the high and medium confidence studies included in the analysis (see Figure 3-55. For additional details see HAWC link.

Histopathology

Histopathological lesions in the liver were reported in four *high* confidence studies using Sprague Dawley rats (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000a) or mice (Chang et al., 2018), two *medium* confidence study using Wistar rats (Ramhøj et al., 2020) or CD-1 mice, and one low confidence study using C57BL/6 mice (He et al., 2022) (see Figure 3-57).

Two short-term studies evaluated histopathological responses male and female SD rats after exposing animals to doses ranging from 2.5 to 10 mg/kg-day for 28 days, and one chronic study evaluated effects in male C57BL/6 mice treated with 60 μg/kg-day PFHxS for 12 weeks. Statistically significant increases in the incidence of hepatocellular hypertrophy[14] (44% to 100%) were observed in male SD rats exposed to PFHxS at doses ≥2.5 mg/kg-day (NTP, 2018a), or 10 mg/k-day (3M, 2000a) (see Figure 3-58). 3M (2000a) also evaluated other histological responses (including hematopoietic cell foci, single cell necrosis, coagulative necrosis, hepatocellular vacuolation, and inflammatory cell foci), but reported no significant exposure-related effects. Both studies also report that female animals did not exhibit the histopathological effects observed in male animals (NTP, 2018a; 3M, 2000a). In male C57BL/6 mice, exposure to 60 μg/kg-day for 12 weeks resulted in increased hepatocyte ballooning, inflammatory infiltration and fibrosis. However, several deficiencies were identified in He et al. (2022) including lack of reporting of histopathological effect incidences, observational bias, and chemical administration (see Figure 3-57, and follow HAWC link for additional details).

PFHxS-induced histopathological effects were also evaluated in two multigenerational toxicity studies. In F0 generation male SD rats or male and female CD-1 mice, exposure to PFHxS caused increased incidence of histopathological effects (see Figure 3-59), primarily hepatocellular hypertrophy. In the rat study, F0 generation animals exposed to PFHxS for 42 days to 3 or 10 mg/kg-day increased the incidence of hepatocellular hypertrophy by 90% and 100%, but other histological responses (including focal necrosis, lipidosis, vacuolation [midzonal or multifocal], and chronic liver inflammation) were not significantly affected (Butenhoff et al., 2009; 3M, 2003). Similar observations were made in male F0 generation CD-1 mice for which exposure to 0.3, 1, or 3 mg/kg-day PFHxS for 42 days increased hepatocellular hypertrophy and cytoplasmic alterations by 80%, 100%, and 100%, respectively when compared with controls (Chang et al., 2018). Furthermore, the incidence of single cell necrosis and microvesicular fatty change were increased (40% and 60% respectively) at the highest dose, but hepatocellular cell necrosis was not affected. Female F0 generation rats or mice used in the Butenhoff et al. (2009) and Chang et al. (2018) studies were exposed to PFHxS for 14 days before cohabitation and continued up to postnatal day 22. F0 generation female rats were nonresponsive to PFHxS exposure (Butenhoff et al., 2009; 3M,

[14]Hepatocellular hypertrophy: a cellular response to chemical-induced stress that is considered indicative of hepatomegaly (Cattley and Cullen, 2018; Thoolen et al., 2010) and characterized by an increase of hepatocyte size (Cesta et al., 2014). It may be caused by increases in mitochondria, peroxisomes, endoplasmic reticulum, or metabolic enzyme induction (Thoolen et al., 2010).

2003). However, in F0 generation female CD-1 mice cytoplasmic vacuolation was increased by 30% at the highest dose (3 mg/kg-day) and hepatocellular hypertrophy and cytoplasmic alterations (ground glass) were increased by 50 to 100% in all treated animals, but these effects were not dose-dependent (Chang et al., 2018). F1 generation CD-1 mice exposed to 3 mg/kg-day PFHxS during gestation and lactation displayed statistically significant increases in cytoplasmic alterations (63% incidence in males and 88% in females) and hepatocellular hypertrophy (83% incidence in males and 88% in females) (see Figure 3-60), but the incidence of hepatocellular necrosis, inflammation, and cytoplasmic vacuolation was not affected in F1 male or female CD-1 mice (Chang et al., 2018). A separate study using CD-1 mice reported no effect on male or female F1 animals exposed to 1 mg/kg-day PFHxS from GD1 to PND20 (Marques et al., 2021). These varying responses in the two studies using CD-1 mice (Marques et al., 2021; Chang et al., 2018) could have been due to differences in experimental exposure durations: Chang, 2018, 4409324@@author-year exposed animals before mating (14 days) and then during gestation and lactation, whereas Marques et al. (2021) only exposed animals during gestation and lactation. Furthermore, a separate study using Wistar rats reported no significant effects in F0 or F1 animals exposed to PFHxS (0.05 to 25 mg/kg-day) from GD7 to PND22 (Ramhøj et al., 2020).

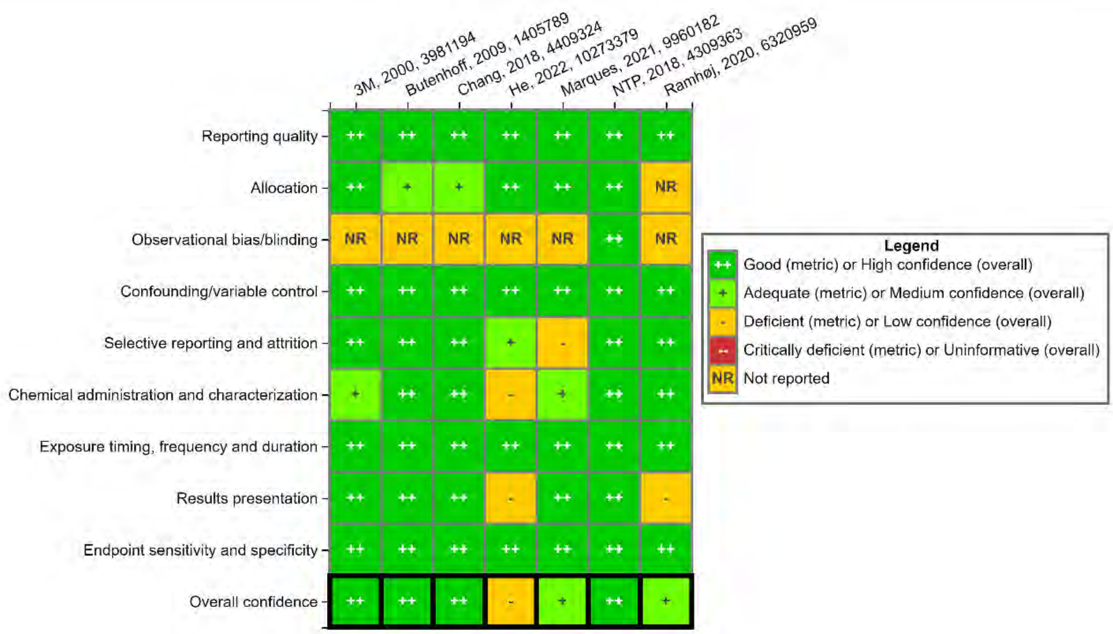

Figure 3-57. Liver histopathology animal study evaluation heatmap. For additional details see HAWC link.

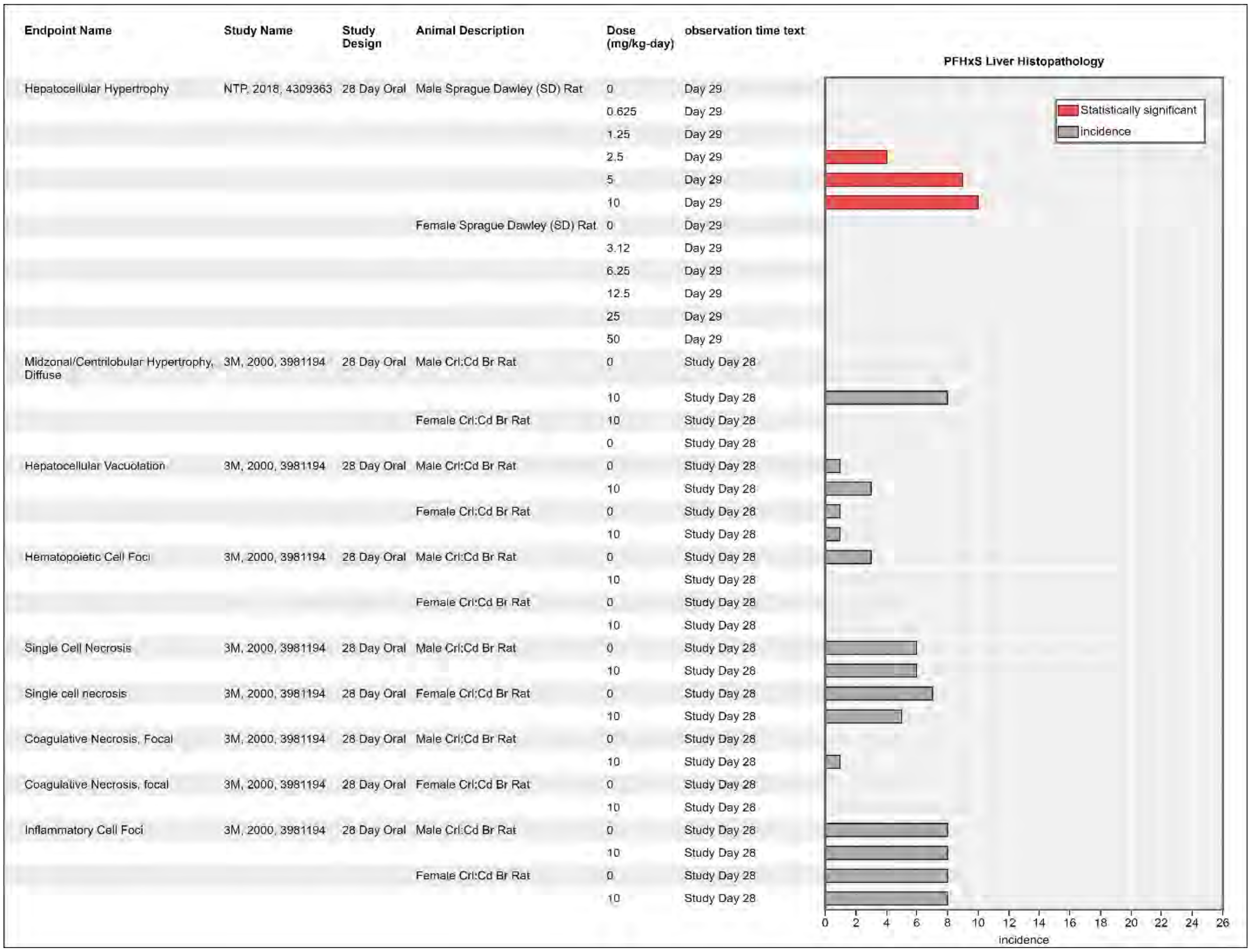

Figure 3-58. Histopathology observations from short-term studies. Figure displays the *high* and *medium* confidence studies included in the analysis. Details on study confidence may be found in Figure 3-57. For additional details see HAWC link.

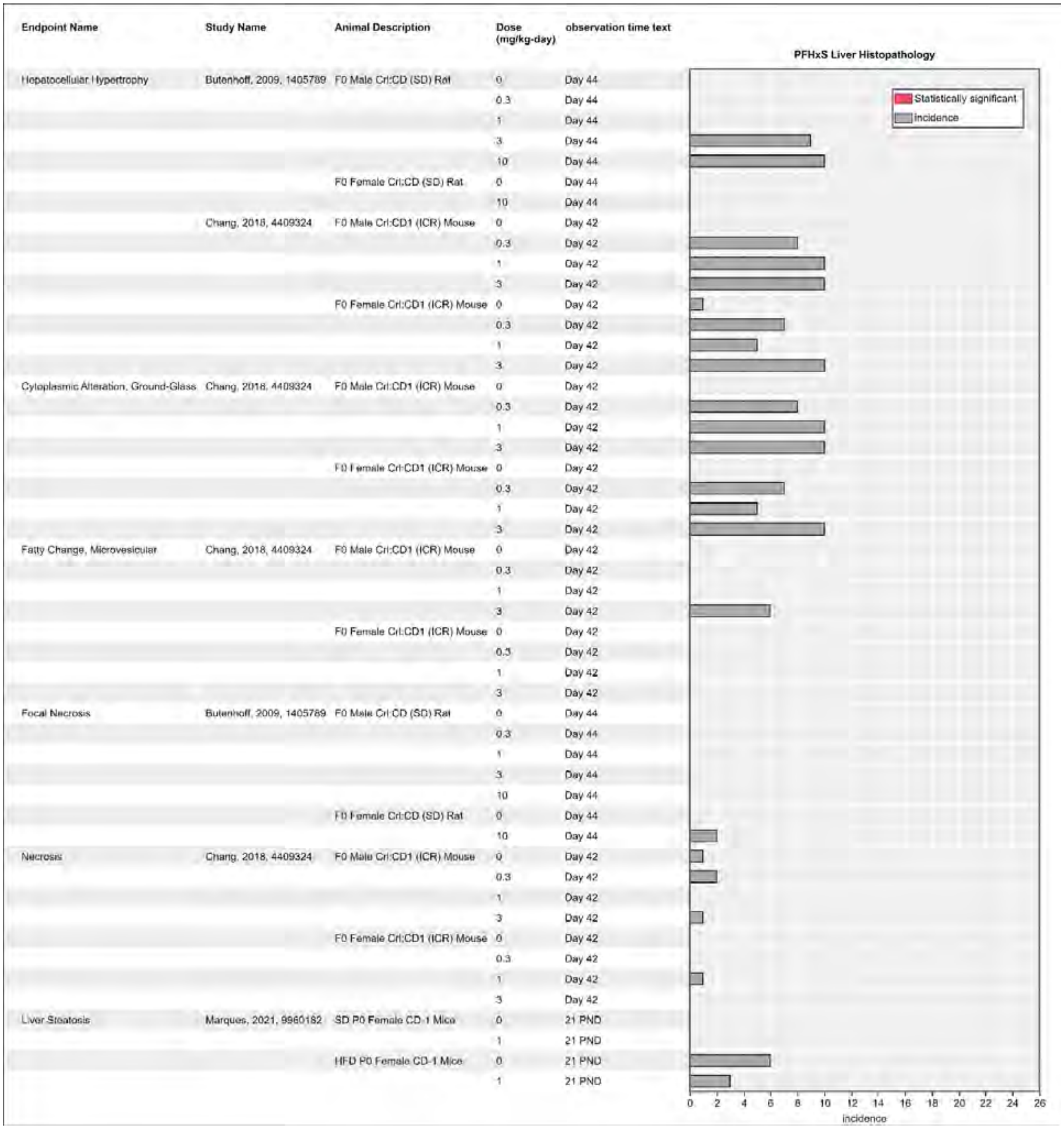

Figure 3-59. Histopathology observations from developmental toxicity studies (F0 generation animals). Figure displays the high and medium confidence studies included in the analysis. Details on study confidence may be found in Figure 3-57. For additional details see HAWC link.

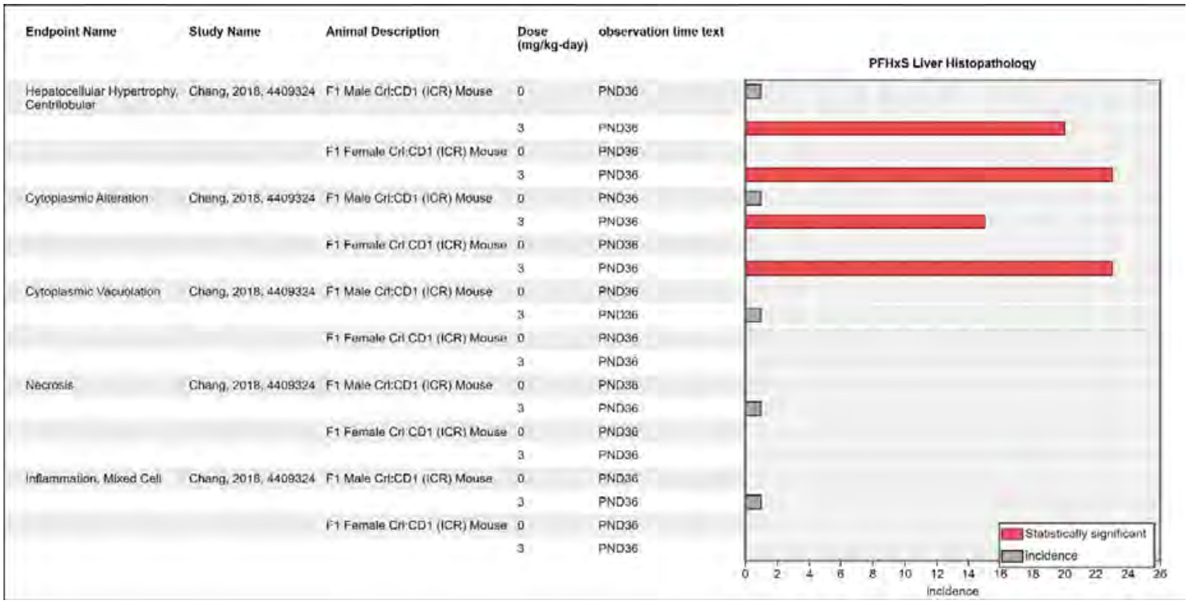

Figure 3-60. Histopathology observations from developmental toxicity studies (F1 generation animals). Figure displays the *high* and *medium* confidence studies included in the analysis. Details on study confidence may be found in Figure 3-57. For additional details see HAWC link.

Serum biomarkers of liver function

Four *high* confidence studies and two *medium* confidence studies measured serum biomarkers indicative of potential liver toxicity (see Figure 3-61). As in epidemiological studies, serum measures of clinical markers which inform of potential liver damage in experimental studies: ALT and AST are markers of hepatocellular function/injury; circulating ALP, bile salts/acids, and bilirubin are routinely used to evaluate hepatobiliary toxicity (Whalan, 2015; Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Changes in albumin and total protein may be indicative of chronic liver disease, as well as damage to other organs such as kidney, pancreas, thyroid, and G.I. tract (Whalan, 2015).

Two multigenerational toxicity studies report that exposure to 3 or 10 mg/kg PFHxS for 24 or 44 days statistically increased ALP[15] in F0 generation male CD-1 mice (133%) and SD rats (37%), respectively (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003) (see Figure 3-62). Albumin was also statistically increased (5%) in F0 male SD rats treated with the highest PFHxS dose (10 mg/kg-day) (Butenhoff et al., 2009; 3M, 2003) and bilirubin was decreased by 60% in male F0 CD-1 mice treated with 3 mg/kg-day PFHxS for 42 days (Chang et al., 2018). These two studies also measured ALP, AST, ALT, and serum bilirubin in female F0 rats and mice but reported no significant exposure-related effects (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). Furthermore, a recent study

[15]Increased serum ALP is considered indicative of cholestasis, and osteoclast activity (Whalan, 2015; Yang et al., 2014). It is produced in liver, but also in bone and intestines (Whalan, 2015) and conditions other than liver injury (e.g., bone disease) are associated with increased ALP (Yang et al., 2014). Thus, ALP is not regarded as a unique biomarker for cholestasis (Yang et al., 2014).

using CD-1 mice treated with 0, or 1 mg/kg-day PFHxS also reported no effects on serum ALT in F0 dams sampled on PND21 or male or female F1 animals sampled on PND5, 21, or 90 (Marques et al., 2021).

Two short-term studies using SD rats and one chronic exposure study using C57BL/6J mice evaluated serum levels of AST, ALT, ALP, and bile salts/acids after exposure to doses ranging from 0.6 to 10 mg/kg-day PFHxS for 28 days (NTP, 2018a; 3M, 2000a). 3M (2000a) reported that ALP was statistically increased by 20% in male SD rats exposed to 10 mg/kg-day, but a similar study by NTP observed no exposure-related effects (NTP, 2018a). Serum levels of ALT or AST were not affected in male or female SD rats in either study (NTP, 2018a; 3M, 2000a). However, a chronic exposure study using male C57BL/6J reported a 42% increase in ALT after exposure to 0.6 mg/kg-day for 12 weeks. (NTP, 2018a) also evaluated serum levels of albumin and total protein in male and female SD rats and reported no significant exposure-related effects (NTP, 2018a). Serum globulin levels were statistically decreased by 14% to 15% in male SD rats exposed to 10 mg/kg-day PFHxS for 28 days (NTP, 2018a; 3M, 2000a), and bilirubin was significantly decreased by 12% to 21% in male SD rats after 28 days of exposure to PFHxS at doses ranging from 2.5 to 10 mg/kg-day (NTP, 2018a). The 3M and NTP studies also evaluated female animals and reported no exposure-related effects.

One study using APOE*3-Leiden.CETP male mice, an animal model that better emulates human lipoprotein profiles, evaluated PFHxS-induced changes in hepatic triglyceride, cholesterol esters, and free cholesterol levels. Exposure to 6 mg/kg-day PFHxS for 42 days resulted in a 67% increase in liver triglyceride levels, but free cholesterol levels were not affected (Bijland et al., 2011). These observations suggest PFHxS exposure may alter hepatic function in a manner relevant to humans and they are supported by mechanistic studies evaluating PFHxS-induced alterations in the liver of wild-type and genetically modified animals (see mechanisms section below).

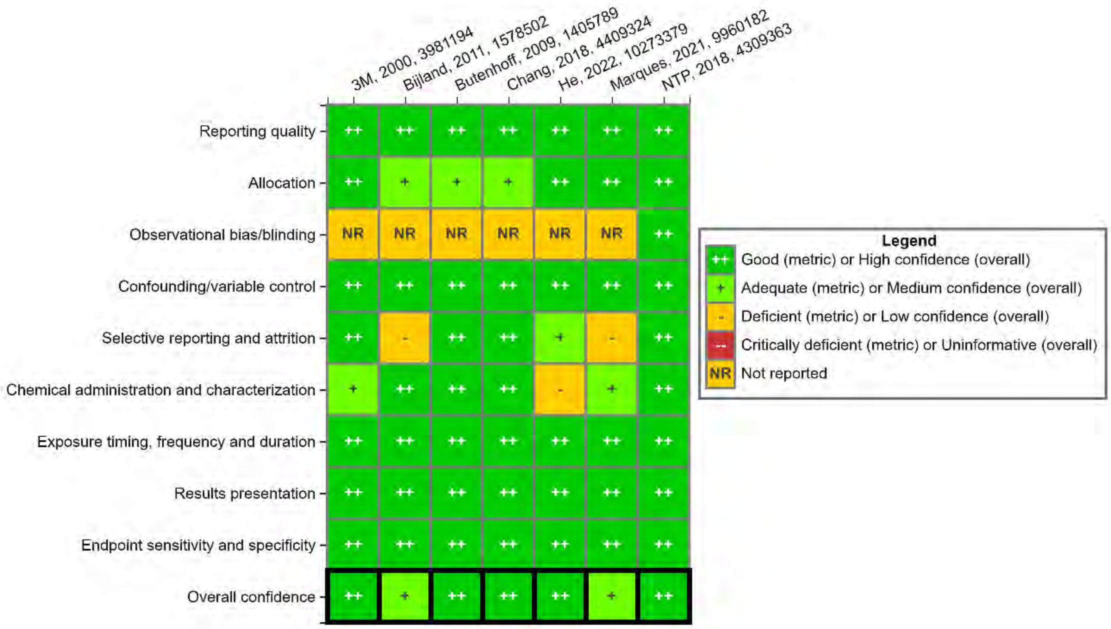

Figure 3-61. PFHxS liver serum biomarkers animal study evaluation heatmap. For additional details see HAWC link.

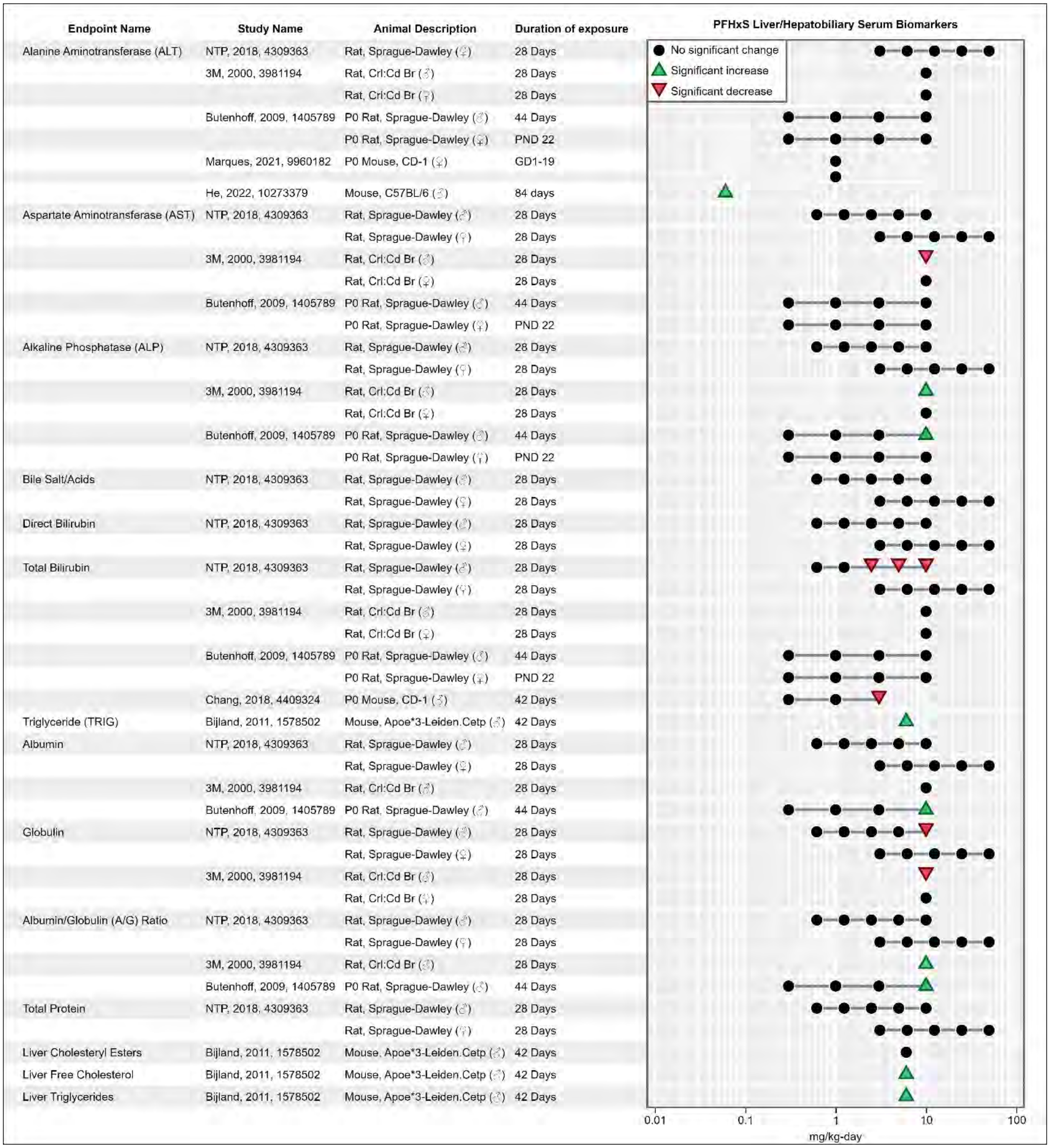

Figure 3-62. PFHxS liver/hepatobiliary serum biomarkers. Figure displays the *high* and *medium* confidence studies included in the analysis (see Figure 3-61). For additional details see HAWC link.

Mechanistic Evidence and Supplemental Information

Mechanistic evidence relevant to PFHxS-induced effects was collected from the peer-reviewed literature and from in vitro high-throughput screening (HTS) assays from the ToxCast and Tox21 databases accessed via EPAs Chemicals Dashboard. The available in vitro and in vivo studies were evaluated based on a proposed mode of action (MOA) for liver injury for PFOS and PFOA, two structural analogs of PFHxS and among the most well-studied PFAS (U.S. EPA, 2019c). Further, an AOP-based approach was employed to organize and discuss the evidence according to the following levels of biological organization: molecular events, cellular effects, organ effects, and organism effects. Reponses informative of later two biological levels of organization are presented in the preceding hazard sections. Refer to Appendix C for more details on the objective and methodology of the mechanistic evaluation undertaken herein, and a description of the proposed MOA for PFAS-induced hepatotoxicity (see Appendix C, Section 2). A detailed summary of the HTS data analysis can be found in Appendix C, Section 3.

Molecular Initiating Events

The available studies have examined several nuclear receptor and cell signaling pathways associated with chemical-induced liver toxicity. Many of the hepatic effects caused by exposure to perfluorinated compounds such as PFHxS have been attributed to activation of the peroxisome proliferator-activated receptor alpha (PPARα[16]) (Das et al., 2017; Gleason, 2017; NJDWQI, 2017; Rosen et al., 2017; U.S. EPA, 2016a, b). In vivo studies using SD rats or several strains of mice report that exposure to PFHxS results in activation of PPARα and increased expression of PPARα-responsive genes (Chang et al., 2018; NTP, 2018a; Das et al., 2017; Rosen et al., 2017; Bijland et al., 2011). Two cell culture studies using rat FaO hepatoma cells or primary mouse hepatocytes also reported altered expression of PPARα-responsive genes (Bjork et al., 2021; Rosen et al., 2013). PFHxS also activates the human PPARα. PFHxS caused PPARα activation in human hepatoma cell lines Rosenmai et al. (2018) and in primary human hepatocytes exposure was associated with increased expression of PPARα-responsive genes (Rosen et al., 2013). Overall, these studies suggest that PFHxS exposure can activate PPARα in animal in vivo and in vitro studies, and in human liver cell culture models.

Animal studies also provide evidence suggesting that additional nuclear receptor pathways may be involved in PFHxS-induced liver effects. Two studies using genetically modified animals reported increases in absolute and relative liver weight in both wild-type and PPARαnull animals (Das et al., 2017; Rosen et al., 2017). However, one study (Rosen et al., 2017) also reported that these effects were reduced in PPARα-null mice. Gene expression analyses in both wild-type and PPARαnull animals report that in addition to PPARα, other hepatocellular receptors that are known to play a role in liver function can be affected by PFHxS exposure. These include: PPARα, the constitutive androstane receptor (CAR), and the pregnane × receptor (PXR) (Chang et al., 2018; Rosen et al., 2017; Bijland et al., 2011; 3M, 2010). A 28-day study using SD rats also reported increased mRNA levels of CAR/PXR-responsive genes (NTP, 2018a), suggesting these molecular

[16]PPARα is a member of the nuclear receptor superfamily that can be activated endogenously by free fatty acid derivatives. PPARα plays a role in lipid homeostasis, but it is also associated with cell proliferation, oxidative stress and inflammation (Li et al., 2017a; Mellor et al., 2016; Hall et al., 2012).

effects are conserved across rodent models. Furthermore, PFHxS was able to activate nuclear receptors other than PPARα, in human cells (including PPARα, RXR, LXR, FOS, and NRF2; see Appendix C). Activation of these hepatic nuclear receptors plays an important role in regulating responses to xenobiotics, energy and nutrient homeostasis, and development of fatty liver disease[17] (Mackowiak et al., 2018; Angrish et al., 2016; Mellor et al., 2016; di Masi et al., 2009).

Cellular Effects

As discussed below, the available studies provide evidence for PFHxS-induced alterations in reactive oxygen species production, cellular stress, and alterations in liver metabolic functions.

Excessive production of reactive oxygen species (ROS) is considered a mechanism associated with PFAS-induced hepatocellular toxicity (Li et al., 2017a; U.S. EPA, 2016a, b) and fatty liver disease (Wahlang et al., 2019; Joshi-Barve et al., 2015). One in-vivo study using C57BL/6J mice reported increased mRNA levels of genes associated with oxidative stress, after exposure to 0.15 mg/kg-day for 25 weeks (Pfohl et al., 2020). Two cell culture studies using HepG2 human hepatocytes present conflicting evidence (Ojo et al., 2021; Wielsøe et al., 2015). While both studies exposed cells for the same duration (24 hours) and similar concentrations (0, 0.02, 0.2, 2, 20, 200 μM in (Wielsøe et al., 2015); and 0, 0.2, 2, 20 μM in (Ojo et al., 2021)) only (Wielsøe et al., 2015) observed increased intracellular ROS production and neither study observed exposure-related changes in cellular antioxidant levels.

PFHxS-induced alterations in hepatic lipid metabolism were evaluated in three in vivo studies using mice and in one cell culture study using primary rat hepatocytes. In mice PPFhX exposure is associated with increased mRNA levels of genes associated with lipid synthesis, metabolism, and transport (Pfohl et al., 2020), and liver cell lipid content and size (Das et al., 2017). Similar have been reported in genetically modified PPARα-null mice (Das et al., 2017). However, PPARα-null animals also had higher (sevenfold) baseline levels of cellular lipids when compared with wild type SV129 control mice (Das et al., 2017). The same study used WY-14643, a PPARα activator, as a positive control and observed no significant effects in hepatic lipid accumulation in WY-14643-exposed PPARα-null animals, suggesting that PFHxS-induced lipid accumulation in genetically modified animals is mediated mostly (or entirely) via a PPARα-independent mechanism (Das et al., 2017). Das et al. (2017) also observed that PFHxS exposure did not have an impact on fatty acid beta-oxidation in wild-type and PPARα-null animals, and a separate in vitro experiment by the same group reported no significant exposure-related effects on rat hepatic mitochondria

[17]Fatty liver (steatosis) is a hepatic response to moderate alcohol consumption, xenobiotic exposure, or other factors that may alter metabolic functions (Roth et al., 2019; Joshi-Barve et al., 2015; Wahlang et al., 2013). It is characterized by excessive lipid accumulation in hepatocytes (Angrish et al., 2016) and is considered a reversible response when the stimulus is temporary (Roth et al., 2019). However, steatosis increases susceptibility to other insults and persistent steatosis is considered a precursor to other forms of liver disease (Bessone et al., 2019; Roth et al., 2019). When combined with inflammation (steatohepatitis) fatty liver can progress to fibrosis and cirrhosis (Roth et al., 2019; Wahlang et al., 2013).

fatty acid beta-oxidation. Two studies evaluated hepatic triglyceride (TG) content and report that PFHxS exposure led to increased liver TG levels in wild-type and APOE*3-Leiden.CETP mice (Das et al., 2017; Bijland et al., 2011), a genetically modified animal model used to investigate cholesterol metabolism and cardiovascular disease. However, Das et al. (2017) also observed that PPARα-null animals appeared to be less sensitive to this effect (Das et al., 2017). Gene expression analysis revealed that in both wild-type and PPARα-null animals PFHxS treatment resulted in altered expression of genes associated with peroxisomal and mitochondrial fatty acid metabolism and increased levels of genes associated with fatty acid and triglyceride transport and synthesis (Das et al., 2017). However, these responses were also attenuated in the PPARα-null mice (Das et al., 2017). The available studies suggest that PFHxS may alter hepatic lipid metabolism in animal models. Experiments using genetically modified animals suggest that PPARα activation plays a role in the metabolic responses described above, but other pathways are likely involved. Overall, the metabolic effects reported in the Das et al. (2017) and Bijland et al. (2011) studies are considered to be potential indicators of toxicant-induced alterations in hepatocyte function, which can result in abnormal metabolism and accumulation of fatty acids leading to steatosis (Wahlang et al., 2019; Angrish et al., 2016). Biological understanding suggests that such changes can, in turn, increase lipotoxicity susceptibility to other hepatic insults or independently progress to steatohepatitis (Roth et al., 2019; Mendez-Sanchez et al., 2018; Yang et al., 2014).

Cytotoxicity induced by PFHxS exposure was evaluated in two cell culture studies using HepG2 human hepatocytes (Ojo et al., 2021; Ojo et al., 2020). Ojo, 2020, 6333436 reported increased cytotoxicity at an effective dose of 183 μM. (Ojo et al., 2021), did not report PFHxS-induced changes in cytotoxicity. However, this was a mixture study designed to evaluate the combined effects of PFHxS with other PFAS and (Ojo et al., 2021) selected concentrations below their previously identified effective dose of 183 μM.

Conclusions from Mechanistic Evidence

Mechanistic evidence from in vivo and in vitro rodent cell models suggests that PFHxS activates several hepatic xenobiotic-sensing nuclear receptors and other cell signaling pathways, namely PPARα, PPARα, CAR, PXR, and LXR. PFHxS exposure was also associated with alterations in hepatic ROS production, cellular stress, and abnormal liver function related to lipid metabolism in animals (including genetically modified mouse models). The molecular and cellular mechanisms induced by PFHxS exposure in these models have been implicated in chemical-induced liver diseases such as steatosis, steatohepatitis, and fibrosis (Angrish et al., 2016; Mellor et al., 2016; Joshi-Barve et al., 2015; Wahlang et al., 2013), and provide support for the biological plausibility of the observed liver effects (i.e., histopathological responses, biomarkers of altered liver function and lipid accumulation, and organ weight changes) in short-term oral studies on PFHxS.

Available mechanistic information in human models is limited to two in vitro studies in the peer-reviewed literature and HTS assays from the ToxCast databases accessed via EPAs Chemicals

Dashboard. As described in Appendix C-3, none of the 54 available assays in the ToxCast database using the human hepatoma HepG2 cells were responsive to PFHxS treatment. These HTS assay findings are inconsistent with the observations from the other two in vitro studies Wielsøe et al. (2015) and Rosenmai et al. (2018), which also used HepG2 cells and reported that PFHxS exposure promotes activation of the human PPARα and increased reactive oxygen species production. Additional studies are needed to resolve these conflicting results.

Overall, the mechanistic evidence on pathways known to be associated with liver toxicity (i.e., increased oxidative stress and altered lipid metabolism) provides biological plausibility for the liver effects observed in animal bioassays. The available mechanistic evidence provides some support for a possible role for both PPARα-dependent and PPARα −independent mechanisms in the hepatic responses to PFHxS exposure, including hepatocellular hypertrophy, increased cellular lipid content, and increased liver weight observed in animal studies. Limited evidence from in vitro studies suggest that some responses may also be activated in human cellular models, including nuclear receptor and transcription factor pathways that regulate liver functions (i.e., PPARα/γ, CAR, PXR, RXR, LXR, FOS, NRF2), and outcomes indicative of oxidative stress and altered metabolism. As described above activation of these nuclear receptor and cell signaling pathways is associated with changes in hepatic functions, lipid accumulation, and progression of fatty liver disease. However, inconsistencies between the available peer-reviewed studies using human cell culture models and HTS assays from the ToxCast database suggest that additional experiments are needed.

Considerations for potentially adaptive versus adverse responses

Increases in liver weight and hepatocyte hypertrophy were observed in rodents with PFHxS administration in short-term oral studies. Enlargement of the liver and/or individual hepatocytes is a common chemical-induced response that can involve lipid accumulation (e.g., micro- or macro-vesicular steatosis), organellar growth and proliferation (e.g., peroxisomes, endoplasmic reticulum), increased intracellular protein levels (e.g., Phase I and II enzymes), and altered regulation of gene expression (e.g., stress response, nuclear receptors) (reviewed by Batt and Ferrari (1995)). Hepatocyte hypertrophy related to organelle growth and proliferation in response to activation of xenobiotic-sensing receptors (primarily PPARα) is often considered an adaptive response (Hall et al., 2012). Histological and clinical effects considered adverse responses in the liver (e.g., increased hepatic inflammation, and elevated serum markers of hepatocyte damage (Hall et al., 2012)) were reported in the study by (He et al., 2022). However, the study by He et al. (2022) was considered *low confidence* due to issues related with evidence reporting and animal allocation to exposure groups, and the other in vivo studies described above also evaluated hepatocellular necrosis, inflammation and serum markers of liver disease and they report no PFHxS-induced changes (see synthesis of histopathology and serum biomarkers of liver function above). In the absence of concordant histopathological evidence of degenerative changes or other changes indicative of adverse responses, the available evidence supports an interpretation that the responses to PFHxS observed in the currently available animal studies are considered adaptive.

Evidence Integration

The available **evidence suggests** but is not sufficient to infer that exposure to PFHxS might cause hepatobiliary system effects in humans given sufficient exposure conditions[18]. This is due to limitations in the available evidence that introduce significant uncertainty (see Table 3-23).

The available evidence on PFHxS-induced hepatic effects in humans is considered *slight*. There is some evidence of an association between PFHxS exposure and hepatic effects in human studies that is based on largely consistent associations with liver biomarkers (primarily small increases in ALT, a specific biomarker of potential liver injury) in the blood in multiple studies of adults. In addition, one study of liver disease found that in children with nonalcoholic fatty liver disease, PFHxS exposure was associated with severe disease. However, there were no additional studies of clinical liver effects available, and so it is not possible to evaluate whether the small changes in liver enzymes observed in the biomarker studies translate into clinical hepatic injury. There is also some unexplained inconsistency across studies and incoherence across liver enzymes other than ALT that further reduces the strength of the evidence.

The available evidence on PFHxS-induced hepatic effects in animal toxicity studies is considered *slight*. The evidence from short-term and multigenerational animal studies provides evidence of PFHxS-induced effects on multiple endpoints relevant to the assessment of liver responses to chemical exposure (including organ weight changes, histopathology [hepatocellular hypertrophy], and lipid accumulation). Alterations in serum biomarkers of liver/hepatobiliary function (ALT, ALP, bile salts/acids, and globulin) were observed in SD rats (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000b), and C57BL/6J and CD-1 mice (Chang et al., 2018). However, as described above, responses such as alterations in ALT, ALP and albumin were not consistently observed in similar short-term (NTP, 2018a; 3M, 2000b), sub-chronic and chronic (He et al., 2022; Chang et al., 2018; Butenhoff et al., 2009), or multigenerational (Marques et al., 2021; Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003) studies, and markers considered indicative of hepatocellular toxicity (ALT and AST) (Hall et al., 2012) were not affected in the available studies (Chang et al., 2018; NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000b).

Increased liver weights were reported in SD rats after 28 to 44 days of exposure (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000b) and in APOE*3-Leiden.CETP and CD-1 mice treated with PFHxS for 42 to 44 days (Chang et al., 2018; Bijland et al., 2011). Alterations in histological responses were also observed in the available studies and responses such as hepatocellular hypertrophy were consistently observed after short-term exposure in male rats and mice (NTP, 2018a; Butenhoff et al., 2009; 3M, 2000b) and F1 generation male and female mice (Chang et al., 2018). He et al. (2022) observed evidence of increased hepatic inflammation, but as described above several issues were identified with this study which lowers our confidence to *low*, and other outcomes indicative of hepatocellular degeneration (e.g., vacuolization) or injury (e.g., necrosis)

[18] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

(Hall et al., 2012) were unaffected in the available short-term and multigenerational studies (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). Responses such as single cell necrosis might progress to more severe effect after continued exposure (Thoolen et al., 2010), but the available information from short-term studies is not sufficient to determine whether the observed histological effects can evolve to clearly adverse hepatic injuries with continued exposure. Exposure to PFHxS also resulted in increased hepatocyte lipid accumulation in exposed APOE*3-Leiden.CETP (Bijland et al., 2011), as well as wild-type and PPARα-null, mice (Das et al., 2017) suggesting that PFHxS exposure may have the potential to promote fatty liver development, including in the absence of PPARα. In general, the responses observed in animals exhibited a dose-response gradient.

Analysis of mechanistic data from in vivo and in vitro rodent models provide biological plausibility for the apical effects reported in the short-term and multigenerational oral studies summarized above. Exposure to PFHxS was associated with the activation of several molecular signaling pathways and altered cellular functions thought to be involved in the MOA for liver toxicity of well-studied PFAS such as PFOA and PFOS (see synthesis of Mechanistic evidence and supplemental information above for more details). Additionally, the evidence for PFHxS-mediated liver effects point to potential PPARα-dependent and -independent pathways, which is consistent with the mechanisms of potential hepatotoxicity for related perfluorinated compounds (ATSDR, 2018b; Li et al., 2017a; U.S. EPA, 2016a, b).

Potential adverse liver effects caused by exposure to PFHxS and other PFAS have been attributed, in part, to activation of PPARα (ATSDR, 2018b; Li et al., 2017a; U.S. EPA, 2016a, b). However, in addition to PPARα, PFHxS exposure appears to promote activation of other nuclear receptor pathways (PPARγ, CAR, PXR, LXR, and transcriptional factors, FOS, and NRF2) and responses indicative of oxidative stress and cellular damage were observed in human liver cell models (see synthesis of Mechanistic studies and supplemental information above for more details). In addition, studies of PFHxS in PPARα-null mice indicate that many of the observed responses are unaffected by loss of PPARα-signaling. Therefore, the available evidence supports the interpretation that PPARα-dependent and -independent mechanisms mediate PFHxS-induced effects in animals.

The available mechanistic evidence supports that PFHxS exposure may induce fatty liver disease, but subchronic and chronic duration studies are not available to inform whether the observed PFHxS-induced effects progress to adverse responses (e.g., steatosis and steatohepatitis) in animal models.

Table 3-23. Evidence profile table for oral PFHxS exposure and liver effects

<table>
<tr><th colspan="5">Evidence stream summary and interpretation</th><th>Evidence integration summary judgment</th></tr>
<tr><td colspan="5">Evidence from studies of exposed humans (see Hepatic Human Studies Section)</td><td rowspan="4">⊕⊙⊙
Evidence suggests, but is not sufficient to infer

Based primarily on small increases in ALT in men and women, and consistent, but possibly not adverse, hepatic effects in rodents

Human relevance:
Limited studies in human in vitro models suggest activation of molecular and cellular responses observed in rodent models are relevant to human toxicity

Cross-stream coherence:
Alterations in serum biomarkers of hepatobiliary injury were reported in animals and in a few epidemiological studies, although the observations are uncertain, and the markers affected differed across species.

Susceptible populations and lifestages:</td></tr>
<tr><th>Studies and confidence</th><th>Factors that increase certainty</th><th>Factors that decrease certainty</th><th>Summary and key findings</th><th>Evidence stream judgment</th></tr>
<tr><td>Serum Biomarkers
10 medium and 2 low confidence studies</td><td>• Most medium confidence studies reported an effect
• Consistency increased ALT in adults
• Precision in three studies</td><td>• Unexplained inconsistency for biomarkers other than ALT
• Lack of coherence across biomarkers
• Unclear biological significance of small changes in ALT</td><td>• Positive associations observed between PFHxS and ALT in multiple studies.
• Direction of association with other liver biomarkers varied within and across studies.
• 1 study of liver disease reported a positive association (p > 0.05) with severe disease.</td><td>⊕⊙⊙
Slight

Based on largely consistent, but uncertain, increases in ALT in adults</td></tr>
<tr><td colspan="5">Evidence from in vivo animal studies (see Hepatic Animal Studies Section)</td></tr>
</table>

Evidence stream summary and interpretation					Evidence integration summary judgment
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	None identified, although those with pre-existing liver disease could potentially be a greater risk
Organ Weight 5 *high* and 3 *medium* and confidence studies in rats and mice • 28-d (×2) • 42-d • 203-d • Gestational (×4)	• *Consistent* increases, across studies • *Dose-response* in studies reporting effects • *Coherence* with histopathology in male rats and mice • *All high* or *medium* confidence studies	• Unclear biological significance (adversity) of the combined hepatic findings in animals across endpoints	• Dose-related increases in liver weights reported at doses ranging from 1.25 to 50 mg/kg-d rat and mouse studies, and a gestational exposure study in mice	⊕⊙⊙ *Slight* Based on consistent, coherent, and dose-dependent increases in organ weight and related histopathology. However, the current evidence is insufficient to support the adversity of the changes.	
Histopathology 4 *high,* 1 *medium*, and 1 *low* confidence studies in rats and mice: • 28-d (×2) • 84-d • Gestational (×3)	• *Consistent* cellular hypertrophy across studies and species • *Coherence* with liver weight effects (especially at high doses) • *Dose response*	• Unclear biological significance (adversity) of histopathological changes (e.g., no necrosis observed) as well as the combined hepatic findings in animals across endpoints	• Hepatocellular lesions observed in rats and mice including hepatocellular hypertrophy in mice exposed to ≥0.3 mg/kg-d and rats exposed to 2.5 mg/kg-d.		

Evidence stream summary and interpretation					**Evidence integration summary judgment**
	• *All high* confidence studies				
Serum Biomarkers 4 *high* confidence studies in rats and mice: • 28-d (×2) • 44-d • 42-d 1 *high* and 2 *medium* confidence studies in mice • 42-d • 84-d • Gestational (×1)	• *Dose response*	• Affected biomarker (ALP) not specific to liver • Inconsistent evidence on ALT levels • No effects on AST) • Unclear biological significance (adversity) of the combined hepatic findings in animals across endpoints	• Dose-related increases in biomarker (ALP) in male mice and rats exposed to 3 or 10 mg/kg-d respectively • Increased serum ALT in 1 mouse study • Increased marker of altered function (tissue triglyceride levels) in mice exposed to 6 mg/kg-d		
Mechanistic evidence and supplemental information (see Mechanistic Studies and Supplemental Information Section)					
Biological events or pathways	**Summary of key findings, interpretation, and limitations**			**Evidence stream judgment**	
Molecular initiating events — PPARα	*Key findings and interpretation:* • Activation of hepatic PPARα in rat and mouse models. Some evidence of PPARα activation in human in vitro models.			Evidence indicates a role for PPARα-dependent and -independent	

Evidence stream summary and interpretation			Evidence integration summary judgment
	• In vivo PFHxS exposure increased expression of PPARα-responsive genes in wild-type and hPPARα mice. *Limitations*: No evidence in humanized in vivo models. Inconsistencies in peer-reviewed and ToxCast/Tox21 studies using human hepatoma HepG2 cells.	pathways in the MOA for noncancer liver effects of PFHxS. Limited in vitro studies suggest some responses may be activated in human molecular/cellular models.	
Molecular initiating events — PPARg	*Key findings and interpretation*: • Activation of PPARγ in mouse (in vivo) and human (in vitro) models. • Increased expression of PPARγ-responsive genes in vivo; and induction of PPARγ transactivation in human hepatoma HepG2 cells. *Limitations*: Few studies and no evidence in humanized in vivo models.		
Molecular initiating events — CAR/PXR	*Key findings and interpretation*: • *Increased expression of CAR/PXR-responsive genes in mice.* *Limitations: No evidence in humanized in vivo or in vitro models.*		
Molecular initiating events — other pathways	*Key findings and interpretation*: • Limited in vivo evidence supports activation of cell signaling pathways related to altered hepatic metabolism and oxidative/cellular stress responses (RXR, LXR, FOS, and Nrf2). *Limitations: Few studies and no evidence in humanized in vivo or in vitro models.*		
Cellular effects	*Key findings and interpretation*: • Increased hepatic lipid content and altered expression of genes associated with fatty acid and triglyceride metabolism. • Increased ROS production and markers of cellular stress/cytotoxicity in HepG2 cells.		

Evidence stream summary and interpretation			Evidence integration summary judgment
	Limitations: Few in vivo studies examining cellular toxicity, functions, other cell signaling pathways, and no evidence in humanized in vivo models. Inconsistencies in the in vivo and in vitro results likely due to differences in experimental model and/or design features.		

3.2.5. Neurodevelopmental Effects

The available database examining potential nervous system effects of PFHxS exposure was composed of 17 epidemiological and 2 animal studies. All the studies in the evidence base examined the effects of PFHxS in children or, in animal studies, exposed animals during early lifestages to examine potential effects on neurodevelopment manifest in later lifestages (i.e., testing in newborn, juvenile, or adult rats). Therefore, this section examines and discusses the evidence on PFHxS-induced effects on the developing nervous system. For information on other developmental effects please see Section 3.2.3.

Human Studies

Twenty-two studies (reported in in 31 publications) examined associations between PFHxS exposure (measured in blood) and neurodevelopmental outcomes. Neurodevelopment is typically assessed with a wide array of neurobehavioral or neuropsychological tests, which makes it difficult to draw clear-cut divisions of neuropsychological categories. For example, a longer mean reaction time (a measure of response time after a stimulus is introduced) on a continuous performance test typically indicates inattention but may also be affected by slower information processing or motor response. For the purposes of this review, and due partly to data availability, tests were organized into the following categories: (1) cognition, (2) Attention Deficit Hyperactivity Disorder (ADHD) or related behaviors, (3) social behavior or autism spectrum disorder, and (4) other outcomes. Nine studies evaluated cognition, which comprised several endpoints including IQ, executive function, language development, and intellectual disability. Seven studies evaluated ADHD or related behaviors, which included ADHD diagnosis, inattention, impulsivity, hyperactivity, and externalizing problems. Five studies evaluated social behavior and included autism spectrum disorder (ASD) diagnosis, and two different autism screening scores, although there is overlap with the behaviors assessed with ADHD. Given the heterogeneity in the tools and age ranges used in the studies, it can be difficult to assess consistency within these categories. Other outcomes included motor effects (three studies) and cerebral palsy (one study).

There were several considerations specific to the use of neuropsychological tests for assessing children. For outcome ascertainment, tests used in a study should be appropriate for the age range being studied and for the culture and language. Other relevant factors, such as time of day of test administration or computer use, should have been considered, and some description of the testing environment should have been provided. If there were multiple raters, this factor should have been considered (e.g., statistical adjustment for rater, or analysis of interrater reliability). While blinding to exposure is ideal, this information was not commonly reported, and it was considered unlikely that participants or the outcome assessors would have knowledge of PFHxS exposure levels during testing. Therefore, no blinding or lack of reporting on blinding was determined to be unlikely to cause outcome misclassification. Evaluation of confounding was based on the approach used by the study authors to identify potential confounders; confounders that were considered potentially relevant across studies included child age and sex, maternal age, socioeconomic status, quality of caregiving environment, prenatal tobacco exposure, and parental mental health and IQ. It was considered preferable for analyses to use the outcome scales as continuous variables to minimize misclassification into artificial categories and improve statistical power (Sagiv et al., 2015), although this does not apply to clinical diagnosis of conditions such as ASD and ADHD.

The majority of available studies were birth cohorts or case-control studies nested in birth cohorts that evaluated maternal exposure to PFHxS during pregnancy (Yao et al., 2022; Dalsager et al., 2021b; Oh et al., 2021; Skogheim et al., 2021; Luo et al., 2020; Spratlen et al., 2020a; Niu et al., 2019; Harris et al., 2018; Liew et al., 2018; Høyer et al., 2017; Jeddy et al., 2017; Oulhote et al., 2016; Vuong et al., 2016; Wang et al., 2015). Some of these studies were considered adequate rather than good for exposure measurement due to variations in the timing during gestation of sample collection across participants within each study. While the half-life of PFHxS is long and exposure levels are unlikely to have changed drastically during pregnancy, changes in hemodynamics during pregnancy may influence levels in the blood at different points during pregnancy. In some cohort studies, childhood exposure was measured as well (Harris et al., 2018; Vuong et al., 2018a; Oulhote et al., 2016). There was one case-control study with measurements from banked maternal samples (Lyall et al., 2018) and one case-control study with maternal samples taken concurrently with outcome measurement (Shin et al., 2020). In addition, there were three cross-sectional studies, based on data from NHANES (Hoffman et al., 2010), the C8 Health Project (Stein and Savitz, 2011), and a survey in the United States (Gump et al., 2011). While the exposures measured in these studies with concurrent exposure and outcome measurement may not represent an etiologically relevant period, particularly for capturing any influence of exposure on the genetic component of ADHD, these studies were considered adequate for exposure measurement due to the long half-life of PFHxS and since exposure levels are generally expected to be fairly stable over time. Reverse causation is not a concern for these outcomes because neuropsychological performance is unlikely to influence PFHxS levels. The study evaluations are summarized in Figure 3-63.

For data extraction and synthesis, when multiple exposure measures from different time points (ages) were available, cross-sectional results were not extracted unless the results were different from results from the prospective measurement.

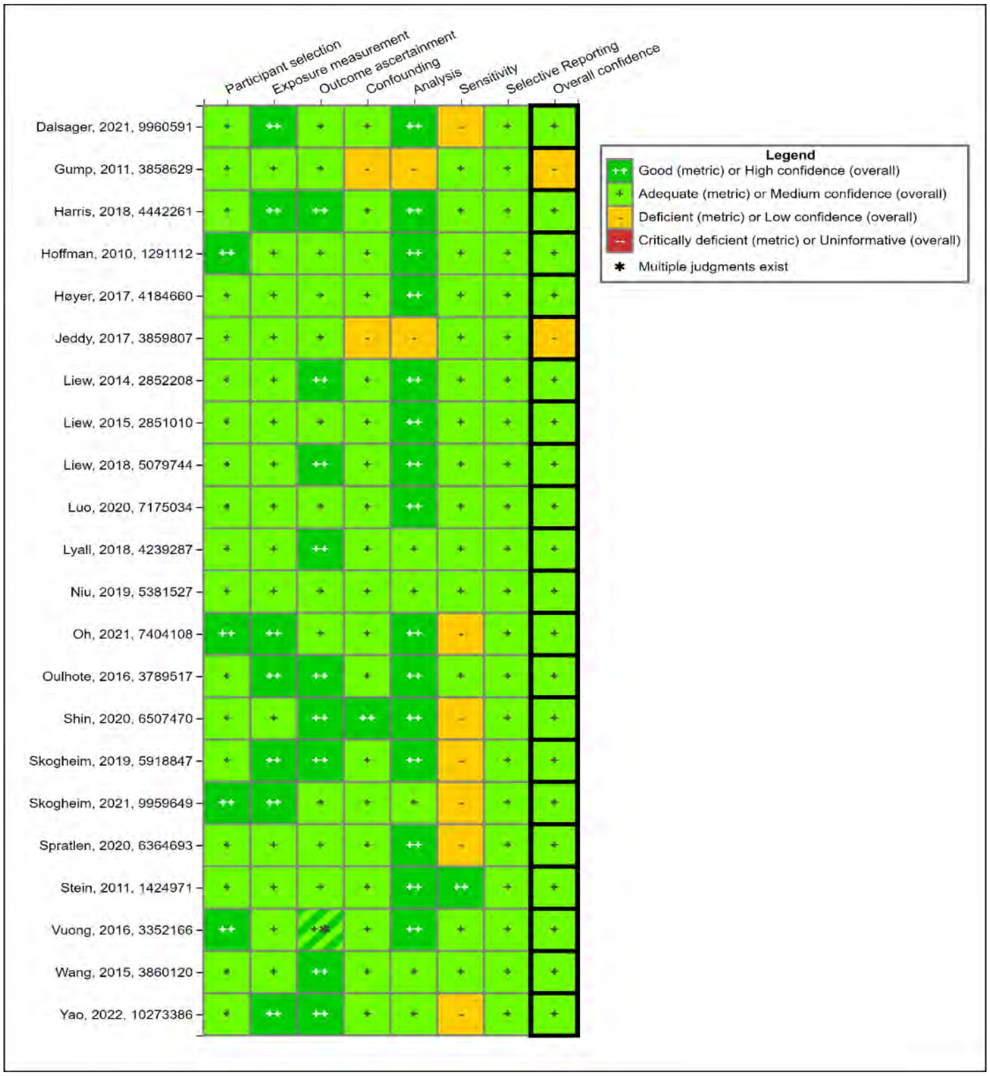

Figure 3-63. Summary of study evaluation for epidemiology studies of neurodevelopment. Multiple publications of the same study: HOME study: Vuong et al. (2016) also includes Vuong et al. (2018b), Vuong et al. (2018a), Vuong et al. (2019), Braun et al. (2014), Zhang et al. (2018a), Vuong et al. (2020), Vuong et al. (2021a), and Vuong et al. (2021b). Project Viva: Harris et al. (2018) also includes Harris et al. (2021). Four publications with data from the Danish National Birth Cohort were evaluated separately due to significantly different procedures but should not be considered independent: Liew et al. (2014), Liew et al. (2015), Liew et al. (2018), Luo et al. (2020). Two publications with data from the Norwegian Mother Father and Child Cohort were evaluated separately due to significantly different selection procedures but should not be considered independent: Skogheim et al. (2020) and Skogheim et al. (2021) For additional detail see HAWC link.

Cognition

Ten studies (13 publications) reported on endpoints related to cognition and PFHxS exposure, including 9 *medium* confidence studies and 1 *low* confidence study. The *medium* confidence studies are presented in Table 3-24. Among the *medium* confidence studies, there was a non-statistically significant inverse association with an exposure-response gradient across quartiles in one study for nonverbal IQ when exposure was measured in mid-childhood (Harris et al., 2018). The same study also reports inverse associations between nonverbal IQ and maternal exposure during pregnancy and between verbal IQ in mid-childhood and both exposure measures, but these are nonmonotonic across the quartiles. Nonmonotonic associations with maternal exposure during pregnancy were also observed for the Full-Scale Intelligence Quotient (FSIQ) at 5 years of age in Liew et al. (2018) and for intellectual disability in Lyall et al. (2018). Other studies reported non-statistically significant inverse associations with in some analyses but positive associations in others (Yao et al., 2022; Skogheim et al., 2020; Niu et al., 2019); Vuong et al. (2019); (Vuong et al., 2016; Wang et al., 2015), with no clear pattern by endpoints, timing of exposure measurement, sex, or any other factor. The remaining *medium* confidence studies did not show decreased cognition with PFHxS exposure. Lastly, the single *low* confidence study (Jeddy et al., 2017) reported associations in opposite directions for multiple measures of language and communication development, and these varied by maternal age. This could be due to social factors associated with age, but since only one *low* confidence study examined this interaction, it should be interpreted with caution. Overall, while there are some inverse associations between cognitive performance and PFHxS exposure, the nonmonotonicity, general imprecision, and inconsistency across sub-analyses within studies make the findings difficult to interpret. It is possible that there are biological reasons for the inconsistencies, but given the heterogeneity in study designs, the data currently do not provide clear support for associations between PFHxS exposure and cognition in children.

Attention Deficit Hyperactivity Disorder (ADHD) or Related Behaviors

Ten studies (13 publications) reported on associations between PFHxS exposure and ADHD or behaviors potentially related to ADHD, including nine *medium* confidence studies and one *low* confidence study. The *medium* confidence studies are presented in Tables 3-25 and 3-26. Six of the ten studies (five of nine *medium* confidence) reported positive associations.

Two *medium* confidence studies examined ADHD diagnosis with PFHxS exposure measured in children cross-sectionally and two studies were cohorts examining maternal exposure. Stein and Savitz (2011) reported statistically significant associations between ADHD diagnosis and diagnosis plus medication in children 5 to 18 years old and exposure-response gradients observed across quartiles. Hoffman et al. (2010) also reported statistically significant positive associations for both outcomes in children 12–15 years of age. Liew et al. (2015) and Skogheim et al. (2021) examined ADHD cases identified from national registries. In Liew et al. (2015), the registry was limited to hospital and psychiatric admissions, which likely represent only severe cases. Neither registry study observed higher likelihood of ADHD with higher PFHxS exposure. All of the studies of ADHD adjusted for sex but did not examine associations stratified by sex.

The remaining seven studies focused on behaviors. While these behaviors are not specific to ADHD, many of them are elevated in individuals with ADHD and are used in its diagnosis. Externalizing problems (consisting of hyperactivity and conduct subscales on the Strengths and Difficulties Questionnaire [SDQ]) were examined in four studies (using the parent version of SDQ). One *medium* confidence study (Høyer et al., 2017) reported a statistically significant positive association for 5- to 9-year-olds with maternal exposure measured during the second trimester of pregnancy modeled as continuous (when exposure was modeled as tertiles, there was an exposure-response gradient across exposure groups, but it was not statistically significant). Another *medium* confidence study using the SDQ reported non-statistically significant positive associations for externalizing, internalizing, and total scores (Luo et al., 2020). The other two study using the SDQ, also *medium* confidence, did not report greater problem behaviors with higher exposure (Harris et al., 2021; Oulhote et al., 2016). The SDQ is a validated instrument, but its sensitivity for ADHD has been inconsistent in different populations (Hall et al., 2019; Pritchard, 2012; Ullebo et al., 2011).

Looking at other neurobehavioral tests, most had only a single study available. One study examined impulsivity and inattention using a different tool (the Conners Continuous Performance Test-II) and also found a non-statistically significant positive association. for inattention but not impulsivity in 8-year-olds with both maternal exposure and exposure measured in the children (Vuong et al., 2018a). In the same study population using a different tool (the Behavioral Assessment System for Children 2 [BASC-2]), positive associations were reported with externalizing problems, hyperactivity, internalizing problems, and attention (statistically significant for all but the latter) when exposure was measured during gestation, but no associations were observed when exposure was measured in children at 3 years. Another *medium* confidence study found no association with behavior problems (measured using the Child Behavior Checklist) using either maternal or childhood exposure measurement. Finally, a *low* confidence cross-sectional study examined inter-response time (IRT) at age 9–11 and found statistically significant decreases in IRT, which indicates poor response inhibition (a primary deficit in children with ADHD) as the test is designed to reward longer response times (Gump et al., 2011).

Taken together, there is some evidence of an association between PFHxS exposure and ADHD or potentially related behaviors. A positive association was observed in most studies (6 of 10) across a variety of populations and diagnostic tests, with an exposure-response gradient in multiple studies. However, there is remaining uncertainty. Associations were inconsistent across *medium* confidence studies. In addition, the only studies reporting an association with ADHD diagnosis are cross-sectional, which may not represent exposure in an etiologically relevant period, while the prospective study of ADHD diagnosis reported an inverse association, although the bias in the cross-sectional studies would likely be toward the null due to nondifferential misclassification.

A few studies examined the possibility of an interaction with sex. Vuong et al. (2018a) reported better performance (lower errors of omission) in boys with higher PFHxS (β = −4.5, 95% CI: −10.0, 1.0), but worse in girls (β = 3.2, 95% CI: −1.1, 7.4). In sex-stratified analyses in Oulhote et al. (2016), most associations were similar in boys and girls, but some had deficits in girls but not boys (cross-sectional analyses at 7 years for externalizing problems and related subscales). Høyer et al. (2017) reported a lack of interaction with sex ($p > 0.1$). There is not adequate evidence to fully assess differences in the association with ADHD or related behaviors by sex.

Social behavior or autism spectrum disorder

Nine studies (10 publications), all *medium* confidence, examined social behaviors or ASD and PFHxS exposure. Five studies examined ASD diagnosis. Two studies (Shin et al., 2020); Liew et al. (2015) reported positive associations. Liew et al. (2015) found a higher risk ratio (RR 1.10, 95% CI: 0.92, 1.33) with PFHxS exposure and Shin et al. (2020) a higher odds ratio (OR 1.36, 95% CI: 0.96, 1.93). The associations in both studies became statistically significant when adjusting for other PFAS. The other three studies ASD diagnosis reported no increase in the odds of ASD diagnosis (Oh et al., 2021; Skogheim et al., 2021; Lyall et al., 2018).

Four *medium* confidence studies (five publications) examined questionnaires for social behavior. Braun et al. (2014) used the Social Responsiveness Scale at 4 and 5 years and reported a nonsignificant positive association (more problem behaviors) (β: 0.4, 95% CI: −1.5, 2.3); in the same study population, Vuong et al. (2021b) used the BASC-2 questionnaire and found similar results with poor social skills. Niu et al. (2019) examined the Ages and Stages questionnaire at 4 years of age and also reported an elevated risk ratio ($p > 0.05$) for personal social skills problems with higher exposure (RR 1.60, 95% CI: 0.92, 2.80 per ln-unit increase in exposure). However, Oulhote et al. (2016) calculated an autism screening score using the peer problems and prosocial subscales on the SDQ at 7 years and reported an inverse association (mean difference: −0.1, 95% CI: −0.3, 0.1). Yao et al. (2022) reported no association with the Social Development Quotient on the Gesell Development Schedules at 1 year. Three of these studies measured PFHxS exposure in maternal serum samples collected during pregnancy (most at 16 weeks gestation for Braun et al. (2014), at 12–16 weeks gestation for Niu et al. (2019), and at 32 weeks gestation for Oulhote et al. (2016)); one study measured exposure in cord blood (Yao et al., 2022), and one study measured exposure in childhood at 3 and 8 years (Vuong et al., 2021b).

Overall, there is some evidence of an association between PFHxS exposure and autism and social behaviors, but there is inconsistency across studies and estimates are generally imprecise. It is feasible that the inconsistency could be explained by timing of exposure measurement, autism measurement tool, or some other factor, but is not possible to determine with the evidence currently available.

Other neurodevelopmental outcomes

Four *medium* confidence studies reported on motor-related behaviors and PFHxS exposure. In (Harris et al., 2018), there was a statistically significant decrease in the visual-motor score from the Wide Range Assessment of Visual Motor Abilities (WRAVMA) test in mid-childhood with higher exposures, when measured cross-sectionally (mean difference (95% CI) versus Q1: Q2: −5.1 (−8.9, −1.3); Q3: −5.0 (−9.0, −0.9), Q4: −5.0 (−9.1, −0.8)). When using a maternal exposure measure during pregnancy, the association was nonmonotonic across the quartiles. No association was observed between the WRAVMA total score and early childhood and maternal exposure measures. In Yao et al. (2022), a statistically significant inverse association was reported with the Gross Motor Development Quotient on the Gesell Development Schedules at 1 year. Conversely, in Spratlen et al. (2020a), positive associations (better motor function on Motor Development Index on Bayleys Scales of Infant Development) were observed with PFHxS exposure at 1, 2, and 3 years of age ($p > 0.05$). An association ($p > 0.05$) with better fine motor skills was also observed in Niu et al. (2019), but no association was observed with gross motor skills using the Ages and Stages Questionnaire. Given the lack of consistency across studies, there is not clear evidence of an association between PFHxS exposure and motor-related behaviors.

One *medium* confidence study examined the association of PFHxS exposure measured during the first or second trimester of gestation with rates of cerebral palsy (Liew et al., 2014). Cases of congenital cerebral palsy were identified from a population-based registry. There was a nonstatistically significant positive association with congenital cerebral palsy in boys (RR 1.2, 95% CI: 0.9, 1.7, exposure-response gradient across quartiles). No association was observed in girls (RR 1.1, 95% CI: 0.6., 1.9), and when limited to girls born at term, a nonsignificant inverse association was observed (RR 0.7, 95% CI: 0.3, 1.6). Given the lack of additional studies and imprecision in the estimate (i.e., wide confidence intervals), there is not clear evidence of an association between PFHxS exposure and cerebral palsy.

Table 3-24. Summary of results for *medium* confidence epidemiology studies of PFHxS exposure and cognitive effects

Study name, country, reference(s)	Measured endpoint (test used)	Exposure measurement timing	Estimate type (adverse direction)[a]	Sub-population / N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
Danish National Birth Cohort, Denmark Liew et al. (2014)	FSIQ at 5 yrs (WPPSI)	Maternal (median 8.7, SD 2.5 wk gestation)	Mean Difference vs. Q1 (↓)	Boys (n = 831)	Q1	<LOQ–0.76	Ref		
					Q2	0.77–1.07	**-4.5***	-8.6	-0.4
					Q3	1.08–1.38	-2.7	-7.0	1.6
					Q4	> = 1.39	-2.0	-7.0	2.9
			Mean Difference vs. Q1 (↓)	Girls (n = 761)	Q1	<LOQ–0.76	Ref		
					Q2	0.77–1.07	2.8	-0.8	6.5
					Q3	1.08–1.38	2.6	-1.1	6.2
					Q4	> = 1.39	-0.7	-5.1	3.6
Health Outcomes and Measures of the Home Environment (HOME), U.S. Vuong et al. (2016) Vuong et al. (2019)	FSIQ at 8 yrs (WISC-IV)	3 yrs	Regression Coefficient (↓)	221	Ln-unit increase in exposure	NR	-0.4	-2.5	1.6
		Maternal (16 ± 3 wks gestation)	Regression Coefficient (↓)	221	Ln-unit increase in exposure	GM 1.4	0.5	-1.8	2.9
	Global executive function score at 5/8 yrs (BRIEF)	Maternal (16 ± 3 wks gestation)	Mean Difference (↑)	219	Ln-unit increase in exposure	1.5 (0.9–2.4)	1.36	-0.41	3.12

Study name, country, reference(s)	Measured endpoint (test used)	Exposure measurement timing	Estimate type (adverse direction)[a]	Sub-population / N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
Vuong et al. (2020)	Reading composite scores at 8 yrs	Maternal	Regression Coefficient (↓)	161	Log10-unit increase in exposure	1.7	4.5	-3.1	12.0
Project Viva, U.S. Harris et al. (2018) Harris et al. (2021)	Word knowledge early childhood[b] (PPVT)	Maternal (5–21 wks gestation)	Mean Difference vs. Q1 (↓)	948	Q1	<0.1–1.6	Ref		
					Q2	1.7–2.4	0.7	-1.6	2.9
					Q3	2.5–3.7	0.1	-2.1	2.4
					Q4	3.8–43.2	0.4	-1.9	2.7
	Verbal IQ mid-childhood[b] (KBIT)	Maternal (5–21 wks gestation)	Mean Difference vs. Q1 (↓)	851	Q1	<0.1–1.6	Ref		
					Q2	1.7–2.4	**-2.8***	-5.1	-0.5
					Q3	2.5–3.7	-1.2	-3.6	1.2
					Q4	3.8–43.2	0.3	-2.2	2.8
		Mid-childhood (6–10 yrs)	Mean Difference vs. Q1 (↓)	631	Q1	<0.1–1.1	Ref		
					Q2	1.2–1.9	-0.8	-3.6	2.1
					Q3	2.0–3.4	-0.2	-3.3	2.8
					Q4	3.5–56.8	-1.7	-4.8	1.5
	Nonverbal IQ mid-childhood[b] (KBIT)	Maternal (5–21 wks gestation)	Mean Difference vs. Q1 (↓)	862	Q1	<0.1–1.6	Ref		
					Q2	1.7–2.4	**-3.9***	-6.9	-0.5
					Q3	2.5–3.7	-1.6	-4.7	1.5
					Q4	3.8–43.2	-1.0	-4.2	2.2
				640	Q1	<0.1–1.1	Ref		

Study name, country, reference(s)	Measured endpoint (test used)	Exposure measurement timing	Estimate type (adverse direction)[a]	Sub-population / N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
		Mid-childhood (6–10 yrs)	Mean Difference vs. Q1 (↓)		Q2	<0.1–1.1	-0.9	-4.4	2.7
					Q3	1.2–1.9	-2.3	-6.1	1.5
					Q4	2.0–3.4	-2.7	-6.6	1.2
	Global executive function score at 6-10 yrs (BRIEF)	Maternal (5–21 wks gestation)	Mean Difference vs Q1 (↑)	921	Q1	<0.1–1.6	Ref		
					Q2	1.7–2.4	-0.3	-1.9	1.3
					Q3	2.5–3.7	0.2	-1.4	1.9
					Q4	3.8–43	-1.1	-2.8	0.6
Taiwan maternal and infant cohort study, Taiwan Wang et al. (2015)	FSIQ at 5 yrs (WPPSI)	Maternal (3rd trimester)	Regression Coefficient (↓)	120	Doubling of exposure	0.7 (0.07–1.09)	0.4	-1.1	1.9
	FSIQ at 8 yrs (WISC)		Regression Coefficient (↓)	120	Doubling of exposure	0.7 (0.07–1.07)	-0.2	-1.8	1.4
WTC cohort, U.S. Spratlen et al. (2020a)	MDI at 1 yr (BSID)	Cord blood/ maternal (1 d post-delivery)	Regression Coefficient (↓)	302	Log-unit increase	GM 0.7 (range <LOQ–15.8)	0.20	-2.06	2.45
				Girls 150			0.03	-2.71	2.77
				Boys 152			0.45	-2.69	3.59
	MDI at 3 yr (BSID)			302			3.30	0.70	5.90
				Girls 150			2.39	-1.0	5.78
				Boys 152			4.62	-5.08	14.3

Study name, country, reference(s)	Measured endpoint (test used)	Exposure measurement timing	Estimate type (adverse direction)[a]	Sub-population / N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
	FSIQ at 4 yr (WPPSI)			302			0.04	-2.78	2.86
				Girls 150			0.35	-3.20	3.90
				Boys 152			-0.41	-4.84	4.02
	FSIQ at 6 yr (WPPSI)			302			-0.34	-3.71	3.03
				Girls 150			0.57	-3.13	4.27
				Boys 152			-1.64	-8.07	4.79
Norwegian Mother, Father and Child cohort, Norway Skogheim et al. (2020)	Verbal working memory at 42 mo (CDI)	Maternal (17 wk gestation)	Regression coefficient (↓)	768	Q2	0.7 (0.5–0.9)	0.03	-0.20	0.26
					Q3		0.10	-0.13	0.33
					Q4		0.20	-0.03	0.44
					Q5		0.21	-0.03	0.45
	Nonverbal working memory at 42 mo (CDI)			934	Q2		-0.18	-0.38	0.03
					Q3		-0.05	-0.26	0.16
					Q4		-0.23	-0.44	-0.02
					Q5		-0.18	-0.40	0.04
Shanghai-Minhang cohort, China Niu et al. (2019)	Communication at 4 yrs (ASQ-3)	Maternal (12–16 wks gestation)	Risk ratio for problems (↑)	533	Ln-unit increase in exposure	2.8 (2.1–0.5)	1.10	0.78	1.54
				Girls 236			1.46	0.79	2.70
				Boys 297			0.90	0.60	1.35
				533			0.85	0.54	1.36

Study name, country, reference(s)	Measured endpoint (test used)	Exposure measurement timing	Estimate type (adverse direction)[a]	Sub-population / N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
	Problem solving at 4 yrs (ASQ-3)			Girls 236			1.06	0.40	2.78
				Boys 297			0.75	0.43	1.32
Early Markers for Autism (EMA), U.S. Lyall et al. (2018)	Intellectual disability at 4–9 yrs (clinical diagnosis)	Maternal (15–19 wks gestation)	Odds Ratio (OR) (↑)	622	Ln-unit increase in exposure	GM 1.33	1.11	0.86	1.42
			Odds Ratio (OR) vs. Q1 (↑)	160	Q1	<0.8	1.0		
				171	Q2	0.8–<1.3	1.43	0.86	2.40
				133	Q3	1.3–<2.0	1.03	0.58	1.85
				157	Q4	> = 2.0	1.30	0.74	2.29
Laizhou Wan Birth Cohort, China Yao et al. (2022)	Adaptive Development Quotient at 1 yr	Cord serum	Regression coefficient (↓)	274	Log10-unit increase in exposure	0.3 (range 0.1-1.1)	-1.40	-6.17	3.37
				Girls 135			-2.02	-9.27	5.23
				Boys 139			-1.22	-7.62	5.18
	Language Development Quotient at 1 yr			274			3.00	-1.67	7.67
				Girls 135			2.05	-4.82	8.93
				Boys 139			4.02	-2.39	10.42

*$p < 0.05$.

[a]The arrows indicate the direction the effect estimate will be if there is an association between PFHxS and reduced cognitive performance. For some tests, a higher score means better performance, while for other tests, a higher score means more problems.

[b]Early childhood median age 3.2 years, range 2.8–6.3; Mid-childhood median age 7.7 years, range 6.6–10.9.

FSIQ: Full-Scale Intelligence Quotient; WPPSI: Wechsler Primary and Preschool Scales of Intelligence, WISC: Wechsler Intelligence Scale for Children, BRIEF: Behavior Rating Inventory of Executive Function, PPVT: Peabody Picture Vocabulary Test, KBIT: Kaufman Brief Intelligence Test, BSID: Bayley Scales of Infant Development, MDI: mental development index.

Table 3-25. Summary of results for *medium* confidence epidemiology studies of PFHxS exposure and attention deficit hyperactivity disorder (ADHD)

Study name	Measured endpoint	Exposure measure-ment timing	Estimate type (adverse direction)[a]	Subpopulation/ N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
C8 Health Project, U.S. (Stein and Savitz, 2011)	ADHD diagnosis at 5–18 yrs (clinical)	Cross-sectional	Odds Ratio (OR) vs. Q1 (↑)	1,0546	Q1	0.25–<2.9 ng/mL	1.0		
					Q2	2.9–<5.2	1.27*	1.06	1.52
					Q3	5.2–<10.1	1.43*	1.21	1.70
					Q4	10.1–276.4	1.53*	1.29	1.83
	ADHD diagnosis + medication at 5–18 yrs (clinical)	Cross-sectional	Odds Ratio (OR) vs. Q1 (↑)	1,0546	Q1	0.25–<2.9 ng/mL	1.0		
					Q2	2.9–<5.2	1.44*	1.09	1.90
					Q3	5.2–<10.1	1.55*	1.19	2.04
					Q4	10.1–276.4	1.59*	1.21	2.08
NHANES (1999–2000, 2003–2004), U.S. Hoffman et al. (2010)	ADHD at 12–15 yrs (clinical)	Cross-sectional	Odds Ratio (OR) (↑)	571	One unit increase in exposure	2.2 (2.9)	1.06*	1.02	1.11
	ADHD+ medication at 12–15 yrs (clinical)					2.2 (2.9)	1.07*	1.03	1.11
Danish National Birth Cohort, Denmark Liew et al. (2015)	ADHD diagnosis (national registry)	Maternal (1st trimester)	Risk ratio (↑)	770	In-unit increase	Controls 0.9 (0.7–1.2)	0.97	0.88	1.08
					Q1	<LOQ–0.68	1.0		
					Q2	0.69–0.92	1.05	0.88	1.26
					Q3	0.93–1.23	0.94	0.78	1.14
					Q4	>1.23	0.67*	0.54	0.83

Study name	Measured endpoint	Exposure measure-ment timing	Estimate type (adverse direction)[a]	Subpopulation/ N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
Norwegian Mother Father Child Cohort, Norway Skogheim et al. (2021)	ADHD diagnosis (national registry)	Maternal (2[nd] trimester, 18 wks gestation)	Odds ratio (↑)	1801	Q1	0.1-0.5	1.0		
					Q2	0.5-0.6	1.08	0.82	1.42
					Q3	0.6-0.9	1.12	0.85	1.49
					Q4	0.9-15	0.89	0.66	1.19

*$p < 0.05$.
[a]The arrows indicate the direction the effect estimate will be if there is an association between PFHxS and reduced behavior. For all the tests included here, higher scores indicate more ADHD diagnosis.

Table 3-26. Summary of results for medium confidence epidemiology studies of PFHxS exposure and behavior

Study name	Measured endpoint	Exposure measure-ment timing	Estimate type (adverse direction)[a]	Subpopulation/ N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
Faroe Island cohort, Denmark Oulhote et al. (2016)	Externalizing problems at 7 yrs (SDQ)	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.6 (0.5–0.9)	0	-0.36	0.37
		Maternal (32-wk gestation)		539		4.5 (2.2–8.4)	-0.19	-0.48	0.11
	Internalizing problems at 7 yrs (SDQ)	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.6 (0.5–0.9)	-0.1	-0.43	0.22
		Maternal (32-wk gestation)		539		4.5 (2.2–8.4)	-0.1	-0.36	0.17
	Total SDQ score at 7 yrs	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.6 (0.5–0.9)	-0.1	-0.66	0.46
		Maternal (32-wk gestation)		539		4.5 (2.2–8.4)	-0.28	-0.75	0.18
INUENDO (Bio persistent organochlorines in diet and human fertility), Greenland, Ukraine, Poland Høyer et al. (2017)	Hyperactivity score at 5–9 yrs (SDQ)	Maternal (median 2nd trimester)	Regression Coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.20*	0.00	0.40
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.15	-0.30	0.60
					High exposure	2.0–18.8	0.41	-0.03	0.86
	Total SDQ score at 5–9 yrs	Maternal (median 2nd trimester)	Regression Coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.45	-0.03	0.92
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.68	-0.04	1.38
					High exposure	2.0–18.8	0.80*	0.06	1.54
Project Viva, U.S. Harris et al. (2021)	Externalizing problems at 6-10 yrs (SDQ)	Maternal (5-21 wks gestation)	Mean Difference vs Q1 (↑)	921	Q1	<0.1-1.6	Ref		
					Q2	1.7-2.4	0.0	-0.5	0.5
					Q3	2.5-3.7	0.6	0.0	1.1
					Q4	3.8-43	0.0	-0.5	0.6

Study name	Measured endpoint	Exposure measure-ment timing	Estimate type (adverse direction)[a]	Subpopulation/ N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
	Internalizing problems at 6–10 yrs (SDQ)				Q1	<0.1-1.6	Ref		
					Q2	1.7-2.4	0.2	-0.3	0.6
					Q3	2.5-3.7	-0.1	-0.5	0.4
					Q4	3.8-43	0.2	-0.3	0.7
	Total SDQ score at 6–10 yrs				Q1	<0.1-1.6	Ref		
					Q2	1.7-2.4	0.2	-0.6	1.0
					Q3	2.5-3.7	0.5	-0.3	1.4
					Q4	3.8-43	0.2	-0.7	1.1
Danish National Birth Cohort, Denmark [Luo et al. (2020)]	Externalizing problems at 7 yrs	Maternal (1st trimester)	OR (↑) (odds of elevated score)	2421	Per doubling of exposure	0.9 (0.7-1.3)	1.11	0.86	1.43
	Internalizing problems at 7 yrs						1.18	0.88	1.58
	Total SDQ score at 7 yrs						1.15	0.94	1.42
Odense Child Cohort, Denmark ([Dalsager et al., 2021b])	Behavior problems (CBC) at 2–5 yrs	Maternal (8–16 wks gestation)	Incidence rate ratio (↑)	1138	Doubling of exposure	0.4	0.98	0.93	1.03
			Odds ratio (↑)				0.95	0.79	1.16
		18 mo	Incidence rate ratio (↑)	817		0.3	0.95	0.88	1.04
			Odds ratio (↑)				1.04	0.79	1.37
Health Outcomes and Measures of the Home	Impulsivity – Commissions at 8 yrs (CPT)	3 yrs	Regression Coefficient (↑)	204	In-unit increase in exposure	1.9 (1.0–3.3)	-0.6	-2.1	1.0
		Maternal (16 ± 3 wk-gestation)				1.3 (0.8–2.3)	-0.5	-1.9	0.9
		3 yrs				1.9 (1.0–3.3)	0.6	-2.3	3.5

Study name	Measured endpoint	Exposure measure-ment timing	Estimate type (adverse direction)[a]	Subpopulation/ N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
Environment (HOME) U.S. [Vuong et al. (2018a)](#) [Vuong et al. (2021a)](#) [Vuong et al. (2021b)](#)	Inattention – Omissions at 8 yrs (CPT)	Maternal (16 ± 3 wk-gestation)				1.3 (0.8–2.3)	2.5	-0.9	6.0
	Externalizing problems (BASC-2) at 5 and 8 yrs	Maternal (16 ± 3 wk-gestation)	Odds ratio (↑)	241	ln-unit increase in exposure	1.5	1.9*	1.1	3.2
	Hyperactivity (BASC-2)						2.5*	1.5	4.3
	Attention (BASC-2)						1.2	0.8	1.9
	Internalizing problems (BASC-2)						2.0*	1.1	3.4
	Externalizing problems (BASC-2) at 8 yrs	3 yrs	Regression Coefficient (↑)	208	Ln-unit increase in exposure	1.9	0.02	-1.6	1.6
	Hyperactivity (BASC-2)						-0.3	-1.9	1.2
	Attention (BASC-2)						-0.1	-1.6	1.4
	Conduct problems (BASC-2)						0.4	-1.3	2.1

*$p < 0.05$.
SDQ: Strengths and Difficulties Questionnaire, CPT: Conners continuous performance test, CBC: Child Behavior Checklist, BASC-2: Behavioral Assessment System for Children 2.
[a]The arrows indicate the direction the effect estimate will be if there is an association between PFHxS and reduced behavior. For all the tests included here, higher scores indicate more difficulties/behavior problems.

Animal Studies

There were three animal studies evaluating neurodevelopmental outcomes and PFHxS exposure: two *medium* confidence studies (Ramhøj et al., 2020; Butenhoff et al., 2009) and one *low* confidence study (Viberg et al., 2013) (see Figure 3-64). Butenhoff et al. (2009) exposed male and female Crl:CD Sprague Dawley rats to 0.3, 1, 3, or 10 mg/kg-day daily via oral gavage starting at 14 days prior to cohabitation (F_0). F_1 pups were not exposed directly but were exposed in utero and through lactation. The study authors then assessed 5 pups per sex per litter from 10 dams using the functional observation battery (FOB)[19] and an automated motor activity assessment tool at PND22. In the second *medium* confidence study, Ramhøj et al. (2020) exposed Wistar dams to 0, 0.05, 5, or 25 mg/k bw-day PFHxS via gavage starting at gestational day 7 (GD7) through postnatal day 22 (PD 22). After weaning, one male and one female pup from each litter subsequently underwent behavioral assessment of motor activity levels[20] at each of three ages: PD 27, PD 115, and PD 340. Additionally, Viberg et al. (2013) evaluated spontaneous locomotor behavior by exposing male and female NMRI mouse pups at postnatal day 10 (PND10) to a single oral dose of PFHxS at 0.61, 6.1, or 9.2 mg/kg-bw PFHxS. Spontaneous locomotor behavior was then evaluated at 2– and 4–months post-exposure, and nicotine-induced behavior was evaluated at 4 months.

19FOB evaluations consisted of assessment of (1) autonomic functions: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, and urination and defecation; (2) reactivity and sensitivity: sensorimotor responses to visual, auditory, tactile and painful stimuli; (3) excitability reactions to handling and behavior in the open field; (4) gait and sensorimotor coordination: gait pattern in the open field, severity of gait abnormalities, air righting reaction and landing foot splay; forelimb and hindlimb grip strength; and (5) abnormal clinical signs including convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance and deposits around the eyes, nose or mouth. (Butenhoff et al., 2009)
20Measured in activity boxes with photocells recording horizontal activity for 30 minutes. Rearing behavior (vertical activity) was not measured by Ramhøj et al. (2020)

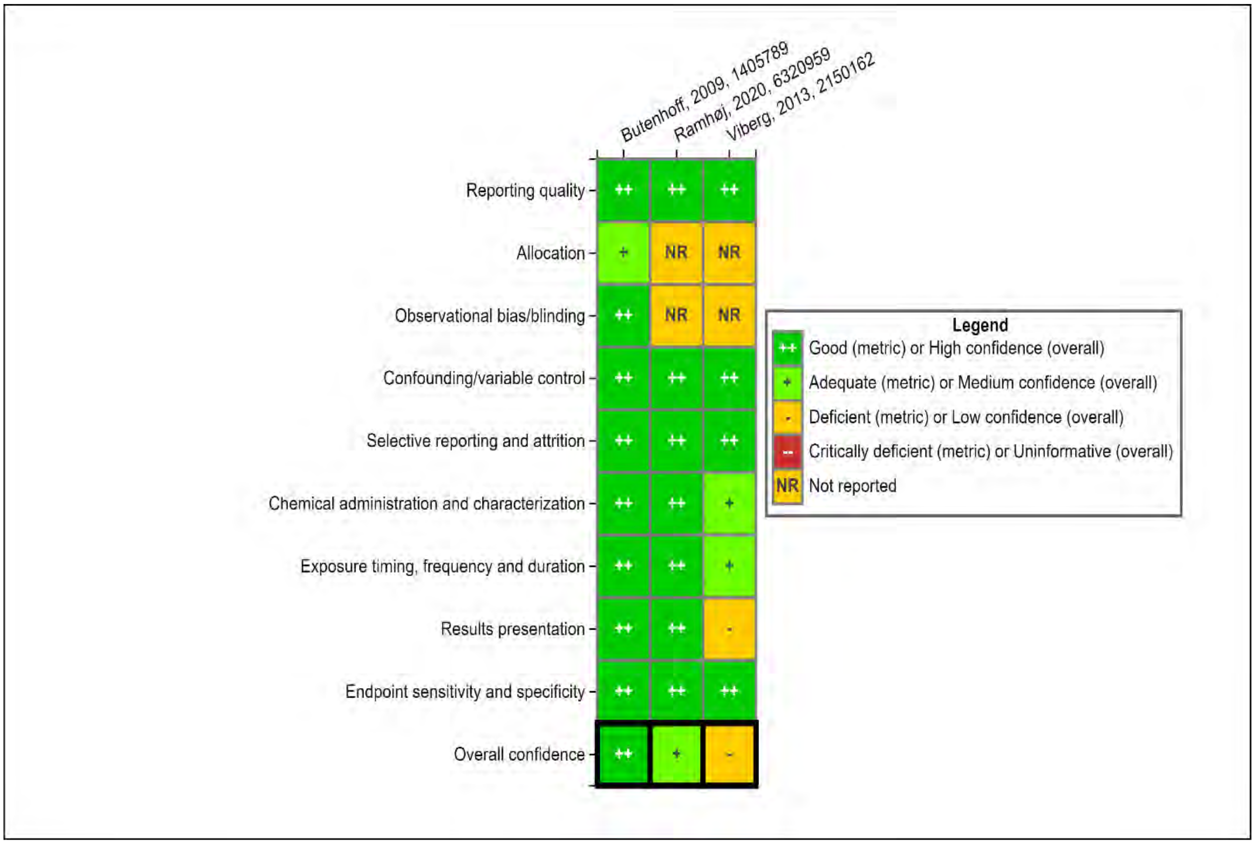

Figure 3-64. Confidence scores of neurodevelopmental system effects from repeated PFHxS dose animal toxicity studies. For additional details see HAWC link.

Functional observation battery (FOB)

One study (Butenhoff et al., 2009) reported on PFHxS effects on FOB assessment on F1 pups. The authors reported no statistically significant differences between control animals and PFHxS treated animals on the assessments of FOB parameters.

Learning and memory

One study (Ramhøj et al., 2020) reported on PFHxS effects on radial arm maze assessments in Wistar male and female rat offspring exposed to PFHxS in utero and through lactation. Assessments were performed at postnatal day (PD) 115 and PD 340. The authors reported that no statistically significant differences between control animals versus PFHxS treated animals.

Motor-related behaviors

Butenhoff et al. (2009), Ramhøj et al. (2020) and Viberg et al. (2013) evaluated and reported on locomotor activity (including anxiety-related behaviors) in response to PFHxS exposure. The two *medium* confidence studies, Butenhoff et al. (2009) and Ramhøj et al. (2020), reported no statistically significant differences in motor activity in either sex with in-utero and lactational PFHxS dosing of dams from 0.05 to 25 mg/kg-day. One *low* confidence study, Viberg et al. (2013) reported decreases in ambulatory (horizontal) activity and rearing behaviors in male and female NMRI pups at 2 and 4 months following a single oral dose of PFHxS at 0.61, 6.1, or 9.2 mg/kg bw PFHxS at postnatal day 10 (PND10) during the habituation (first 20 minutes) and end (minutes 40–60) periods of observation at 2 and 4 months after a single exposure to 9.2 mg/kg-day PFHxS on PND9; however, the authors did not account for the potential impact of litter effects In their experimental design, and they allocated pups to dosing groups from 3–4 litters in an unclear fashion, reducing confidence in these findings. Taken together, the potential effects of PFHxS exposure on motor-related behaviors in rodents remain unknown.

Mechanistic evidence and supplemental information

Seven mechanistic studies were identified relating to the potential for PFHxS to elicit neurodevelopmental effects. Two of these studies were performed in vivo and five were performed using in vitro models. Of the two in vivo studies, one was a follow-up to the Viberg et al. (2013) study described above. Using the same study design as Viberg et al. (2013), and thus possessing the same methodological limitations, Lee and Viberg (2013) examined changes in proteins[21] involved in a variety of neuronal functions in the cerebral cortex and hippocampus in NMRI male and female mice at both 24 hours and 4 months following a single dose of PFHxS on PND9 at either 6.2 mg/kg bw or 9.2 mg/kg bw. While the authors observed significant changes in protein levels at 24 hours in PFHxS-exposed animals the majority of these changes had resolved at the 4-month timepoint. At 4 months the only significant change was an increase in Tau protein expression ($p < 0.01$) in the cerebral cortex of male mice at the 6.1 mg/kg bw dose.

PFHxS was also shown to produce a significant repression of long-term potentiation (LTP) ($p < 0.05$), which is associated with learning and memory formation processes, in adult Sprague Dawley rats exposed via intracerebroventricular injection at the CA1 region of the hippocampus both 10 and 100 μM PFHxS (Zhang et al., 2016a). However, the authors noted no remarkable changes in field excitatory postsynaptic potential (fEPSP) amplitude (decreased LTP would be expected to represent weaker synaptic strength and reduced fEPSP) between control and PFHxS treated groups (Zhang et al., 2016a). In addition, this study was performed in adult rats therefore making it difficult to determine how relevant the effects observed by Zhang et al. (2016a) are to human neurodevelopment.

[21]**BNDF**: brain derived neurotrophic factor; protein involved in canonical nerve growth (Huang and Reichardt, 2001); **CaMKII:** Ca^{2+}/calmodulin dependent protein kinase II; a serine-threonine-specific protein kinase that is regulated by Ca^2/calmodulin. Involved in a variety of neuronal processes including learning and memory (Yamauchi, 2005). **GAP43:** Growth Associated Protein 43; Protein expressed at high levels in neural growth cones during development and axonal regeneration (Rosskothen-Kuhl and Illing, 2014) **Synaptophysin:** protein present in the neuroendocrine cells involved in synaptic transmission (Mcmahon et al., 1996); **Tau:** Tau proteins are a group of 6 highly soluble protein isoforms that are produced by alternative splicing. Tau proteins play a role in the stability of microtubules in axons and are present in abundance in CNS neurons (Barbier et al., 2019).

Evidence from animals prenatally exposed to other per and polyfluoroalkyl substances (PFAS) such as PFOA and PFOS, suggest that PFAS may affect neurodevelopment (Kawabata et al., 2017; Shrestha et al., 2017; Salgado et al., 2016; Zhang et al., 2016b; Fuentes et al., 2007; Lau et al., 2003). PFAS-related effects relevant to neurodevelopment include decreased choline acetyltransferase activity in the prefrontal cortex of exposed rats postnatally (Lau et al., 2003), delayed neuromotor maturation (e.g., decreased resistance to backward pull-on postnatal day [PND] 10 and 11) (Fuentes et al., 2007).

Evidence Integration

Taken together, the available human studies were interpreted to provide *slight* evidence. Specifically, five *medium* confidence epidemiological studies that reported some evidence of positive associations between PFHxS exposure and ADHD or behaviors potentially related to ADHD at median blood concentrations in the study populations of 1–5 ng/mL. In addition, several epidemiology studies examined whether PFHxS exposure has the potential to affect the following neurodevelopmental outcomes: cognition, social behavior and autism, and other outcomes such as motor-related behaviors and cerebral palsy. However, associations with these neurodevelopmental outcomes were inconsistent across studies and generally imprecise, and thus did not contribute to the overall judgment for potential neurodevelopmental effects.

Th animal evidence base consisted of three studies examining PFHxS effect on FOB and motor function, and a single study on PFHxS effects on learning and memory. PFHxS-related effects in these studies were null or of *low* confidence. Additional animal studies potentially relevant to interpreting the outcomes examined in the epidemiology studies of PFHxS were unavailable. Thus, the overall animal evidence was considered *indeterminate* (see Table 3-27).

The endocrine and nervous systems work in harmony during early development. To this end, evidence from the endocrine evidence base was also examined to see if any of the studies in the endocrine database could help inform PFHxS neurotoxicity. While no studies evaluated both endocrine and neurological outcomes as part of their study designs, the prior judgment that PFHxS exposure is likely to result in decreased levels of serum thyroxine (T4)—particularly the evidence after developmental PFHxS exposure (for more details please see Section 3.2.1), is of potential relevance. In rats, decreased serum T4 is correlated with adverse neurodevelopmental outcomes (Crofton, 2004), and, in humans, a link between prenatal maternal T4 and decreased cognitive function in children has been observed (Finken et al., 2013; Henrichs et al., 2013; Li et al., 2010; Haddow et al., 1999; Man et al., 1971). The lack of neurological outcome measurements in the available endocrine studies examining PFHxS-related toxicity highlights an important data gap.

The available **evidence suggests** but is not sufficient to infer whether exposure to PFHxS might cause neurodevelopmental effects in humans given sufficient exposure conditions[22] (see

[22] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-27). This conclusion is based on *slight* epidemiological evidence primarily from four *medium* confidence epidemiological studies that reported some evidence of positive associations between PFHxS exposure and ADHD or behaviors potentially related to ADHD at median blood concentrations in the study populations of 1–5 ng/mL.

Table 3-27. Evidence profile table for PFHxS neurotoxicological effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Nervous System Human Studies Section)					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary of key findings**	**Evidence stream judgment**	
• ADHD or related behaviors • 9 *medium*, 1 *low* confidence studies	• Exposure-response gradients in multiple studies • Mostly *medium* confidence studies, with positive associations in 5 of 9	• *Unexplained inconsistency* • Unclear biological relevance of etiologic window in cross-sectional studies reporting associations	5 *medium* and 1 *low* confidence studies reported positive associations between PFHxS exposure and ADHD or behavior consistent with ADHD.	⊕⊙⊙ *Slight* Based on some evidence of an association between PFHxS exposure or ADHD and related behaviors, although uncertainty remains. Other outcomes did not contribute to this judgment.	⊕⊙⊙ **Evidence suggests, but is not sufficient to infer** *Primary basis*: Based on human evidence for decreased ADHD and related behaviors at median blood concentrations of 0.9–5 ng/mL *Human relevance*: Evidence comes from epidemiological studies (see Nervous System Human Studies Section) *Cross-stream coherence*: NA: animal evidence is indeterminate *Susceptible populations*: In utero or childhood exposure.
Cognition • 9 *medium* and 1 *low* confidence studies	• No factors noted	• Unexplained inconsistency, including by timing of exposure measurement.	Inverse associations between cognition and PFHxS exposure were observed in multiple studies, but there were inconsistencies across studies and in sub-analyses within studies.		

Evidence stream summary and interpretation					Evidence integration summary judgment
Social behavior or ASD • 9 medium confidence studies	• No factors noted	• Unexplained inconsistency • Imprecision	Of 5 studies of ASD, 2 reported higher likelihood of diagnosis. Other studies of social behavior were similarly inconsistent.		
Other neurodevelopmental effects • 5 medium confidence studies	• No factors noted.	• Unexplained inconsistency for motor-related behaviors • Imprecision for cerebral palsy	2*medium* confidence studies reported a decrease in motor scores with higher PFHxS exposure, while improved motor function was observed in two *medium* confidence studies. A *medium* confidence study reported a non-statistically significant positive association with cerebral palsy in boys.		
Evidence from In vivo Animal Studies (see Nervous System Animal Studies Section)				**Evidence stream judgment**	
Studies and confidence	**Factors that increase strength**	**Factors that decrease strength**	**Summary of key findings**		
Behavioral • 2 *medium* 1 *low* confidence studies	• No factors noted	• *Low* confidence study is only one to observe an effect	2 *medium* confidence studies reported no effects on FOB parameters, motor activity, or learning and memory. The *low* confidence study observed decreases in spontaneous behaviors.	⊙⊙⊙ *Indeterminate*	

3.2.6. Cardiometabolic Effects

Cardiometabolic risk refers to the likelihood of developing diabetes, heart disease, or stroke. Contributors to this risk include a combination of metabolic dysfunctions mainly characterized by insulin resistance, dyslipidemia, hypertension, and adiposity (obesity).

Human Studies

Serum lipids

High cholesterol, specifically low-density lipoprotein (LDL) cholesterol, is one of the major controllable risk factors for cardiovascular disease, including coronary heart disease, myocardial infarction, and stroke. Cholesterol levels are typically measured in the blood. Thirty-eight studies evaluated the relationship between PFHxS exposure and blood lipids (i.e., cholesterol, LDL cholesterol, and triglycerides).

Multiple outcome-specific considerations for study evaluation influenced the ratings. First, for outcome ascertainment, collection of blood during a fasting state is preferred for all blood lipid measures (NIH, 2020; Nigam, 2011) but lack of fasting was considered deficient for triglycerides and LDL cholesterol (which is typically calculated using triglycerides). This is because triglyceride levels remain elevated for several hours after a meal (Nigam, 2011), which is likely to result in substantial outcome misclassification if there is not standardization across study participants. Self-reported high cholesterol was also considered deficient for outcome ascertainment due to the high likelihood of misclassifying cases as controls (Natarajan et al., 2002). Both of these issues are likely to result in nondifferential outcome misclassification and to generally bias results toward the null. It is also important for studies to account for factors that meaningfully influence serum lipids, most notably use of cholesterol lowering medications and pregnancy. Studies that did not consider these factors by exclusion, stratification, or adjustment were considered deficient for the participant selection domain. All of the available studies analyzed serum lipids and PFHxS in serum or plasma using standard, appropriate methods. As described in the Endocrine Effects section, reverse causation was considered based on binding of lipophilic chemicals (such as PFAS) to serum lipids (Chevrier, 2013), but this is unlikely to significantly bias the results because PFAS, including PFHxS, do not preferentially bind to serum lipids (Forsthuber et al., 2020), so exposure measurements in blood, including cross-sectional, were considered adequate for this outcome.

A summary of the study evaluations is presented in Figure 3-65, and additional details can be obtained from HAWC. Five studies were excluded from further analysis as *uninformative* due to critical deficiencies confounding in four studies (Seo et al., 2018; Yang et al., 2018; Rotander et al., 2015b; Tao et al., 2008) and selection bias in two studies (Sinisalu et al., 2021; Yang et al., 2018). Twenty-four studies were classified as *medium* confidence for at least one serum lipid measure (Cakmak et al., 2022; Dunder et al., 2022; Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Dalla Zuanna et al., 2021; Gardener et al., 2021; Li et al., 2021a; Tian et al., 2021; Canova et al., 2020; Jensen et al., 2020a; Liu et al., 2020a; Spratlen et al., 2020b; Yang et al., 2020b; Dong et al., 2019; Lin et al., 2019; Jain and Ducatman, 2018; Kang et al., 2018; Mora et al., 2018; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015; Starling et al., 2014b), although 11 of these were *low* confidence for triglycerides (and LDL cholesterol when calculated from triglycerides), as described above (Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015; Starling et al., 2014b). Nine studies were classified as *low* confidence for all serum lipid endpoints (Batzella et al., 2022; Varshavsky et al., 2021; Khalil et al., 2020; Li et al., 2020b; Chen et al., 2019a; Khalil et al., 2018; Koshy et al., 2017; Christensen et al., 2016).

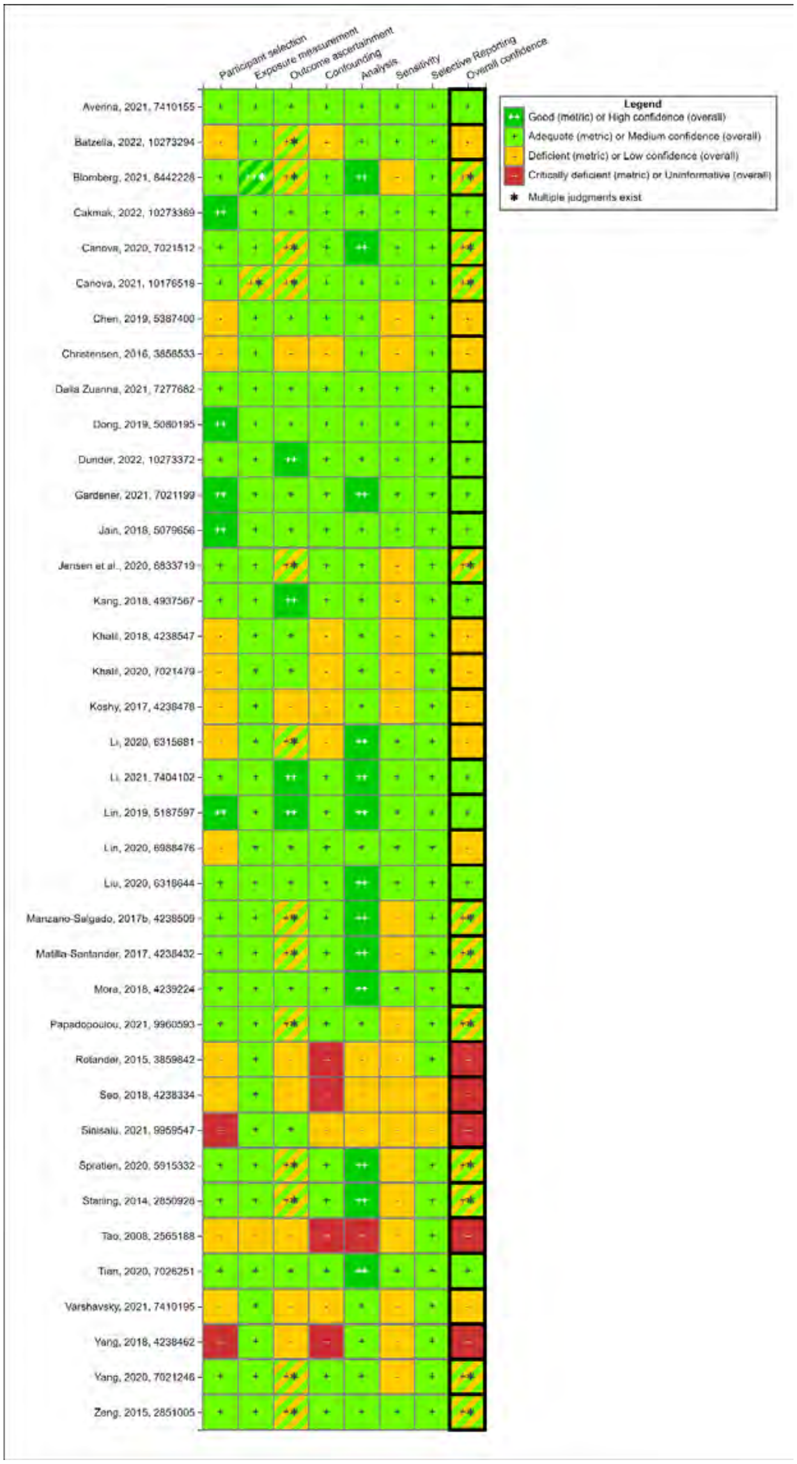

Figure 3-65. Study evaluation results for epidemiology studies of PFHxS and blood lipids. For additional details see HAWC. Multiple publications of the same study: **Canova et al. (2020)** also includes **Zare Jeddi et al. (2021)**; **Cakmak et al. (2022)** also includes **Fisher et al. (2013)**.

The results for the association between PFHxS exposure and blood lipids are presented in Table 3-28. It is difficult to directly compare the magnitudes of effect across studies due to the different analyses and data transformations (e.g., log transformations of PFHxS levels and/or lipid levels), so the synthesis is focused primarily on direction of association.

In adults, all six *medium* confidence studies (reported in eight publications) examining total cholesterol reported positive associations between total cholesterol and PFHxS exposure (Cakmak et al., 2022; Dunder et al., 2022; Canova et al., 2020; Liu et al., 2020a; Dong et al., 2019; Lin et al., 2019), with statistical significance in four (Cakmak et al., 2022; Dunder et al., 2022; Canova et al., 2020; Lin et al., 2019). In the four studies that additionally examined exposure modeled as quartiles, three reported a monotonic exposure-response gradient (Canova et al., 2020; Liu et al., 2020a; Fisher et al., 2013), while one reported the strongest association in the third quartile (Lin et al., 2019). While the direction of association was mostly consistent across studies, in the NHANES data reported in Dong et al. (2019), the direction of association was not consistent across NHANES study cycles. The association was inverse (not statistically significant) in 2003–2004 and 2005–2006, but positive (not statistically significant) in 2007–2008, 2011–2012, and 2013–2014, despite similar exposure levels across cycles. Further, in the two studies with prospective exposure measurement, only one found a positive association (Dunder et al., 2022), while the other found an association in cross-sectional but not prospective analyses (Lin et al., 2019). This raises the possibility that the observed associations across mostly cross-sectional studies could be due to reverse causality.

Two *low* confidence studies (Li et al., 2020b; Chen et al., 2019a) in general population adults also observed positive associations with total cholesterol, with the latter being statistically significant, while a third *low* confidence study (Lin et al., 2020c) found no association in older residents (55–75 years). The populations in both Lin et al. (2020c) and Li et al. (2020b) were living in high contamination areas (in Taiwan and Sweden, respectively). In addition, two studies examined occupational populations with PFAS exposure. These studies were *low* confidence due to concerns for potential selection bias and residual confounding. Batzella et al. (2022), examining PFAS production workers in Italy, and Khalil et al. (2020) examining firefighters in the U.S., both reported positive, but not statistically significant associations between PFHxS and total cholesterol.

In pregnant women, two studies (Yang et al., 2020b; Starling et al., 2014b) out of five (see Table 3-28) reported higher total cholesterol with higher PFHxS exposure, with statistical significance in Yang et al. (2020b) and an exposure-response gradient across quartiles in Starling et al. (2014b). In a *low* confidence study of high cholesterol (Christensen et al., 2016), no association was observed (OR 1.01, 95% CI: 0.91, 1.13), but the study is expected to be biased toward the null due to nondifferential outcome misclassification.

Three of the *medium* confidence studies additionally reported analyses of dichotomous hypercholesterolemia (Canova et al., 2020; Lin et al., 2019; Fisher et al., 2013). Cutoffs for high cholesterol differed across studies: in Fisher et al. (2013) the cutoff for total cholesterol was 5.2

mmol/L; in Canova et al. (2020), the cutoff was 190 mg/mL, and in Lin et al. (2019), the outcome was initiation of cholesterol lowering medication, or total cholesterol of 240 mg/mL/LDL cutoff of 160 ng/mL). Significantly higher odds of high cholesterol (OR of 1.4–1.6 in the highest quartiles) were reported in both Fisher et al. (2013) and Canova et al. (2020), with a monotonic exposure-response gradient across quartiles. In Lin et al. (2019), higher odds (not statistically significant) were observed in an analysis of high cholesterol at baseline, but not when risk of high cholesterol was analyzed prospectively.

Results for LDL cholesterol and triglycerides in adults were less consistent than total cholesterol in the *medium* confidence studies, with most studies showing similar results across the different outcome markers, but a few reporting inverse associations for LDL and/or triglycerides (Cakmak et al., 2022; Dalla Zuanna et al., 2021; Matilla-Santander et al., 2017).

In adolescents and children, there was very limited evidence of an association, with 4 of 12 *medium* confidence studies reporting higher total cholesterol with higher PFHxS exposure (Canova et al., 2021; Kang et al., 2018; Mora et al., 2018; Zeng et al., 2015), and only one reporting statistically significance, but without an exposure-response gradient across quartiles (Canova et al., 2021). The other *medium* confidence studies reported no association (Averina et al., 2021; Blomberg et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020a; Jain and Ducatman, 2018; Manzano-Salgado et al., 2017b). For triglycerides, 4 of 12 studies reported positive associations (Blomberg et al., 2021; Spratlen et al., 2020b; Manzano-Salgado et al., 2017b; Zeng et al., 2015). Of note, both Spratlen et al. (2020b) and (Blomberg et al., 2021) reported statistically significant positive associations in neonates, though the third study in neonates found no association (Tian et al., 2020). Looking at the two studies of *low* confidence in adolescents (Koshy et al., 2017) and children (Khalil et al., 2018), both reported higher total cholesterol with higher exposure, with the difference being statistically significant in Koshy et al. (2017), but both had serious limitations.

Overall, there is some evidence that higher PFHxS exposure is associated with higher total cholesterol levels in adults, with less consistent evidence for parallel changes in triglycerides. The majority of studies in adults, including pregnant women, support this association, though there are remaining uncertainties, including less consistent evidence for LDL cholesterol and triglycerides. Additionally, a possible explanation for the observed associations is the presence of residual confounding. It is plausible that an association between PFAS exposure and consumption of high cholesterol foods, as suggested in some studies (Susmann et al., 2019; Schaider et al., 2017), could induce a positive association with serum lipids; however, the currently available evidence does not allow for evaluation of this possibility as most studies that adjusted for dietary habits were in children, where the evidence was less consistent. In addition, there is potential for confounding across the PFAS. In the studies with stronger association, there were similar associations with other PFAS, including PFOS, PFOA, and PFNA, and PFHxS is moderately positively correlated with them. With the available evidence, it is not possible to rule this out, but the association with cholesterol was still present in a study with weak correlations (~0.3) between PFHxS and PFOS and PFOA

(Cakmak et al., 2022). Given the overall consistency across studies and the observation of exposure-response gradients across quartiles in multiple studies, there is reasonable support for a positive association with this outcome.

Table 3-28. Associations between PFHxS exposure and blood lipids in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	Total cholesterol[a]	LDL[a]	Triglycerides[a]
General population, adults						
Dong et al. (2019)	NHANES cross-sectional (2003–2014 pooled), U.S.; 8,950 adults	1.6	β (95% CI) for 1 unit increase	0.98 (−0.89, 2.85)	0.72 (−1.63, 3.06)	NR
Fisher et al. (2013)	Canadian Health Measures Survey (2007–2009) cross-sectional, Canada; 2,345 adults	2.2 (1.2–3.6)	β (95% CI) for 1 log-unit increase	0.03 (0.01,0.05)*	0.06 (0.01,0.11)*	0.02 (−0.02,0.06)
Cakmak et al. (2022)			OR (95% CI) for high cholesterol vs. Q1	Q2: 1.05 (0.69,1.61) Q3: 1.43 (0.85,1.4) Q4: 1.57 (0.93, 2.64) *p*-trend: 0.001*	NR	NR
	(2007–2017); 6,045 participants	1.5 (GM)	% change for increase equivalent to GM	2.8 (1.1, 4.5)*	-3.8 (-9, 1.7)	-1.4 (-5.0, 2.3)
Lin et al. (2019)	Participants from randomized trial of diabetes prevention, U.S.; 888 overweight and pre-diabetic adults	2.3 (1.4–3.8)	Mean diff (95% CI) for twofold increase	2.24 (0.15, 4.33)*	1.32 (−0.59, 3.22)	3.91 (−1.77, 9.59)
			quartiles vs. Q1	Q2: 3.87 (−2.89, 10.63) Q3: 9.28 (2.38, 16.19)* Q4: 7.43 (0.53, 14.33)*	Q2: 1.22 (−4.94, 7.38) Q3: 6.22 (−0.06, 12.52) Q4: 3.88 (−2.39, 10.17)	Q2: 9.64 (−8.75, 28.03) Q3: 16.43 (−2.34, 35.22) Q4: 11.23 (−7.52, 29.99)
			Cross-sectional OR (95% CI) for high lipids	1.08 (0.94, 1.25)	NR	1.03 (0.90, 1.18)
			Prospective HR (95%) for high lipids	Total: 1.00 0.92 (1.09) Placebo: 1.02 (0.89, 1.17) Lifestyle: 1.02 (0.90, 1.15)	NR	Total: 1.14 (1.00, 1.28)* Placebo: 1.23 (1.03, 1.47)* Lifestyle: 1.19 (0.98, 1.44)

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	Total cholesterol[a]	LDL[a]	Triglycerides[a]
Liu et al. (2020a)	Cross-sectional analysis from randomized clinical trial of weight loss; 326 overweight adults	2.4 (1.6–3.6)	Means ± SE for tertiles	T1: 181.6 ± 7.8 T2: 189.3 ± 7.6 T3: 192.5 ± 7.8 *p*-trend = 0.15	NR	T1: 119.4 ± 11.2 T2: 133.6 ± 11.0 T3: 130.8 ± 11.2 *p*-trend = 0.37
Dunder et al. (2022)	Cohort study (2001–2004), Sweden; 864 older adults (70 yrs at baseline)	3.1 (2.0–5.8)	β (95% CI) for ln-unit increase (for lipids over 10 years)	0.08 (0.01, 0.15)*	0.04 (-0.01, 0.10)	0.04 (0.01, 0.07)*
Canova et al. (2020)	Cross-sectional study in highly contaminated area (2017–2019), Italy; 15,720 young adults (20-39 yrs)	3.6 (1.6–7.8)	β (95% CI) for ln-unit increase	2.02 (1.45, 2.58)* (exposure-response gradient across quartiles)	1.31 (0.81, 1.8)*	0.02 (0.01, 0.02)*[b]
			OR (95% CI) vs Q1 for abnormal lipids	Q2: 1.18 (1.06, 1.30)* Q3: 1.19 (1.07, 1.32)* Q4: 1.41 (1.25, 1.58)*	Q2: 1.21 (1.08, 1.35)* Q3: 1.15 (1.02, 1.29)* Q4: 1.37 (1.20, 1.55)*	Q2: 1.11 (0.93, 1.32) Q3: 1.17 (0.98, 1.40) Q4: 1.22 (1.02, 1.46)*[b]
Pregnant women						
Yang et al. (2020b)	Pregnancy cohort (2013–2014), China, 436 women	0.3 (0.2–0.5)	β (95% CI) for ln-unit increase	0.18 (0.05, 0.32)*	0.09 (0.001, 0.19)*	0.07 (-0.1, 0.24)[b]
Gardener et al. (2021)	Pregnancy cohort (2009), U.S., 433 women	0.5 (0.3-0.9)	Means ± CI for quartiles	No clear association (reported only on figure)	NR	No clear association (reported only on figure)
Starling et al. (2014b)	Norwegian Mother and Child cross-sectional analysis (2003–2004), Norway; 891 women	0.6 (0.4–0.9)	β (95% CI) for ln-unit increase	3.00 (−1.75,7.76)	1.92 (−2.50, 6.33)[b]	−0.01 (−0.05, 0.03)[b]
			quartiles vs. Q1	Q2: 0.65 (−6.87,8.17) Q3: 1.62 (−6.08,9.32) Q4: 4.25 (−3.88,12.39)	Q2: 0.44 (−6.19, 7.08) Q3: 0.50 (−6.15, 7.16) Q4: 1.48 (−5.89, 8.85)[b]	Q2: −0.04 (−0.11, 0.02) Q3: −0.02 (−0.10, 0.05) Q4: −0.02 (−0.09, 0.05)[b]
Matilla-Santander et al. (2017)	INMA cross-sectional analysis (2003–2008), Spain; 1,240 women	0.6 (0.4–0.8)	% change (95% CI) for log-unit increase	−0.09 (−8.25, 1.45)	NR	−4.90 (−9.16, −0.72)*[b]
			quartiles vs. Q1	Q2: 1.21 (−1.05, 3.45) Q3: 0.60 (−1.69, 2.94) Q4: 0.70 (−1.86, 3.38)	NR	Q2: −7.69 (−14.3, −1.00)

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	Total cholesterol[a]	LDL[a]	Triglycerides[a]
						Q3: −3.92 (−10.9, 3.05) Q4: −7.69 (−13.9, 1.40)[b]
Dalla Zuanna et al. (2021)	Cross-sectional study in highly contaminated area (2017–2020), Italy; 319 women	2.1 (1.1–4.1)	β (95% CI) for ln-unit increase	-4.91 (-10.06, 0.24)	-8.17 (-12.54, -3.81)*	NR
Adolescents and children						
Blomberg et al. (2021) **(additional results with different timing of exposure and outcome measurement are available in the publication)**	Birth cohort (2007–2009), Faroe Islands, 459 children (followed to 9 yrs)	0.2 (0.1-0.2)	β (95% CI) for doubling PFAS and lipids at birth	Overall 0.03 (-0.04, 0.09) Girls 0.05 (-0.03, 0.14) Boys -.003 (-0.1, 0.09)	Overall 0.01 (-0.03, 0.05) Girls 0.019 (-0.03, 0.07) Boys -0.01 (-0.06, 0.05)	Overall 11 (5.9, 17)* [b] Girls 13 (5.5, 21)* Boys 9.7 (1.9, 18)*
			PFAS at birth and lipids at 18 mo	Overall -0.04 (-0.18, 0.1) Girls -0.03 (-0.22, 0.17) Boys -0.05 (-0.24, 0.15)	Overall -0.05 (-0.15, 0.06) Girls -0.05 (-0.2, 0.1) Boys -0.04 (-0.19, 0.12)	Overall 3.5 (-3.9, 11) Girls 7.9 (-2.5, 19) Boys -0.87 (-11, 9.9)
			PFAS and lipids at 9 yrs	Overall -0.02 (-0.14, 0.1) Girls -0.05 (-0.21, 0.1) Boys 0.02 (-0.15, 0.19)	Overall -0.06 (-0.14, 0.03) Girls -0.06 (-0.18, 0.06) Boys -0.05 (-0.18, 0.08)	Overall -1.8 (-8.3, 5.2) Girls 2.6 (-6.3, 12) Boys -6.8 (-16, 3)
Jensen et al. (2020a)	Birth cohort (2010–2012), Denmark; 612 children (followed to 18 mo)	0.3 (5^{th}-95^{th}: 0.1–0.7)	β (95% CI) for 1 unit increase	3 mo -0.08 (-0.33, 0.17) Girls -0.11 (-0.37, 0.16) Boys 0.13 (-0.58, 0.85) 18 mo -0.06 (-0.32, 0.21) Girls -0.05 (-0.32, 0.21) Boys -0.10 (-1.41, 1.21)	3 mo 0.01 (-0.24, 0.26) Girls 0.05 (-0.22, 0.32) Boys -0.28 (-1.01, 0.44) 18 mo -0.06 (-0.35, 0.22) Girls -0.08 (-0.37, 0.21) Boys 0.37 (-1.02, 1.76)[b]	3 mo 0.18 (-0.07, 0.44) Girls 0.21 (-0.06, 0.48) Boys -0.02 (-0.75, 0.71) 18 mo -0.24 (-0.51, 0.04) Girls -0.22 (-0.50, 0.06) Boys -0.62 (-1.95, 0.70)[b]
Papadopoulou et al. (2021)	Six birth cohorts, Europe, 1,301 children (6–11 yrs)	prenatal 0.5 (0.3–0.9)	β (95% CI) for doubling exposure	NR	0.03 (-0.03, 0.09)[b]	0.02 (-0.05, 0.08)[b]
		Children 0.3 (0.2-0.6)		NR	0.02 (-0.06, 0.10)[b]	0.00 (-0.08, 0.08)[b]

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	Total cholesterol[a]	LDL[a]	Triglycerides[a]
Manzano-Salgado et al. (2017b)-	INMA cohort (2003–2008), Spain; 627 children (4 yrs)	prenatal 0.6 (0.4–0.8) (GM (IQR))	β (95% CI) for doubling exposure and cholesterol z-score	0.02 (−0.09,0.12) Boys: −0.02 (−0.17,0.13) Girls: 0.04 (−0.12,0.20)	-0.01 (−0.12, 0.09)[b] Boys: −0.04 (−0.18, 0.10) Girls: 0.00 (−0.15, 0.15)	0.11 (−0.01, 0.21)[b] Boys: 0.16 (0.03, 0.30)* Girls: 0.07 (−0.08, 0.22)
Spratlen et al. (2020b)	WTC cohort (2001–2002), U.S.; 222 newborns	cord blood 0.7 (0.5–1.0)	% difference for 1% increase	0.03 (−0.02, 0.08)	NR	0.13 (−0.04, 0.23)
			Mean ratio vs. Q1	Q2: 1.03 (0.94, 1.12) Q3: 1.06 (0.98, 1.16) Q4: 1.07 (0.98, 1.16) *p*-trend 0.5	NR	Q2: 1.08 (−.92, 1.28) Q3: 1.22 (1.04, 1.45) Q4: 1.26 (1.07, 1.49) *p*-trend 0.002
Kang et al. (2018)	Korea Environmental Health Survey in Children and Adolescents cross-sectional analysis (2012–2014), Korea, 150 children (3–18 yrs)	0.8 (0.6–1.0)	β (95% CI) for ln-unit increase	0.99 (−9.53, 11.50)	-4.22 (−13.98, 5.53)	0.08 (−0.09, 0.25)
Averina et al. (2021)	Cross-sectional study (2010–2011), Norway, 940 adolescents (~16 yrs)	Girls 0.8, Boys 1.0 (GMs)	β (95% CI) for log-unit increase	"No association" (data not shown)	"No association" (data not shown)	"No association" (data not shown)
Jain and Ducatman (2018)	NHANES cross-sectional (2013–2014), U.S.; 458 children (6–11 yrs)	0.9	Means (95% CI)	Q1: 154 (149–159) Q2: 159 (155–163) Q3: 153 (145–161) Q4: 158 (153–164) *p* = 0.4	NR	NR
Zeng et al. (2015)	Genetic and Biomarkers study for Childhood Asthma cross-sectional analysis (2009–2010), Taiwan; 225 adolescents (12–15 yrs)	1.2 (range 0.2–10.3) (boys)	β (95% CI) for 1 unit increase	1.10 (−0.71,2.92)	0.99 (−0.41, 2.39)[b]	1.80 (−0.67, 4.27)[b]
Li et al. (2021a)	HOME cohort (2003–2006);	prenatal 1.3 (0.8–2.3)	Difference for IQR increase	NR	NR	0.1 (0.0, 0.2)

Reference	Population	Median exposure (IQR) or as specified (ng/mL)	Effect estimate	Total cholesterol[a]	LDL[a]	Triglycerides[a]
	U.S.; 186 adolescents (12 yrs)	birth 0.6 (0.4–1.0)		NR	NR	0.1 (-0.1, 0.3)
Mora et al. (2018)	Project Viva cohort (1999–2002), U.S.; 682 children (7–8 yrs)	prenatal 2.4 (1.6–3.8)	β (95% CI) for IQR increase	0.5 (−1.1,2.2) similar for boys and girls	0.5 (−0.9,1.9) similar for boys and girls	-0.6 (−2.0,0.8) Boys: 0.6 (−1.9,3.1) Girls: −1.1 (−3.1,0.1)
		child 1.9 (1.2–3.4)		-0.3 (−1.0,0.5) Boys: −0.5 (−1.5,0.4) Girls: 0.2 (−1.0,1.3)	-0.2 (−0.9,0.4) Boys: −0.5 (−1.4,0.3) Girls: 0.3 (−0.6,1.3)	-0.4 (−1.0,0.3) similar for boys and girls
Tian et al. (2021)	Birth cohort (2012), China; 306 newborns	prenatal 2.7 (2.0–3.5)	β (95% CI) for ln-unit increase	0.05 (-0.07, 0.16)	0.03 (-0.11, 0.18)	0.02 (-0.11, 0.15)
Canova et al. (2021)	Cross-sectional study in highly contaminated area (2017–2019), Italy; 6,669 adolescents (14–19 yrs) and 2,693 children (8–11 yrs)	adolescents 2.8 (1.6–4.8)	β (95% CI) for ln-unit increase	1.49 (0.60, 2.37)	1.44 (0.68, 2.19)	0.01 (-0.01, 0.02)[b]
			β (95% CI) vs Q1	Q2: 1.96 (0.20, 3.73)* Q3: 1.72 (-0.10, 3.54) Q4: 3.80 (1.83, 5.77)*	Q2: 2.03 (0.52, 3.55)* Q3: 1.60 (0.05, 3.16)* Q4: 3.65 (1.97, 5.33)	Q2: 0.01 (-0.02, 0.04) Q3: 0.00 (-0.03, 0.03) Q4: 0.02 (-0.02, 0.05)
		children 1.9 (1.2–2.8)	β (95% CI) for ln-unit increase	1.30 (-0.28, 2.88)	0.54 (-0.87, 1.96)	-0.01 (-0.03, 0.01)
			β (95% CI) vs Q1	Q2: 0.46 (-0.73, 1.65) Q3: 1.68 (0.44, 2.91)* Q4: 1.32 (0.07, 2.56)*	Q2: -1.70 (-4.19, 0.8) Q3: -1.22 (-3.81, 1.38) Q4: 0.76 (-1.86, 3.39)	Q2: 0 (-0.04, 0.04) Q3: 0 (-0.04, 0.04) Q4: -0.02 (-0.07, 0.02)

*$p < 0.05$.
NR: not reported.
[a]Units and transformations of outcome variables varied across studies.
[b]*Low* confidence endpoint within *medium* confidence study.

Other risk factors for cardiovascular disease

Twenty-seven studies report on the association between PFHxS exposure and other risk factors for cardiovascular disease, including blood pressure in the general population (18 studies), blood pressure and hypertensive disorders during pregnancy (6 studies), atherosclerosis (2 studies), abdominal aortic calcification (1 study), and ventricular geometry (1 study). The study evaluations for these outcomes are summarized in Figure 3-66. One study was considered high confidence, 18 were medium confidence, and 7 were low confidence. One study (Yang et al., 2018) evaluating blood pressure was excluded from further analysis (uninformative) due to critical deficiencies in participant selection and confounding.

Considering blood pressure in the general population, the majority of studies reported no association between PFHxS exposure and higher blood pressure. A few positive associations with hypertension or higher blood pressure were observed in studies of adolescents and young adults (see Table 3-29). Statistically significant associations were reported in a cross-sectional study of 16-year-olds in Norway (Averina et al., 2021) and a cohort with follow-up to 12 years of age in the U.S. (Li et al., 2021a), though the association was not monotonic across quartiles in Averina et al. (2021). In a region of Italy with high PFAS contamination, a positive association was observed in young adults aged 20–39 years (Pitter et al., 2020) but not adolescents aged 14–19 years (Canova et al., 2021). Studies in non-age restricted adults (Lin et al., 2020b; Chen et al., 2019a; Christensen et al., 2019; Liu et al., 2018; Bao et al., 2017; Christensen et al., 2016) and children (Papadopoulou et al., 2021; Khalil et al., 2018; Manzano-Salgado et al., 2017b) reported null findings with blood pressure and/or odds of hypertension, and there is not a clear biological explanation for this pattern of results by age.

Results for hypertensive disorders of pregnancy are summarized in Table 3-30. One of four studies of gestational hypertension Borghese et al. (2020) and two of four studies of preeclampsia (Birukov et al., 2021; Borghese et al., 2020) reported positive associations, with statistical significance in one. Conversely, two studies reported inverse associations (statistically significant in one) with gestational hypertension (Liu et al., 2021a; Huang et al., 2019c). The other one study of gestational hypertension (Birukov et al., 2021) and two studies of preeclampsia (Huang et al., 2019c; Starling et al., 2014a) reported no association. One *low* confidence study reported no association between PFHxS and continuous blood pressure during pregnancy (Varshavsky et al., 2021).

No association with PFHxS exposure was observed in studies of atherosclerosis in adults (Lind et al. (2017), medium confidence) and markers of atherosclerosis/arterial wall stiffness in adolescents (Koshy et al. (2017), low confidence). One study examining abdominal aortic calcification, a marker of subclinical atherosclerotic disease, reported a positive, though not statistically significant, association in men but not women (Koskela et al., 2022). Lastly, no association was observed in a single medium confidence study of ventricular geometry (Mobacke et al., 2018).

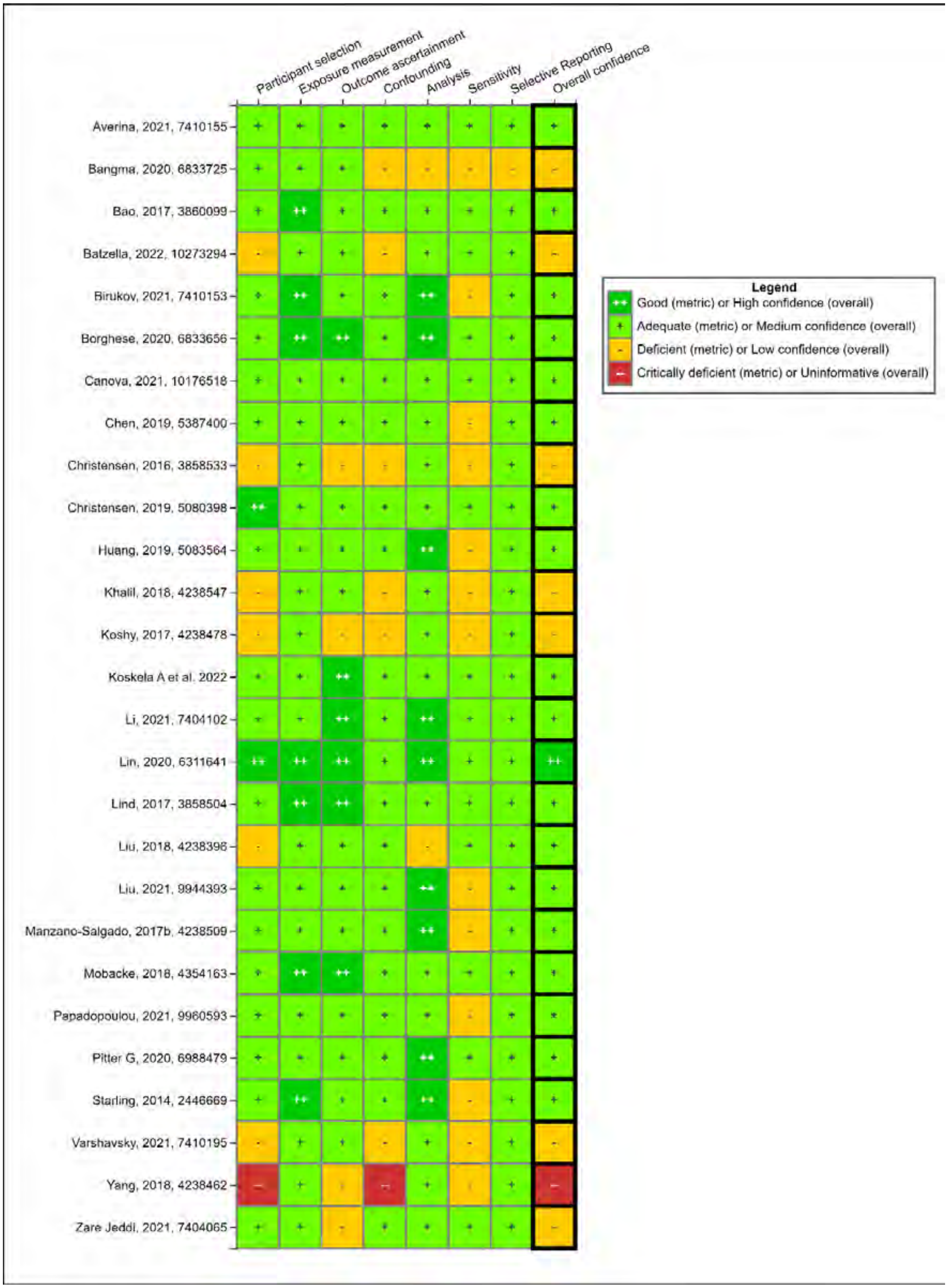

Figure 3-66. Study evaluation results for epidemiology studies of PFHxS and cardiovascular disease risk factors. For additional details see HAWC link. Multiple publications of the same study: **Christensen et al. (2019)** also includes **Liao et al. (2020)**.

Table 3-29. Associations between PFHxS exposure and hypertension in *medium* confidence epidemiology studies in adolescents and young adults

Reference confidence	Population	Median exposure (IQR) or as specified (ug/mL)	Effect estimate	Hypertension
Averina et al. (2021)	Cross-sectional study in Norway; 940 adolescents (~16 yrs)	0.8 (GM in girls)	OR (95% CI) for quartiles vs Q1	Q2: 1.63 (0.90, 2.94) Q3: 1.25 (0.69, 2.28) Q4: 2.06 (1.16, 3.65)*
Li et al. (2021a)	Cohort in U.S.; 221 adolescents (follow-up through 12 yrs)	1.2 (0.9, 1.8) at 8 yrs	Difference for IQR increase (outcome continuous blood pressure z-score)	Systolic BP 0.2 (0.0, 0.4)*
Canova et al. (2021)	Cross-sectional study in highly PFAS exposed region, Italy; 6,669 adolescents (14–19 yrs)	2.8 (1.6-4.8)	β (95% CI) for ln-unit increase (outcome continuous blood pressure)	Systolic BP -0.22 (-0.65, 0.21) Diastolic BP -0.15 (-0.45, 0.16)
Pitter et al. (2020)	Cross-sectional study in highly PFAS exposed region, Italy; 15,786 adults (20–39 yrs)	6.0 (mean)	OR (95% CI) for quartiles vs Q1	Q2: 1.01 (0.86, 1.19) Q3: 1.08 (0.92, 1.27) Q4: 1.19 (1.00, 1.41)

* p <0.05.

Table 3-30. Associations between PFHxS exposure and gestational hypertension and preeclampsia in *medium* confidence epidemiology studies

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Gestational hypertension	Preeclampsia
Liu et al. (2021a)	Nested case-control study within cohort in China; 544 women	0.1 (0.03, 0.1)	OR (95% CI) for tertiles vs T1	T2: 0.41 (0.25, 0.67)* T3: 0.29 (0.17, 0.50)*	NR
Huang et al. (2019c)	Cross-sectional study in China; 674 women at delivery	0.2 (0.1–0.2)	OR (95% CI) for tertiles vs T1	T2: 0.83 (0.31, 2.22) T3: 0.48 (0.16, 1.43)	T2: 1.10 (0.36, 3.38) T3: 0.80 (0.25, 2.60)
Birukov et al. (2021)	Cohort in Denmark; 1,436 women	0.4 (0.3–0.5)	HR (95% CI) for doubling of exposure	0.97 (0.66, 1.43)	1.14 (0.91, 1.42)
Starling et al. (2014a)	Nested case-control study within cohort in Norway; 1,046 women	0.7 (0.5–1.0)	HR (95 CI) for quartiles vs Q1	NR	Q2: 0.86 (0.59, 1.26) Q3: 1.01 (0.69, 1.49) Q4: 0.93 (0.64, 1.36)
Borghese et al. (2020)	Cohort in Canada; 1,739 women	1.0 (0.7–1.6)	OR (95% CI) for tertiles vs T1	T2: 1.03 (0.64, 1.67) T3: 1.39 (0.87, 2.20)	T2: 1.40 (0.54, 3.63) T3: 3.06 (1.27, 7.39)*

*p <0.05.

Cardiovascular disease

Five studies report on the association between PFHxS and cardiovascular disease, including coronary heart disease, myocardial infarction (heart attack), and congestive heart failure. The study evaluations are summarized in Figure 3-67. Two studies, an analysis of NHANES data for 1999–2014 and a prospective cohort of farmers and other rural residents, were *medium* confidence (Huang et al., 2018; Mattsson et al., 2015). The other three were *low* confidence (Graber et al., 2019; Honda-Kohmo et al., 2019; Christensen et al., 2016). These cross-sectional studies were focused on very specific populations—participants in litigation over PFAS exposure (Graber et al., 2019; Honda-Kohmo et al., 2019) or anglers (Christensen et al., 2016). There were concerns about confounding in all of these studies, and for sensitivity in Graber et al. (2019) and Christensen et al. (2016) due to small sample size. Additionally, all the studies except Mattsson et al. (2015)—which used a national register of disease—classified cardiovascular disease based on self-report on questionnaires, which is likely to suffer from misclassification and which could be differential in studies wherein exposure was known due to litigation (Graber et al., 2019; Honda-Kohmo et al., 2019) but is likely nondifferential and thus toward the null in the other studies (Huang et al., 2018; Christensen et al., 2016).

In the two *medium* confidence studies, no association between PFHxS exposure and coronary heart disease (Huang et al., 2018; Mattsson et al., 2015) or total cardiovascular disease, congestive heart failure, coronary heart disease, angina pectoris, myocardial infarction, or stroke (Huang et al., 2018) was observed. In the *low* confidence studies, one reported higher odds of cardiovascular conditions with higher exposure (Graber et al., 2019) and two reported lower odds of coronary heart disease (Honda-Kohmo et al., 2019; Christensen et al., 2016), although only results in Honda-Kohmo et al. (2019) were statistically significant. An exposure-response gradient was observed in Honda-Kohmo et al. (2019) across quantiles.

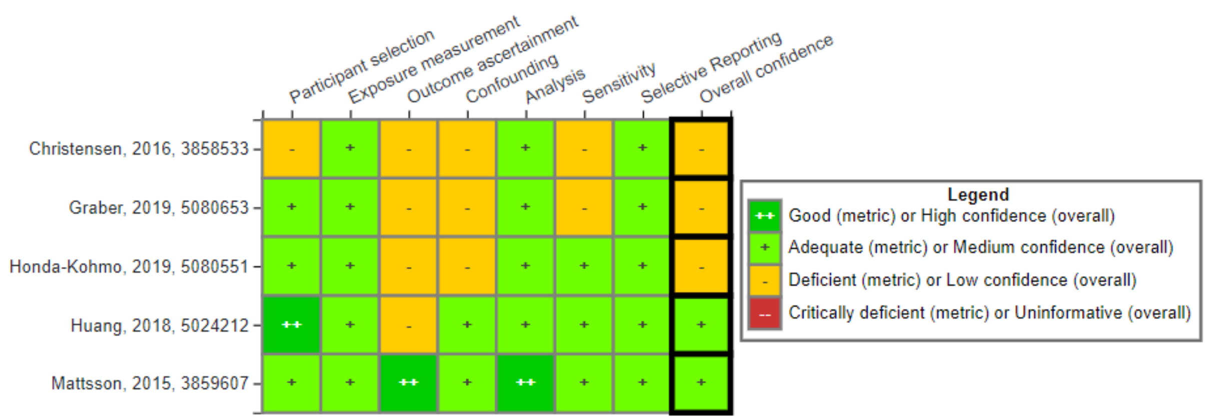

Figure 3-67. Study evaluation results for epidemiology studies of PFHxS and cardiovascular disease. For additional details see HAWC link.

Summary of cardiovascular effects

Overall, there is some evidence of an association between PFHxS exposure and serum lipids. However, the evidence for other cardiovascular-related effects is mostly null, which raises questions about the adversity of the observed lipids changes. It is possible that cholesterol is a more sensitive measure and that the exposure contrasts in the available studies of disease risk were inadequate to detect differences.

Metabolic effects

Diabetes

Seven studies (reported in seven publications) report on the relationship between PFHxS exposure and diabetes (i.e., type 2 diabetes). In cross-sectional studies of PFHxS and diabetes outcomes, there is some concern for reverse causality. Metabolic changes related to diabetes (e.g., impairments of renal function) may affect the amount of PFHxS measured in blood. Four out of the seven available studies were cross-sectional and were considered *low* confidence studies due to temporality and other deficiencies as noted in HAWC. Three studies (Charles et al., 2020; Sun et al., 2018; Cardenas et al., 2017) had prospective exposure measurement prior to development of diabetes. Sun et al. (2018) and Charles et al. (2020) used nested case-control study designs and Cardenas et al. (2017) used a multicenter randomized clinical trial of a diabetes prevention lifestyle intervention. Thus, these three studies were evaluated as *medium* confidence. A summary of the study evaluations for PFHxS and diabetes is presented in Figure 3-68, and additional details of the studies can be obtained from HAWC.

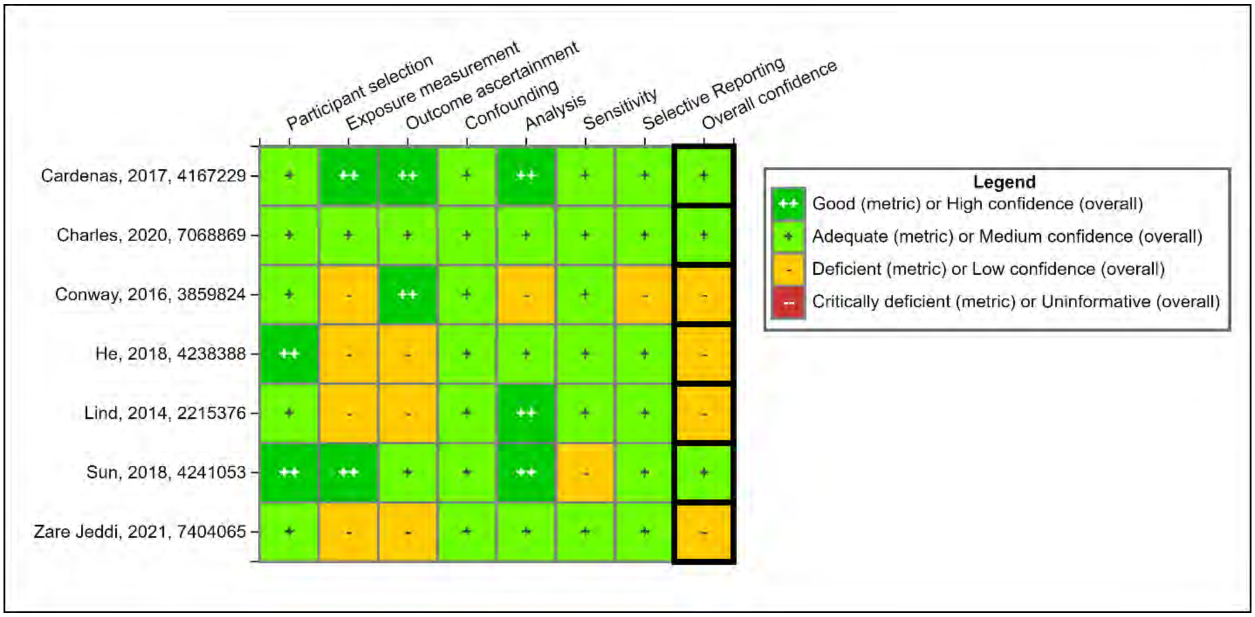

Figure 3-68. Summary of study evaluation for PFHxS and type 2 diabetes in epidemiology studies. For additional details see HAWC link. Multiple publications of the same study: **He et al. (2018)** also includes **Jain (2020)** and **Jain (2021b)**.

The results for the association between PFHxS exposure and diabetes are presented in Table 3-31. All the studies evaluated exposure and outcome associations in adults; in Conway et al. (2016), both adults and children were included in study population. In the three studies of *medium* confidence, one reported higher odds of incident diabetes with higher PFHxS exposure (Sun et al., 2018), although not statistically significant, while one reported an inverse association (also not statistically significant) (Charles et al., 2020) and the other reported no association (Cardenas et al., 2017). In the *low* confidence studies, one study reported higher odds of diabetes with higher exposure in men (He et al., 2018) and one in women (Zare Jeddi et al., 2021). On the other hand, there was an inverse association with PFHxS exposure in Conway et al. (2016) with higher exposure associated with lower odds of diabetes. The third *low* confidence study (Lind et al., 2014) reported no association.

Overall, the evidence for the association between PFHxS exposure and diabetes is mixed. There is some indication of higher odds of diabetes in three studies, one *medium* and two *low confidences*, but other studies of similar confidence and design reported null or inverse findings, and there was inconsistency in sex differences across the two *low* confidence studies reporting an effect.

Table 3-31. Associations between PFHxS exposure and type 2 diabetes in epidemiology studies

Reference, study confidence	Population	Median exposure (IQR) or as specified	Effect estimate exposure change	Diabetes OR (95% CI)
Charles et al. (2020), medium	Prospective nested case-control study of Norwegian Women and Cancer Study (2001–2006), Norway; 88 women (30–70 yrs)	0.9 (5^{th}-95^{th}: 0.4–4.3) Controls	IQR change	0.80 (0.54, 1.20)
Sun et al. (2018), medium	Prospective nested case-control study of Nurses Health Study II (1995–2000), U.S.; 793 adults (32–52 yrs)	2.0 (1.3–3.5) controls	tertiles vs. T1	Incident type 2 T2: 1.15 (0.79, 1.67) T3: 1.26 (0.86, 1.86)
Lind et al. (2014), low	PIVUS study cross-sectional (2001–2004), Sweden; 1,016 adults (70 yrs)	2.1 (1.6–3.4)	ln-unit change	1.00 (0.74, 1.35)
Cardenas et al. (2017), medium	Diabetes Prevention Program (1996–1999), U.S.; 957 adults (25+ yrs)	Geometric mean (IQR) 2.4 (2.4)	log2-unit change	Incident type 2 0.98 (0.86, 1.12)[b]
He et al. (2018), low	NHANES cross-sectional (2003, 2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012), U.S.; 7,904 adults (20+ yrs)	Mean ± SE Male 2.9 ± 0.1 Female 1.9 ± 0.04	quartiles vs. Q1	Men Q2: 1.99 (1.19, 3.33)* Q3: 1.87 (1.15, 3.05)* Q4: 2.31 (1.37, 3.91)* Women Q2: 0.65 (0.41, 1.03) Q3: 0.87 (0.52, 1.43) Q4: 1.22 (0.71, 2.11)
Zare Jeddi et al. (2021), low	Cross-sectional study in region with high PFAS contamination (2017–2019), Italy; 15,876 young adults (20–39 yrs)	3.5 (1.7–7.8)	quartiles vs. Q1	Q2: 0.97 (0.76, 1.24) Q3: 1.23 (0.97, 1.57) Q4: 1.06 (0.82, 1.37) Men Q2: 1 (0.69, 1.46) Q3: 1.22 (0.86, 1.72) Q4: 0.99 (0.7, 1.4) Women Q2: 1 (0.72, 1.39) Q3: 1.39 (1.01, 1.91)* Q4: 1.12 (0.8, 1.58)
Conway et al. (2016), low	C8 Health Project cross-sectional (2005–2006), U.S.; 66,889 children and adults	Mean ± SD 5.2 ± 10.4 no diabetes	Unit change (No transformation)	0.74 (0.71, 0.77)

Gestational diabetes

Six studies report on the relationship between PFHxS exposure and gestational diabetes. The quality of gestational diabetes ascertainment was based on how screening of gestational diabetes mellitus (GDM) was performed (e.g., defined by a study protocol versus doctor's diagnosis at individual clinics). Another important consideration is that GDM associations with exposure are not interpretable in the presence of diabetes. Thus, for participant selection, it was important for studies to account for the diabetic status and/or the use of diabetic medications. Studies that did not consider these factors by exclusion or stratification were considered deficient for the participant selection domain. Overall, there were five studies that examined the association between PFHxS exposure and gestational diabetes that were of *medium* confidence (Yu et al., 2021; Rahman et al., 2019; Wang et al., 2018; Valvi et al., 2017; Shapiro et al., 2016) and one study of *low* confidence (Matilla-Santander et al., 2017). A summary of the study evaluations for PFHxS and gestational diabetes is presented in Figure 3-69, and additional details of the studies can be obtained from HAWC.

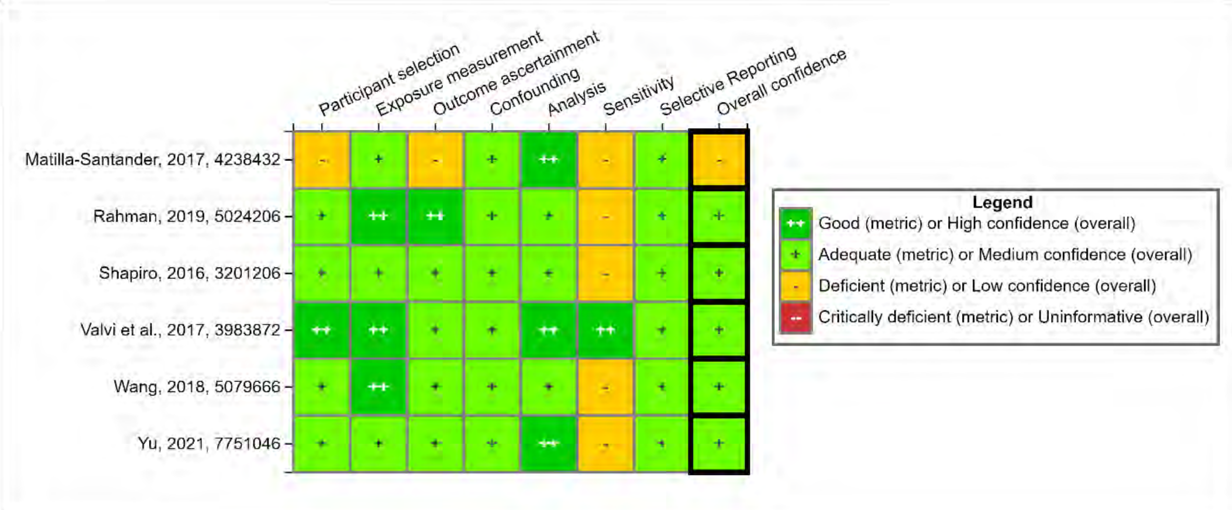

Figure 3-69. Heatmap of study evaluations for PFHxS and gestational diabetes.
For additional details see HAWC link.

The results for the association between PFHxS exposure and gestational diabetes for all studies are presented in Table 3-32. Two medium confidence studies (Yu et al., 2021; Shapiro et al., 2016) reported higher odds of GDM with PFHxS exposure, but neither was statistically significant, and in Shapiro et al. (2016), the exposure-response gradient was nonmonotonic, with the odds ratio highest in the second quartile. The results were generally null in the three other medium confidence studies (Rahman et al., 2019; Wang et al., 2018; Valvi et al., 2017). In the low confidence study (Matilla-Santander et al., 2017), there were higher odds of GDM with PFHxS exposure, although the exposure-response gradient was again nonmonotonic. Overall, there is no clear association between PFHxS exposure and GDM.

Table 3-32. Associations between PFHxS exposure and gestational diabetes in epidemiology studies

Reference, study confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate exposure change	Gestational diabetes mellitus (GDM) OR (95% CI)
Yu et al. (2021), medium	Population-based birth cohort study in Shanghai, China (2013–2016); 2,747 pregnant women	0.5 (0.3) in controls	Log-unit change	1.15 (0.86, 1.54)
Wang et al. (2018), medium	Haidian Maternal & Child Health Hospital in Beijing, China (2013); 84 pregnant women with GDM and 168 healthy pregnant women	0.5 (0.3–0.7) in controls	Unit change	1.07 (0.86, 1.35)
Matilla-Santander et al. (2017), low	Population-based birth cohort study INMA (2003–2008); Spanish regions of Valencia, Sabadell, and Gipuzoka; 2,150 pregnant women (recruited during first trimester of pregnancy)	Geometric mean (Geometric SD) 0.6 (2.0)	Quartiles	Q2: 1.25 (0.51, 3.03) Q3: 1.81 (0.76, 4.28) Q4: 1.15 (0.42, 3.12)
Rahman et al. (2019), medium	NICHD Fetal Growth Study, Singletons (2009–2013); 2,334 pregnant women (8–13 wks of gestation)	Geometric mean (95% CI) Overall cohort 0.8 (0.7–0.8) GDM 0.7 (0.6–0.9)	SD increment	Overall cohort[a] 0.95 (0.73, 1.23) With family history of type 2 diabetes[a] 1.03 (0.92, 1.16)
Shapiro et al. (2016), medium	Longitudinal birth cohort study MIREC (2008–2011); Canada; 1,274 pregnant women (recruited <14 wks of gestation)	Geometric mean (SD) GDM 1.1 (2.0) Non-GDM 1.0 (2.3)	Quartiles	Q2: 1.6 (0.7, 3.8) Q3: 1.4 (0.6, 3.5) Q4: 1.2 (0.4, 3.5)
Valvi et al. (2017), medium	National Hospital in Torshavn (1997 and 2000); Faroe Islands; 604 mother-child pairs (recruited at 34 wks of gestation)	Median (IQR) 4.5 (2.2, 8.5)	Doubling	1.03 (0.80, 1.33)

Blood glucose and insulin resistance

Homeostatic model assessment (HOMA) is a method for assessing insulin resistance and β-cell function from fasting glucose and insulin measured in the plasma (Matthews et al., 1985). The HOMA of insulin resistance (HOMA-IR) is often used in studies evaluating future risk of diabetes. It is important to consider that blood glucose and insulin levels and HOMA-IR are difficult to interpret in the presence of diabetes, especially if diabetes is treated with hypoglycemic medication since the treatment will affect insulin production and secretion. Thus, for participant selection, the studies should account for the diabetic status and/or the use of diabetic medications in participants. Studies that did not consider these factors by exclusion or stratification were considered deficient for the participant selection domain, and *low* confidence overall.

Twenty-eight studies (reported in 31 publications) report on the relationship between PFHxS exposure and blood glucose and/or insulin resistance. Of these, 15 were considered *medium* confidence (Cakmak et al., 2022; Gardener et al., 2021; Goodrich et al., 2021; Li et al., 2021a; Valvi et al., 2021; Yu et al., 2021; Duan et al., 2020; Ren et al., 2020; Alderete et al., 2019; Christensen et al., 2019; Jensen et al., 2018; Kang et al., 2018; Wang et al., 2018; Cardenas et al., 2017; Starling et al., 2017) and ten were *low* confidence. Many of these studies did not account for diabetic status of the participants and were thus deficient for participant selection. In addition, three studies were *uninformative* due to critical deficiencies in at least one domain and are not considered further (Zhang et al., 2019a; Yang et al., 2018; Jiang et al., 2014). Study evaluation results are summarized in Figure 3-49 and additional details are available in HAWC. Fifteen studies reported on general population adults and adolescents, one examined occupational exposure in firefighters, six studies reported on pregnant women, and five studies reported on children.

The results for the association between PFHxS exposure and these outcomes for all studies are presented in Table 3-33. For insulin resistance, two of the *medium* confidence studies in adults (Cardenas et al., 2017) and pregnant women (Jensen et al., 2018) reported higher HOMA-IR with higher PFHxS exposure (both statistically significant). The association in Jensen et al. (2018) was observed primarily in women with high GDM risk based on predefined risk factors (BMI ≥ 27 kg/m^2, family history of diabetes mellitus, present multiple pregnancy, glucosuria during pregnancy, previous GDM, or delivery of macrosomic child). The association in women without GDM risk was in the same direction but much smaller, which may suggest an interaction between PFAS exposure and metabolic vulnerability, but this cannot be assessed further using the available data. The other studies indicated no increase in insulin resistance with higher exposure. For blood glucose, three of the *medium* confidence studies in pregnant women (Yu et al., 2021; Jensen et al., 2018) and 6 weeks postpartum (Wang et al., 2018) reported statistically significantly elevated blood glucose with higher PFHxS exposure. One study in adolescents and young adults also reported a positive association in post-puberty girls undergoing an oral glucose tolerance test, with a significant association at the 1-hour post glucose test, but an inverse association was reported in boys and results at other ages did not show an association (Goodrich et al., 2021). Results in other studies were generally null.

Overall, there is not a clear association between PFHxS exposure and insulin resistance or blood glucose. Some positive associations were observed in *medium* confidence studies, but this was not consistently observed across studies, including other *medium* confidence studies of similar design and power. It is possible that exposure contrast was not adequate to observe an association in these studies, but the positive associations were observed in studies with exposure levels similar to the null studies.

Figure 3-70. Heatmap of study evaluations for insulin resistance and blood glucose [a]. For additional details see HAWC link.

[a]Multiple publications of the same study: Lin et al. (2009a) also includes Nelson et al. (2010); Christensen et al. (2019) also includes Jain (2020); Cakmak et al. (2022) also includes Fisher et al. (2013).

Table 3-33. Associations between PFHxS exposure and insulin resistance or blood glucose in epidemiology studies

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
Adults and adolescents					
Duan et al. (2020), Medium	Cross-sectional study in China in 2017; 294 adults	0.3 (<LOD–0.8)	% change for 1% increase in exposure	NR	0.004 (−0.001, 0.009)
Koshy et al. (2017); Low	World Trade Center Health Registry (WTCHR) who resided in NYC and were born between Sept. 11, 1993 and Sept. 10, 2001; U.S.; 402 adolescents	Control 0.5 (0.5) WTCHR 0.7 (0.7)	Beta coefficient (95% CI) for ln-unit change	−0.09 (−0.18, −0.003)*	NR
Chen et al. (2019a); Low	Cross-sectional study in Croatia in 2007–2008; 123 adults	GM (range) 0.8 (0.3–2.4)	Beta coefficient (95% CI) for ln-unit change	0.64 (−1.27, 2.56)	−0.16 (−0.37, 0.04)
Valvi et al. (2021); Medium	Prospective cohort (1986–1987); Faroe Islands; 699 young adults (28 yrs) with follow-up since birth	0.9 (0.7–1.2)	Beta coefficient (95% CI) for doubling	Exposure in gestation 0.00 (-0.03, 0.04) 7 years 0.01 (-0.04, 0.05) 28 years 0.03 (-0.02, 0.07)	Exposure in gestation 0.00 (-0.01, 0.01) 7 years 0.01 (-0.01, 0.01) 28 years 0.01 (-0.00, 0.02)
Heffernan et al. (2018); Medium	Prospective cohort of women with and without polycystic ovarian syndrome (PCOS) performed within the Hull IVF Unit (United Kingdom); 59 adults (20–45 yrs)	GM (95% CI) Control 0.9 (0.8, 1.2) PCOS 1.1 (0.9–1.4)	Beta coefficient (SE) for ln-unit change	Controls 0.03 (0.10) PCOS −0.15 (0.08)	Controls 0.17 (0.09) PCOS −0.05 (0.09)
Lin et al. (2009a); Low	NHANES cross-sectional (1999–2000, 2003–2004a); U.S.; 1,443 adolescents and adults (12–20 yrs, >20 yrs)	Log mean ± SEM Adolescents 1.0 ± 0.1 Adults 0.6 ± 0.04	Mean ± SEM[b] for log-unit change	Adolescents 0.05 ± 0.03 Adults 0.00 ± 0.04	Adolescents −0.01 ± 0.03 Adults −0.02 ± 0.06
Goodrich et al. (2021), Medium	SOLAR cohort (2001–2012), U.S.; 328 children (8–13 years) with 2 years follow-up Children's Health Study cross-sectional analysis within cohort (2002), U.S.; 137 young adults (17–22 years)	1.1 (GM) in SOLAR cohort; 0.8 in CHS cohort girls	Differences with high vs low PFHxS levels	NR	SOLAR Puberty Girls Fasting: 1 (-9, 12) OGTT 1 hr: 3 (-8, 13) Boys Fasting: 0 (-12, 13) OGTT 1 hr: -7 (-19, 5) Postpuberty

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
					Girls Fasting: 6 (-8, 19) OGTT 1 hr: 25 (12, 39)* Boys Fasting: -5 (-20, 11) OGTT 1 hr: -25 (-40, -9)* CHS young adult Girls Fasting 3 (-17, 23) OGTT 1 hr: 26 (6, 46) Boys Fasting: 1 (-12, 13) OGTT 1 hr: 3 (-10, 17)
Li et al. (2021a); Medium	Prospective cohort (2003–2006); U.S.; 221 adolescents (12 yrs, followed from pregnancy)	1.9 (1.0-3.3) at age 3	Adjusted difference for IQR increase	NR	Exposure in gestation -0.3 (-1.4, 0.9) 3 years 0.4 (-0.6, 1.5) 12 years 0.5 (-0.7, 1.8)
Christensen et al. (2019); Medium	NHANES cross-sectional (2007–2014); U.S.; 2,975 adults (>20 yrs)	2007–2008 2.0 (1.1, 3.5) 2009–2010 1.7 (0.9, 2.9) 2011–2012 1.3 (0.8, 2.3) 2013–2014 1.4 (0.8, 2.6)	Odds ratio (95% CI) for quartiles vs. Q1	NR	Q2: 0.88 (0.61, 1.27) Q3: 0.87 (0.59, 1.29) Q4: 0.85 (0.55, 1.31)
Cakmak et al. (2022); Medium	Canadian Health Measures Survey cross-sectional (2007–2017); Canada; 6,024 all ages	1.5 (GM)	% change for GM increase	-0.1 (-4.1, 4.6)	0.3 (-0.6, 1.3)
Lind et al. (2014); Low	PIVUS study cross-sectional (2001–2004), Sweden; 1,016 adults (70 yrs)	2.1 (1.6–3.4)	Beta coefficient (95% CI) for ln-unit change	−0.085 (−0.14, −0.03)*	NR
Cardenas et al. (2017); Medium	Diabetes Prevention Program (1996–1999), U.S.; 957 adults (25+ yrs)	GM (IQR) 2.4 (2.4)	Beta coefficient (95% CI) for doubling	0.34 (0.12, 0.55)[a]	0.29 (−0.13, 0.70)
Lin et al. (2020c); Low	Cross-sectional study in high contamination area (2016–2017),	2.7	Beta coefficient (95% CI) for quartiles vs. Q1	NR	Q2: 2.42 (-4.91, 9.75) Q3: -3.22 (-10.78, 4.35)

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
	Taiwan; 397 older adults (55–75 yrs)				Q4: 2.54 (-5.13, 10.21)
Khalil et al. (2020); Low	Cross-sectional study of firefighters (2009), U.S. 38 men	3.1 (GM)	Beta coefficient (95% CI) for log-unit change	NR	no association (figure only)
Liu et al. (2018); Low	POUNDS clinical trial (2003–2007), U.S.; 621 adults (30–70 yrs)	Male 3.1 (2.3–4.4) Female 1.9 (1.2–3.0)	Spearman correlation	0.07	Change in glucose 0–6 mo in trial: 0.02 6–24 mo: −0.02
Pregnant women					
Jensen et al. (2018); Medium	Odense Child Cohort (OCC) (2010–2012), Denmark; 649 pregnant women (15–49 yrs), outcome measured at 28 wks gestation	0.3 (0.1–0.6)	% Change (95% CI) for doubling	High GDM risk 9.5 (1.0, 18.8)* Low GDM risk 2.8 (−7.5, 14.3)	High GDM risk 1.7 (0.2, 3.2)* Low GDM risk 0.2 (−1.3, 1.7)
Yu et al. (2021), medium	Population-based birth cohort study in Shanghai, China (2013–2016); 2,747 pregnant women	0.5 (0.3) in controls	Beta coefficient (95% CI) for log-unit change	NR	0.003 (-0.04, 0.05) OGTT 1 hr 0.22 (0.06, 0.37)* OGTT 2 hr 0.08 (-0.06, 0.21)
Gardener et al. (2021); Medium	Vanguard Pilot Study of the National Children's Study cross-sectional (2009); U.S.; 425 pregnant women in 3rd trimester	0.5 (0.3-0.9)	Means (95% CI) for quartilers	Non-significant, non-monotonic increase (figure only)	NR
Wang et al. (2018); Medium	Haidian Maternal & Child Health Hospital in Beijing, China (January–March 2013); 84 pregnant women as GDM and 168 healthy pregnant women, outcome measured at 6 wks postpartum	GDM 0.5 (0.3 – 0.8) Non-GDM 0.5 (0.3 – 0.7)	Odds ratio (95% CI) for categories of blood glucose (3.2–4.74; 4.75–5.04; 5.06–6.84 mmol/L)	NR	GDM/non-GDM pooled (adjusted for status) Medium vs. Lowest 1.32 (0.72, 2.42) Highest vs. Lowest 2.29 (1.22, 4.29)*
Starling et al. (2017); Medium	Health Start cohort at the University of Colorado Hospital (2009–2014); U.S.; 1,410 pregnant women (>16 yrs), outcome measured at mid-pregnancy	0.8 (0.5, 1.2)	% Change (95% CI) for categories of exposure	NR	Group 1 −0.009 (−0.029, 0.010) Group 2 −0.023 (−0.044, −0.002)

Reference and confidence	Population	Median exposure (IQR) in ng/mL or as specified	Effect estimate	Insulin resistance (HOMA-IR)	Blood glucose
Ren et al. (2020); Medium	Shanghai-Minhang Birth Cohort (2012); China; 856 pregnant women (outcome measured at 20–28 weeks gestation)	2.8 (2.1-3.6)	OR (95% CI) for high glucose	NR	0.89 (0.51, 1.55)
Children					
Kang et al. (2018); Medium	Korea Environmental Health Survey in Children and Adolescents (KorEHS-C) subcohort (2012–2014); South Korea; children (3–18 yrs)	Geometric mean (SD) 0.8 (1.6)	Beta coefficient (95% CI) for ln-unit change	NR	0.925 (−1.779, 2.164)
Khalil et al. (2018); Low	Cross-sectional study of obese children from Lipid Clinic at Dayton's Children Hospital (April–Oct. 2016); U.S.; children (8–12 yrs)	1.1 (1.4)	Beta coefficient (95% CI) for unit change	−0.11 (−0.10, 0.78)	0.00 (−2.10, 2.09)
Goodrich et al. (2021), Medium	SOLAR cohort (2001–2012), U.S.; 328 children (8–13 years) with 2 years follow-up	1.1 (GM) in SOLAR cohort; 0.8 in CHS cohort girls	Differences with high vs low PFHxS levels	NR	Prepuberty Girls Fasting -2 (-16, 12) OGTT 1 hr -4 (-18, 10) Boys Fasting -7 (-15, 0) OGTT -7 (-15,0)
Alderete et al. (2019); Medium	Study of Latino Adolescents at Risk of type 2 Diabetes (SOLAR) cohort (2001–2011); U.S.; children (8–14 yrs)	Geometric mean (SD) 1.7 (2)	Beta coefficient (95% CI) for ln-unit change	−0.4 (−1.7, 0.8)	0.9 (−2.5, 4.2)
Fleisch et al. (2017); Low	Project Viva prospective cohort (1992–2002); U.S.; 665 mother–children pairs	Geomean (25%, 75%) Prenatal 2.5 (1.6, 3.8) Mid-childhood 2.2 (1.2, 3.4)	% Change (95% CI) for quartiles vs Q1	Prenatal Q2: −6.7 (−23.7, 14.2) Q3: −13.5 (−29.6, 6.3) Q4: −17.1 (−32.3, 1.6) Mid-childhood Q2: −5.1 (−20.9, 13.8) Q3: −6.7 (−22.7, 12.6) Q4: −16.8 (−31.4, 0.8)	NR

[*] *P*-value or *p*-trend $\leq$ 0.05.
NR = not reported; OGTT = oral glucose tolerance test

Metabolic syndrome

Metabolic syndrome is defined using criteria related to waist circumference, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure, and elevated fasting glucose. Three abnormal findings out of the five factors classify a person with metabolic syndrome (Alberti et al., 2009).

Six studies reported on the association between PFHxS exposure and metabolic syndrome. One study was *uninformative* due to critical deficiencies in participant selection, outcome ascertainment, and confounding (Yang et al., 2018). The other five studies were cross-sectional (Zare Jeddi et al., 2021; Christensen et al., 2019; Fisher et al., 2013; Lin et al., 2009b; Lin et al., 2009a) and considered *medium* confidence. A summary of the study evaluations for PFHxS and metabolic syndrome is presented in Figure 3-71, and additional details of the studies can be obtained from HAWC.

There was little indication of increased odds of metabolic syndrome with higher exposure to PFHxS. One study in older adults in an area with high PFAS contamination (Lin et al., 2020c) reported a positive association in the fourth quartile (OR [95% CI]: 1.22 [0.66, 2.25]), but this association was non-monotonic across quartiles and not statistically significant. The other four studies reported results that were null (Zare Jeddi et al., 2021; Fisher et al., 2013; Lin et al., 2009a) or inverse (Christensen et al., 2019).

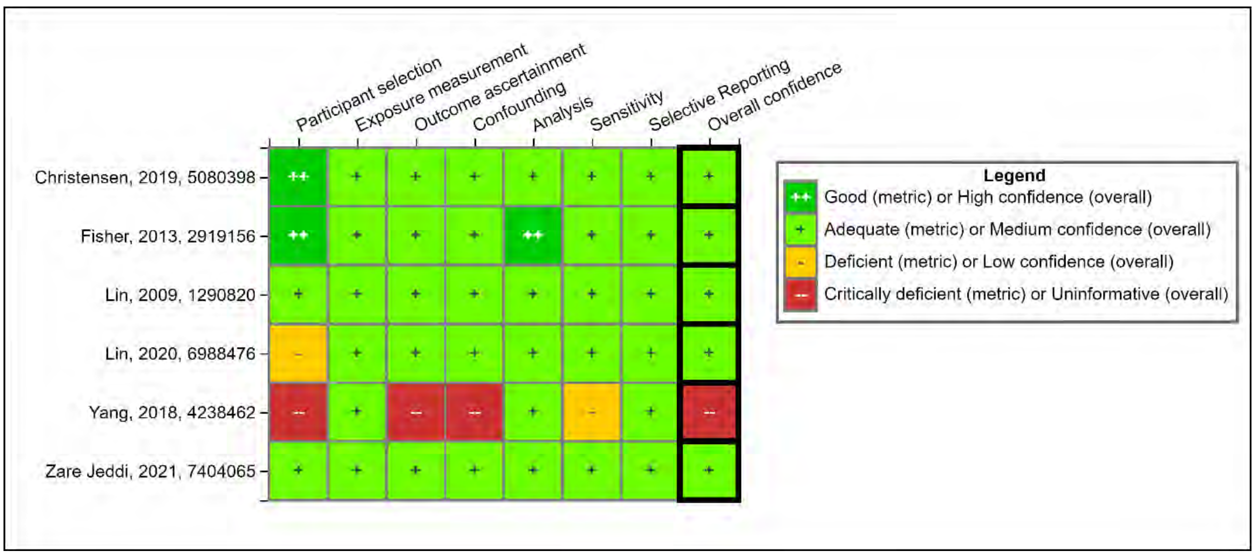

Figure 3-71. Summary of study evaluations for epidemiology studies of PFHxS and metabolic syndrome. For additional details see HAWC link.

Adiposity

Twenty-five studies (29 publications) reported on the association between PFHxS exposure and obesity, BMI, and/or other measures of adiposity. Two studies were excluded as *uninformative* due to lack of consideration of potential confounding (Zhang et al., 2019a; Yang et al., 2018). Of the

23 remaining studies, ten were cross-sectional studies (Lind et al., 2022; Canova et al., 2021; Thomsen et al., 2021; Zare Jeddi et al., 2021; Domazet et al., 2020; Scinicariello et al., 2020a; Chen et al., 2019a; Christensen et al., 2019; Khalil et al., 2018; Nelson et al., 2010) and were classified as *low* confidence because of concern that the timing of exposure measurement was not relevant to development of this chronic outcome, similar to concerns described for diabetes. Thirteen studies had prospective exposure measurement, including nine that examined the association between prenatal or early-life exposure measurements and adiposity during childhood, one cohort of people living near a uranium processing plant, one clinical trial of weight loss diets that examined weight change, and two studies of gestational weight gain. All of the prospective studies, where exposure was measured prior to the outcome, were classified as *medium* confidence. The evaluations for these studies are summarized in Figure 3-72.

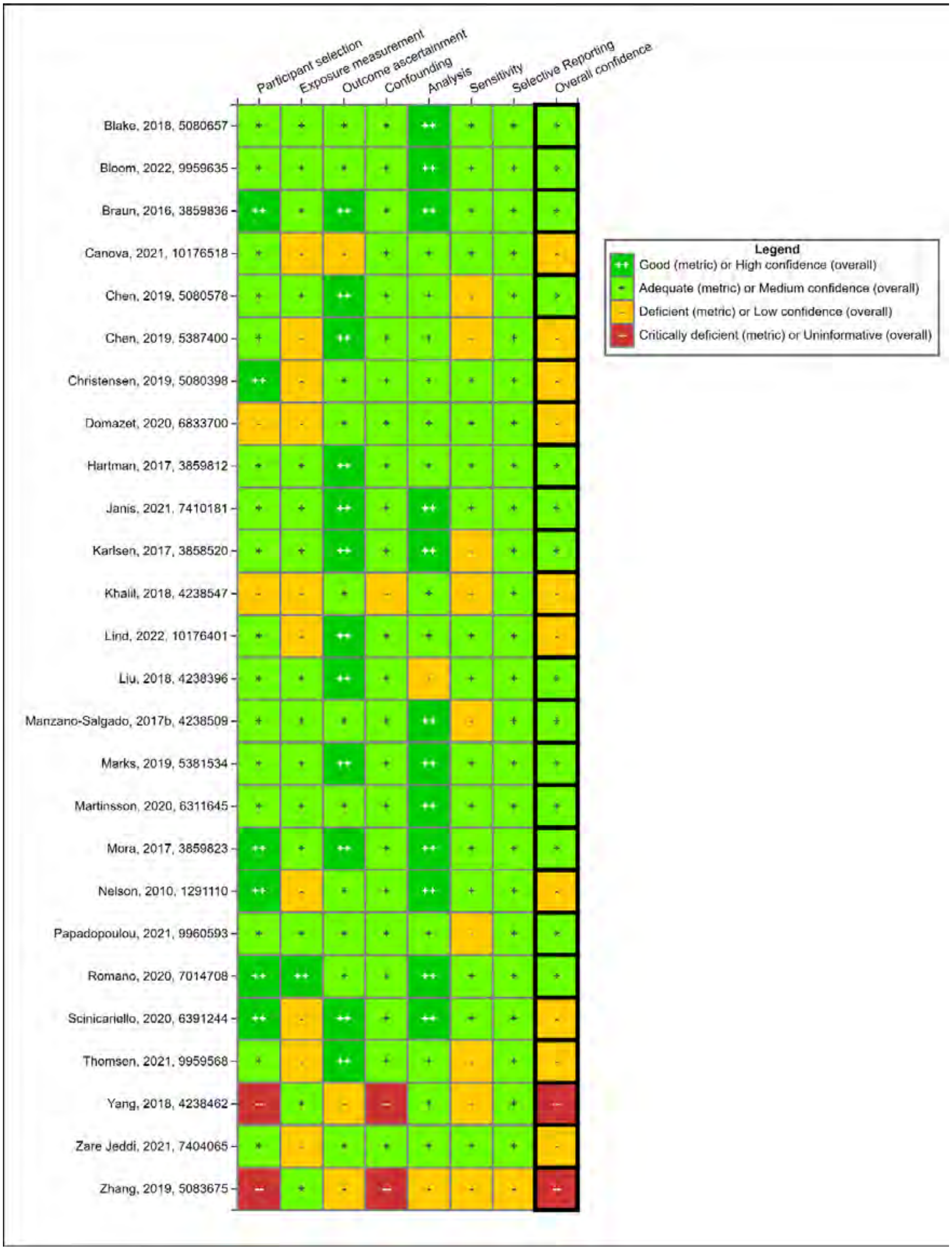

Figure 3-72. Summary of study evaluations for epidemiology studies of adiposity. For additional details see HAWC link. Multiple publications of the same study: **Braun et al. (2016)** also includes **Braun et al. (2020)**; **Liu et al. (2020c)**, and **Li et al. (2021a)**. **Mora et al. (2017)** also includes **Janis et al. (2021)**.

The results from the studies of adiposity in children are summarized in Tables 3-34 and 3-35, which contain the continuous outcome measures and dichotomous outcome (overweight), respectively. Most studies report null results for the associations between PFHxS and BMI, waist circumference, or direct measures of body fat. In analyses of overweight/obesity as a dichotomous outcome, three *medium* confidence studies (four publications) reported positive associations (Liu et al., 2020c; Martinsson et al., 2020; Braun et al., 2016) with odds ratios or relative risks ranging 1.16 to 1.71. However, only one study was statistically significant (Liu et al., 2020c) and the association in Martinsson et al. (2020) was non-monotonic across quartiles, with an inverse association in the third quartile and a positive association in the fourth quartile. n addition, as described in the Developmental Effects section, one *medium* confidence study by Gyllenhammar et al. (2018) was null for weight standard deviation scores over time from 3 to 60 months of age.

In adults, one *medium* confidence prospective study (Liu et al., 2018) reported no difference in weight loss associated with PFHxS exposure but found a statistically significant increase in weight gain associated with PFHxS exposure in women following the weight loss trial (changes in body weight: tertile 1: 2.7 ± 0.8, tertile 2: 3.6 ± 0.9, tertile 3: 4.9 ± 0.9, *p*-trend: 0.009). The second *medium* confidence prospective study (Blake et al., 2018) and the *low* confidence cross-sectional studies (Lind et al., 2022; Zare Jeddi et al., 2021; Chen et al., 2019a; Christensen et al., 2019) reported no difference in adiposity with higher PFHxS exposure. Additionally, two *medium* confidence studies examined gestational weight gain. Marks et al. (2019b) and Romano et al. (2020) reported no association with absolute gestational weight gain (stratified by baseline weight categories under/normal weight and overweight/obese).

Overall, there is very limited evidence of an association between PFHxS exposure and adiposity. The strongest evidence comes from a weight loss trial in adults that observed higher weight gain following the trial, but the lack of coherence with related outcomes in the remaining studies decreases the strength of the evidence.

Table 3-34. Associations between maternal exposure to PFHxS and adiposity in children

Reference, study confidence	Population	Median exposure (IQR) (μg/mL)	Effect estimate	BMI	Waist circumference	Body fat
Chen et al. (2019b), medium	Prospective birth cohort in China; 404 children at 5 yrs	0.2 (range 0.1–0.9)	β (95% CI) for log-unit change	Girls: −0.5 (−1.1, 0.2) Boys: 0.4 (−0.3, 1.1)	Girls: −1.2 (−3.1, 0.7) Boys: 0.6 (−1.3, 2.5)	Body fat percent Girls: −1.9 (−4.9, 1.0) Boys: 1.8 (−0.7, 4.3)
			β (95% CI) for tertiles (ref T1)	Girls T2: 0.2 (−0.8, 0.3) T3: −0.2 (−0.8, 0.3) Boys T2: 0.1 (−0.5, 0.7) T3: 0.2 (−0.4, 0.8)	Girls T2: −0.4 (−2.1, 1.2) T3: −0.4 (−2.1, 1.3) Boys T2: −0.2 (−1.8, 1.4) T3: 0.5 (−1.1, 2.1)	Girls T2: −0.8 (−3.4, 1.7) T3: −1.9 (−4.4, 0.7) Boys T2: 0.2 (−2.0, 2.3) T3: 0.7 (−1.4, 2.8)
Karlsen et al. (2017), medium	Birth cohort (2007–2009), Faroe Islands; 444 children with follow-up at 18 mos	0.2 (0.1–0.3)	β (95% CI) for log-unit increase; T2 and T3 vs. T1	0.10 (−0.01,0.21) T2: −0.03 (−0.23,0.17) T3: 0.18 (−0.03,0.38)	NR	NR
	371 children with follow-up at 5 yrs			0.04 (−0.07,0.15) T2: −0.02 (−0.22,0.19) T3: 0.07 (−0.14,0.28)	NR	NR
Papadopoulou et al. (2021), medium	Six birth cohorts, Europe, 1,301 children at 6–11 yrs	prenatal 0.5 (0.3–0.9)	β (95% CI) for Quartiles vs Q1	NR	Q2: -0.02 (-0.22, 0.17) Q3: 0.05 (-0.18, 0.28) Q4: 0.03 (-0.23, 0.30)	NR
		Children 0.3 (0.2–0.6)		NR	Q2: -0.12 (-0.31, 0.06) Q3: 0.10 (-0.13, 0.32) Q4: 0.04 (-0.22, 0.29)	NR
Thomsen et al. (2021), low	Cross-sectional analysis within birth cohort (2009), Denmark, 109 boys at ~12 yrs	0.5 (0.4–0.7)	β (95% CI) for log-unit increase	NR	NR	Abdominal fat 0.03 (-0.15, 0.20) Visceral fat 0.02 (-0.11, 0.14) Total fat 0.01 (-0.22, 0.23)
Manzano-Salgado et al. (2017b), medium	INMA birth cohort (2003–2008), Spain; 1,230 children with follow-up at 4 yrs	0.6 (GM) (0.4–0.8)	β (95% CI) for doubling exposure	-0.02 (−0.10,0.07)	-0.04 (−0.14,0.05)	NR
	1,086 children with follow-up at 7 yrs			-0.04 (−0.14,0.06)	-0.04 (−0.12,0.04)	NR
Domazet et al. (2020), low	Cross-sectional analysis within multi-center cohort	0.9 (0.7–1.1)	% change (95% CI) for 10% increase	NR	NR	Fat mass -1.07 (-1.99, -0.15)*

Reference, study confidence	Population	Median exposure (IQR) (μg/mL)	Effect estimate	BMI	Waist circumference	Body fat
	(1997), Europe; 242 children at 9 yrs					
Bloom et al. (2022), low	ECHO cohort (2017–2019), U.S. 803 children at 4–8 yrs	0.9 (0.5-1.5)	β (95% CI) for log-unit increase	BMI z-score Without obesity -0.06 (-0.17, 0.05) With obesity 0.01 (-0.22, 0.24)	Without obesity -0.06 (-0.15, 0.04) With obesity 0.16 (-0.09, 0.40)	Fat mass Without obesity -0.08 (-0.42, 0.25) With obesity 0.63 (-0.68, 1.93) Percent body fat Without obesity -0.003 (-0.01, 0.01) With obesity 0.01 (-0.02, 0.04)
Scinicariello et al. (2020a), low	NHANES cross-sectional study (2013–2014), U.S. 600 children at 3–11 yrs	0.9 (GM)	β (95% CI) for tertiles vs T1	BMI z-score T2: -0.17 (-0.47, 0.13) T3: -0.26 (-0.57, 0.04)	Weight for age T2: -0.30 (-0.67, 0.07) T3: -0.42 (-0.76, -0.08)*	NR
Khalil et al. (2018), low	Cross-sectional study (2016), U.S. 48 children with obesity at 8–12 yrs	1.1 (1.4)	β (95% CI) for unit change	0.32 (-0.76, 1.39)	NR	NR
Braun et al. (2016); Liu et al. (2020c); Braun et al. (2020); Li et al. (2021a), medium	HOME birth cohort (2003–2006), U.S.; 204 children with follow-up at 8 yrs	1.4 (0.8–2.3)	Difference (95% CI) Tertiles vs. T1	T2: 0.22 (−0.10,0.54) T3: 0.12 (−0.21,0.45)	T2: 2.7 (0.0,5.4) T3: 1.1 (−1.7,3.9)	Body fat percent T2: 2.3 (0.3,4.2) T3: 1.1 (−0.9,3.1)
	212 children with follow-up at 12 yrs		β (95% CI) for IQR increase	BMI z-score Prenatal exposure 0.10 (-0.08, 0.28) 12 year old exposure 0.09 (-0.14, 0.31)	Prenatal exposure 1.73 (-0.87, 4.33) 12 year old exposure 0.55 (-2.48, 3.57)	Fat mass index Prenatal exposure 0.10 (-0.07, 0.26) 12 year old exposure 0.08 (-0.11, 0.27) Body fat percent Prenatal exposure 0.94 (-0.35, 2.22) 12 year old exposure 0.68 (-0.79, 2.15)
	214 children with follow-up at 12 yrs		β (95% CI) for IQR increase	T2: -0.65 (-1.90, 0.65) T3: -0.50 (-1.78, 0.76)	NR	NR
			Difference (95% CI) Tertiles vs. T1	Rate of BMI change from 8–12 yrs T2: -0.06 (-0.20, 0.09) T3: -0.01 (-0.15, 0.13)	NR	NR
	186 children with follow-up at 12 yrs		Difference (95% CI)	NR	Prenatal exposure 0.03 (-0.01, 0.08)	Visceral fat Prenatal exposure

Reference, study confidence	Population	Median exposure (IQR) (μg/mL)	Effect estimate	BMI	Waist circumference	Body fat
			for IQR change		12 year old exposure 0.02 (-0.04, 0.07)	0.09 (-0.01, 0.20) 12 year old exposure 0.10 (-0.05, 0.26)
Hartman et al. (2017), medium	ALSPAC birth cohort (1991–1992), United Kingdom; 359 children with follow-up at 9 yrs)	1.6 (1.3–2.2)	β (95% CI) for 1 unit increase	-0.02 (−0.08,0.03)	-0.08 (−0.22,0.06)	DXA total body fat -0.06 (−0.21,0.09) DXA trunk fat -0.01 (−0.11,0.08)
Mora et al. (2017); Janis et al. (2021), medium	Project Viva birth cohort (1999–2002), U.S.; 1,006 children with follow-up at median 3 yrs	2.4 (1.6–3.8)	β (95% CI) for IQR increase	0.01 (−0.03,0.05)	0.03 (−0.10,0.16)	Sum of subscapular and triceps skinfold thickness 0.16 (0.01,0.31)
	876 children with follow-up at median 7 yrs			0.01 (−0.03,0.05)	0.11 (−0.22,0.43)	Sum of subscapular and triceps skinfold thickness 0.25 (−0.14,0.64) DXA total fat mass index 0.04 (−0.04,0.13) DXA trunk fat mass index 0.02 (−0.02,0.06)
	531 children with follow-up at 13 yrs		β (95% CI)	BMI z-score -0.05 (-0.09, 0.00)	NR	Total fat mass index -0.22 (-0.35, -0.08)* Truncal fat mass index -0.09 (-0.16, -0.03)*
Canova et al. (2021), low	Cross-sectional study in highly contaminated area (2017–2019), Italy; 6,669 adolescents (14–19 yrs) and 2,693 children (8–11 yrs)	adolescents 2.8 (1.6–4.8)	β (95% CI) vs Q1	BMI z-score Q2: -0.08 (-0.15, 0) Q3: 0.01 (-0.07, 0.09) Q4: 0.03 (-0.05, 0.12) Similar for boys and girls	NR	NR
		children 1.9 (1.2–2.8)	β (95% CI) for ln-unit increase	BMI z-score Q2: 0.06 (-0.08, 0.2) Q3: -0.20 (-0.34, -0.06)* Q4: -0.18 (-0.32, -0.03)*	NR	NR

*$p < 0.05$.
T: tertile, GM: geometric mean, DXA: dual-energy X-ray absorptiometry, NR: not reported.

Table 3-35. Associations between maternal exposure to PFHxS and overweight status in children in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) (μg/mL)	Effect estimate	Overweight
Karlsen et al. (2017)	Birth cohort (2007–2009), Faroe Islands; 444 children with follow-up at 18 mos	0.2 (0.1–0.3)	OR (95% CI) for log-unit increase; Tertiles vs. T1	1.12 (0.97, 1.30) T2: 1.06 (0.82, 1.38) T3: 1.24 (0.97, 1.58)
	371 children with follow-up at 5 yrs			1.11 (0.77, 1.59) T2: 0.86 (0.47, 1.55) T3: 1.22 (0.73, 2.04)
Manzano-Salgado et al. (2017b)	INMA cohort (2003–2008), Spain; 1,230 children with follow-up at 4 yrs	0.6 (GM) (0.4–0.8)	RR (95% CI) for doubling exposure	0.96 (0.87, 1.07)
	1,086 children with follow-up at 7 yrs			0.94 (0.84, 1.05)
Martinsson et al. (2020)	Case-control study (2003–2008), Sweden; 1,048 children at 4 yrs	0.7 (0.5–1.0)	OR (95% CI); Quartiles vs. Q1	Q2: 0.95 (0.66, 1.37) Q3: 0.66 (0.44, 0.97) Q4: 1.16 (0.81, 1.66)
Braun et al. (2016); Liu et al. (2020c)	HOME birth cohort (2003–2006), U.S.; 204 children with follow-up at 8 yrs	1.4 (0.8–2.3)	RR (95% CI); Tertiles vs. T1	T2: 1.33 (0.72, 2.48) T3: 1.48 (0.75, 2.96)
	212 children with follow-up at 12 yrs		RR (95% CI) for IQR increase	1.71 (1.08, 2.73)*
Mora et al. (2017)	Project Viva birth cohort (1999–2002), U.S.; 1,006 children with follow-up at median 3 yrs	2.4 (1.6–3.8)	RR (95% CI) for IQR increase	Overweight: 1.03 (0.94, 1.13) Obese: 1.02 (0.89, 1.17)
	876 children with follow-up at median 7 yrs			Overweight: 1.04 (0.92, 1.17) Obese: 1.07 (0.94, 1.22)

Animal Studies

There are two 28-day gavage studies in SD rats (NTP, 2018b; 3M, 2000a), one 4- to 6-week oral gavage exposure study using genetically modified mice (Bijland et al., 2011), and two reproductive/developmental studies using CD-1 mice (Chang et al., 2018) or Sprague Dawley rats (Butenhoff et al., 2009; 3M, 2003) that measure effects relevant to the assessment of the cardiovascular or metabolic systems after repeated oral dose exposure to PFHxS. The studies report on heart weight and histopathology, and alterations of cardiometabolic endpoints such as fasting levels of serum lipids which are considered indicative of potential cardiotoxicity (Gad, 2015). Overall study confidence was high for cardiometabolic endpoints evaluated in these studies (Chang et al., 2018; NTP, 2018b; Bijland et al., 2011; Butenhoff et al., 2009; 3M, 2003, 2000a).

Studies reporting on heart weight and histopathology were considered of *low* confidence due to experimental design uncertainties (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003) (see Figure 3-73). Specifically, the exposure duration of less a month was not considered sufficient for evaluation of injury to the cardiovascular system (Daugherty et al., 2017), raising significant concerns for insensitivity.

Heart weight and histopathology

There is no clearly preferred measurement for evaluating heart weights (absolute or relative). Some data show that heart weight is nonproportional to body weight (Bailey et al., 2004), other data reports that heart weight in strongly correlated with body weight, with better correlation in males (Nirogi et al., 2014). Thus, both absolute and relative heart weights are considered biological relevant metric for this endpoint. Absolute and relative heart weights were not altered in SD rats exposed to PFHxS for 28 days at 0.625 to 10 mg/kg-day (NTP, 2018a; 3M, 2000a). However, one reproductive/developmental toxicity study reported decreased relative heart/brain weight (by 8%) in F0 generation male SD rats exposed to PFHxS for 44 days (Butenhoff et al., 2009; 3M, 2003); the biological significance of this 8% change is unclear. Importantly, the same study also reports that absolute and heart-to-body weight ratios were not affected in males or females exposed to PFHxS.

Heart histopathology was evaluated in a 28-day study (NTP, 2018a) and a reproductive/developmental toxicity study (Butenhoff et al., 2009; 3M, 2003), both in SD rats. Exposure to PFHxS from 0.625 to 10 mg/kg-day did not cause a significant effect on the incidence of nonneoplastic cardiovascular injury in male or female rats (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003). As noted above, there is concern that the exposure duration of these studies (<1 month) was too short to expect to see histological manifestations of cardiac injury.

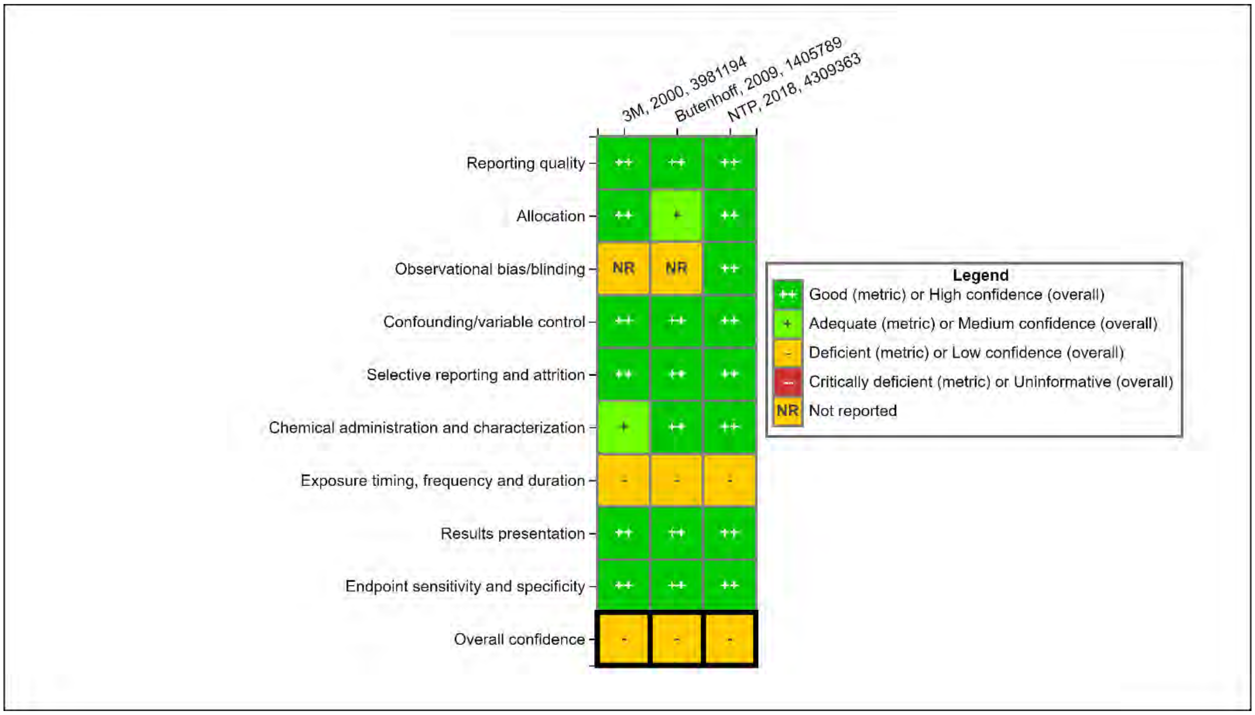

Figure 3-73. Cardiometabolic effects, heart weight/histopathology – animal study evaluation heatmap. For additional details see HAWC link.

Serum lipids

Levels of plasma cholesterol (Gad, 2015) were evaluated in two reproductive/developmental toxicity studies (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003), and in four short-term exposure studies (He et al., 2022; NTP, 2018a; Bijland et al., 2011; 3M, 2000a), and one chronic exposure study (Pfohl et al., 2020) (see Figure 3-74). In the high confidence, short-term studies, exposure to PFHxS for 28 days resulted in a 12% to 51% reduction in serum cholesterol at doses ranging from 1.25 to 10 mg/kg-day in male and female rats in one study (3M, 2000a) and in males only in the other (NTP, 2018b). Likewise, a separate study using male APOE*3-Leiden CETP23 mice reported that exposure to 6 mg/kg-day PFHxS decreased total cholesterol, HDL and non-HDL cholesterol (Bijland et al., 2011). Two reproductive/developmental toxicity studies report that PFHxS exposure for 42 to 44 days decreased serum cholesterol by 19% to 42% in male F0 SD rats at doses ranging from 0.3 to 10 mg/kg-day (Butenhoff et al., 2009; 3M, 2003), whereas F0 CD-1 male mice treated with 10 mg/kg-day displayed a 27% reduction in cholesterol (Chang et al., 2018). However, these effects were not observed in female Sprague Dawley rats or CD-1 mice (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003), or male C57BL/6J mice exposed to 12 or 29 weeks in high (He et al., 2022) or medium confidence studies (Pfohl et al., 2020) exposed to 0.06 or 0.15 mg/kg-day, respectively.

[23]APOE*3-Leiden.CETP mice is a genetically modified animal model which better emulates human lipoprotein profiles and is used to investigate cholesterol metabolism and cardiovascular disease (Veseli et al., 2017).

PFHxS exposure-induced effects on serum lipid levels and production were also measured in rats and mice. In a *high* confidence study of SD rats, short-term oral exposure for 28 days decreased serum triglyceride levels by 22% to 46% after exposures ranging from 2.5 to 10 mg/kg-day (NTP, 2018a; 3M, 2000a), and a *medium* confidence study using APOE*3-Leiden.CETP mice reported decreased serum-free fatty acids (43%) and VLDL-triglyceride production rate (74%) , very-low-density lipoprotein (VLDL) half-life, and VLDL apolipoprotein production in animals treated with 6 mg/kg-day PFHxS (Bijland et al., 2011). The same study reported a 75% increase in lipoprotein lipase in exposed mice (Bijland et al., 2011). Two *high* confidence reproductive/developmental toxicity studies also evaluated PFHxS-induced alterations in other serum lipids. In SD rats, exposure to 10 mg/kg-day, decreased serum triglycerides by 27% in F0 males (Butenhoff et al., 2009; 3M, 2003), but a similar study using CD-1 mice did not observe significant treatment-related changes in serum triglycerides in male or female F0 animals at PFHxS levels up to 3mg/kg-day (Chang et al., 2018). *Medium* and *high* confidence studies exposing using C57BL/6J mice to 0.15 or 0.06 mg/kg-day PFHxS for 29 or 12 weeks respectively report no significant effect on serum tryglycerides (He et al., 2022; Pfohl et al., 2020). Overall, a consistent pattern of dose-dependent decreases in cholesterol and other lipids in the blood of animals exposed to PFHxS were observed across *high* and *medium* confidence studies of varied design in both rats and mice, although effects were largely absent in female rodents and studies that exposed mice to PFHxS at lower doses. However, as described below there are limitations in using animal models (including the APOE-modified mice) to emulate human lipid regulation.

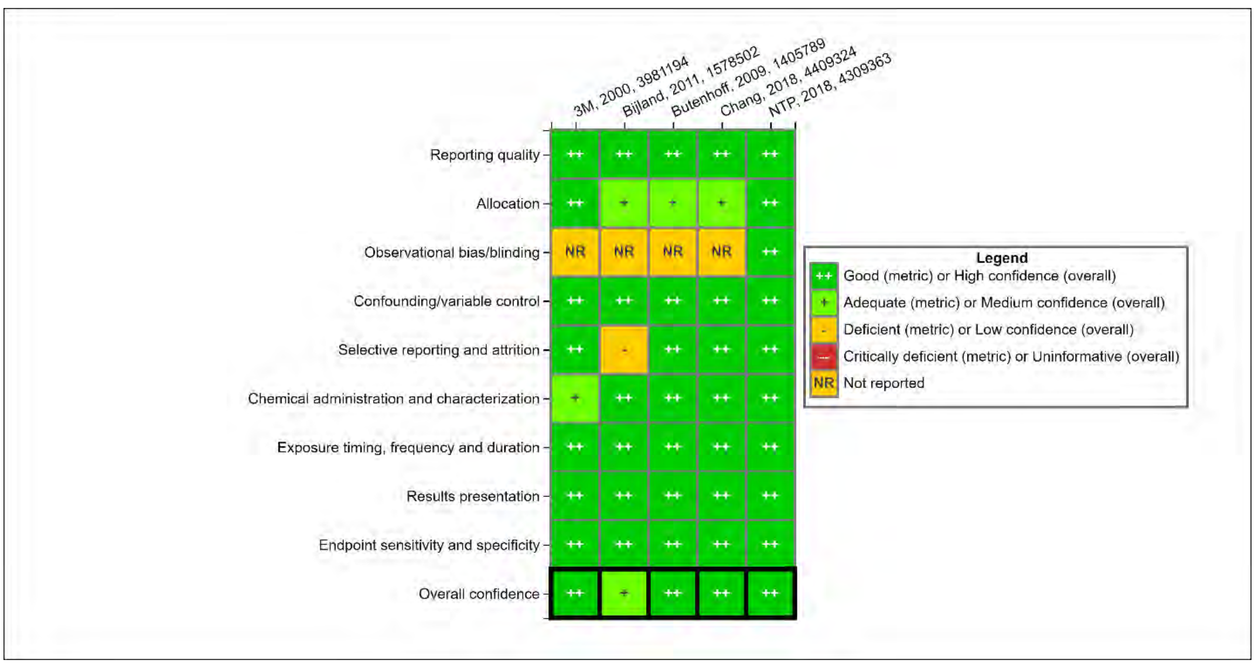

Figure 3-74. Cardiometabolic effects, serum lipids – animal study evaluation heatmap. For additional details see HAWC link.

Considerations for interpreting the human relevance of the animal cardiometabolic evidence

The results from the available animal studies should be interpreted with caution because of known cardiometabolic differences between humans and laboratory animal models commonly used in toxicological studies (Getz and Reardon, 2012). This section briefly highlights what is currently known regarding cardiometabolic differences between humans and laboratory animal models commonly used in toxicological studies to inform potential future studies. The pathophysiology of cardiovascular disease in humans is a complex process driven by multiple risk factors (e.g., diabetes, hyperlipidemia, hypertension, and aging), which lead to metabolic and pro-inflammatory alterations. Unfortunately, there is no single animal model that completely recapitulates all the features of human disease (Oppi et al., 2019). Furthermore, there are significant differences between rodent and human cardiovascular systems that should be taken into consideration. Murine plasma cholesterol is approximately threefold lower, the major lipoprotein in mice is HDL, not LDL (Getz and Reardon, 2012), and differences in bile acid composition contribute to lower intestinal absorption of cholesterol and higher cholesterol excretion (Oppi et al., 2019). These differences contribute to significantly lower cholesterol levels in mice when compared with humans and having lower cholesterol levels in turn confers protection from cardiovascular injuries such as atherosclerosis (Oppi et al., 2019).

Although the available animal **evidence suggests** the cardiovascular system may be responsive to PFHxS-induced responses, additional studies using experimental models and designs that better emulate human disease would help to fully characterize the pathology of potential cardiometabolic responses to this chemical. Future studies should focus on the use of genetically manipulated or experimentally induced rodent models that can emulate human metabolic and pathological conditions (Kodavanti et al., 2015). For example, studies aimed at evaluating vascular injuries such as atherosclerosis should focus on the use of animal models that can generate non-HDL-based hypercholesterolemia such as LDL Receptor or apolipoprotein E (ApoE) null mice (Getz and Reardon, 2012) and expose animals for sufficient time to develop of arterial injuries (Daugherty et al., 2017). Furthermore, future studies focused on potential effects to the cardiovascular system should include analysis of physiological and biochemical parameters (e.g., heart rate, blood pressure, blood gases, and oxygen consumption), which are considered indicative of adverse responses in the cardiovascular system (Gad, 2015).

Evidence Integration

The available evidence on PFHxS-induced cardiometabolic effects in humans is considered *slight* (see Table 3-36). There is some evidence of an association between PFHxS exposure and cardiometabolic effects in humans, specifically an indication of higher serum cholesterol levels. A similar association has been noted for some other long-chain PFAS, including PFOA and PFOS (U.S. EPA, 2016a, b). However, there is little evidence of an association between PFHxS exposure and cardiovascular disease, functional endpoints of cardiovascular function (e.g., blood pressure), or other related cardiovascular risk factors. It is possible that cholesterol is a more sensitive measure to PFHxS exposure and that the exposure levels and contrast were inadequate to detect differences in disease risk. However, without additional evidence, the lack of coherence across outcomes reduces confidence in the evidence of the association with cardiovascular effects and indicates that the observed changes in serum lipids may not be adverse.

The evidence from animal toxicity studies on PFHxS-induced cardiometabolic effects is considered *indeterminate*. Animal studies report dose-related decreases in serum cholesterol and triglyceride levels in male, but not female (largely), rats and mice. The direction of the observed responses in animals is different from the observations made in human studies (e.g., decreased serum lipids in animals versus reported increases in humans) and these effects may be caused by PFHxS-induced alterations in hepatic lipoprotein metabolism (see Serum Biomarkers of Liver Function Section 3.2.5). Heart weights and histopathology were not affected in exposed animals, although these *low* confidence experiments were potentially insensitive. The downstream effects of the metabolic alterations observed in the available studies are unclear in the absence of additional experiments and measures of adverse responses in the cardiovascular system. Further, interpretation of such results is not possible due to major limitations in the animal toxicity database. As described above, commonly used laboratory rodent species are relatively resistant to cardiotoxicity effects in part due to differences in lipid profiles (Veseli et al., 2017). Furthermore, the available evidence on PFHxS-induced cardiometabolic effects consists of short-term and developmental exposure studies, whereas longer study durations (between 10 to 12 weeks in mice Daugherty et al. (2017)) are generally preferred for evaluations cardiovascular system functions and disease (e.g., atherosclerosis). These experimental design and database deficiencies limit the interpretation of observed cardiometabolic changes in rodents and their applicability for informing human health hazard.

The available animal and epidemiological **evidence suggests** but is not sufficient to infer whether exposure to PFHxS might cause cardiometabolic effects in humans given sufficient exposure conditions[24]. This judgement is based primarily on consistent increases in cholesterol in humans, but with limitations in the available epidemiological studies that introduce uncertainty (see description above) and also reflects an inability to interpret the available epidemiology evidence on PFHxS-induced cardiovascular disease as well as the animal evidence available to inform this health effect.

[24] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-36. Evidence profile table for PFHxS exposure and cardiometabolic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Serum Lipids 25 *medium* and 9 *low* confidence studies	• *Consistency* in direction of association for cross-sectional analyses in adults • *Medium confidence studies* reporting an effect • *Exposure-response gradient* observed in five studies	• Potential for residual confounding across PFAS • Unexplained inconsistency among studies with prospective exposure measurement and for all studies of LDL cholesterol and triglycerides	Majority of studies in adults report higher serum cholesterol with higher PFHxS exposure, including 40–60% increases in the odds of high cholesterol.	⊕⊙⊙ *Slight* Generally consistent findings for total cholesterol in adults. Evidence for other related outcomes and age groups is inconsistent.	⊕⊙⊙ **Evidence suggests, but is not sufficient to infer** *Primary Basis:* based primarily on consistent increases in cholesterol in humans, but with limitations in the available epidemiological studies that introduce uncertainty. *Human relevance*: The animal models used are considered inadequate to inform potential human cardiometabolic responses with confidence. *Cross-stream coherence*: Evidence in animals is indeterminate
Other Cardiovascular Risk Factors 1 *high,* 18 *medium,* and 7 *low* confidence studies	• No factors noted	• Unexplained inconsistency	Positive associations reported for hypertension in adolescents and young adults, but not other adults or children. One of four studies of gestational hypertension and two of four studies of preeclampsia reported a positive association. No association between PFHxS exposure		

Evidence stream summary and interpretation					Evidence integration summary judgment
			atherosclerosis or ventricular geometry		
Cardiovascular Disease 2 *medium* and 3 *low* confidence studies	• No factors noted	• *Lack of coherence* across outcomes in *low* confidence studies • *Unexplained inconsistency* – No associations in the two *medium* confidence studies	No association with cardiovascular disease in medium confidence studies. Low confidence studies report higher odds of cardiovascular conditions and lower odds of coronary heart disease		
Evidence from in vivo animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Heart Weight / Histopathology 3 *low* confidence studies in adult rats: • 28-d (×2) • 44-d	• *High* and *medium* confidence studies of serum lipid measures	• Inconsistent findings across studies reporting on serum lipids. • Unclear biological significance of decreases in serum lipids.	• No observed PFHxS-induced effects on heart weight or histopathology in short-term, potentially insensitive studies. • Dose-dependent decreases in serum cholesterol and triglycerides.	⊙⊙⊙ *Indeterminate*	
Serum Lipids 5 *high* confidence studies in adult rats: • 28-d (×2) • 42-d • 44-d • 84-d					

Evidence stream summary and interpretation					Evidence integration summary judgment
2 *medium* quality study: • 42-d • 203-d					

3.2.7. Hematopoietic Effects

Human Studies

One epidemiology study (Jiang et al., 2014) examined the association between PFHxS exposure and hematopoietic system effects, specifically the parameters from a complete blood count (white and red blood cells, hemoglobin, platelets). This study was considered *uninformative* due to lack of consideration of confounding, and thus no human studies were synthesized for hematopoietic effects.

Animal Studies

The toxicity database for PFHxS-induced hematopoietic system effects consists of two 28-day studies (NTP, 2018a; 3M, 2000a) in Crl:Cd Br and Sprague-Dawley (SD) rats, respectively; and one multigenerational study in Sprague Dawley rats (Butenhoff et al., 2009). All studies exposed the animals orally via gavage. Hematopoietic system-related outcomes evaluated by these studies included non-immune blood cells counts and clotting parameters.

Evaluation of the available animal studies showed that these were well conducted for most hematopoietic-related endpoints. All were considered *high* confidence. The available studies generally examined PFHxS hematopoietic effects using doses that ranged between 0 and 10 mg/kg-day in rats (Butenhoff et al., 2009; 3M, 2000a) with the exception of NTP (2018a) in which a range of 0–50 mg/kg-day in female rats and 0–10 mg/kg-day in male rats was used. This approach was to account for the pharmacokinetic (PK) sex differences that have been observed in rats, in which PFHxS appears to have a lower mean half-life in female rats versus their male counterparts (20.7 and 26.9 days respectively (Kim et al., 2016b)). No overt toxicity was observed at any of the highest doses tested in any of the available studies. 3M (2000a) and NTP (2018a) measured PFHxS related hematopoietic effects using the following parameters: hematocrit, hemoglobin, platelet counts, prothrombin time, and red blood cell counts. NTP (2018a) also measured PFHxS effects on reticulocyte counts. The study by Butenhoff et al. (2009) measured hematocrit, hemoglobin, prothrombin time, and red blood cell counts in P0 males and females after 44 days of PFHxS (Butenhoff et al., 2009).

Figure 3-75 below summarizes the results of animal study evaluations, and Figure 3-76 summarizes the experimental studies and their findings.

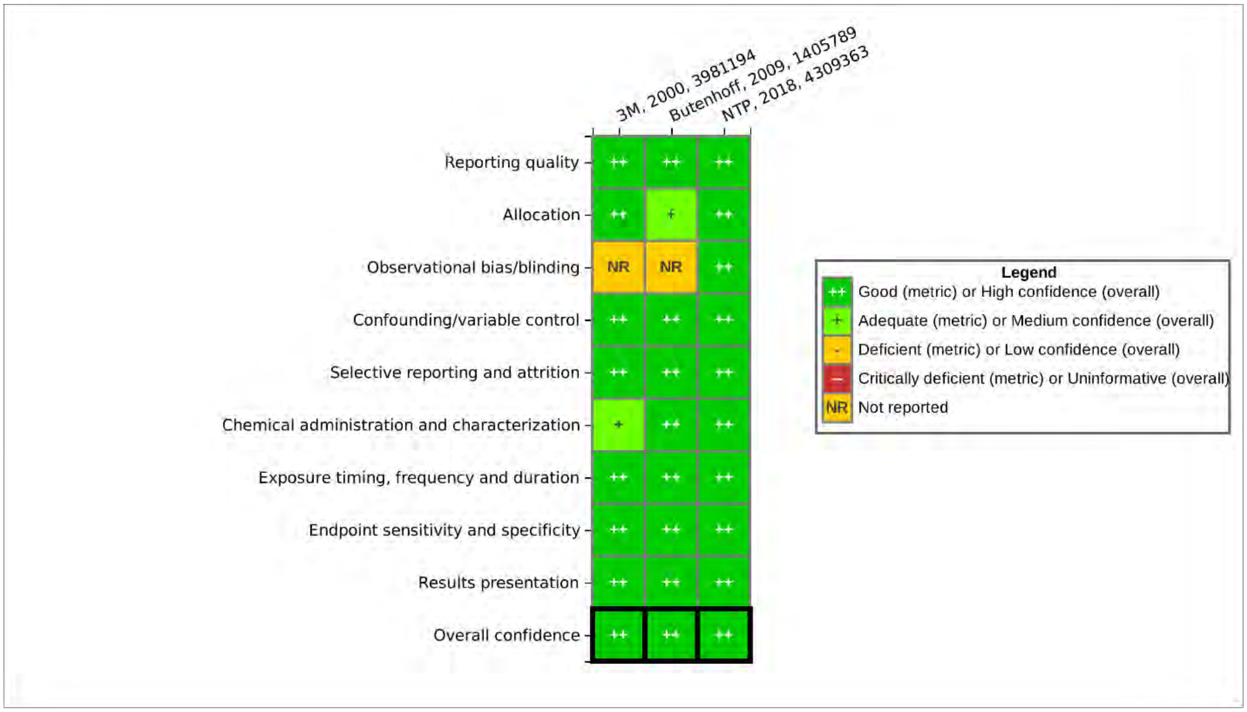

Figure 3-75. Hematological animal study confidence scores from repeated PFHxS dose animal toxicity studies. For additional details see HAWC link.

Hemostasis, the physiological process of blood coagulation after injury, is dependent on interactions between the vasculature and circulating plasma, platelets, blood cells and their related molecules (Harris et al., 2012; Gale, 2011). Clinical hematology assays like those available in the PFHxS evidence based provide insight into bone marrow[25] health as well as to assess blood clotting function. Due to the dynamic interactions between hematopoietic cells and their related molecules, information on the hematopoietic health of an organism is gained by the interpretation of the collective battery of assays, rather than individual assay results (Harris et al., 2012). Therefore, the collective information from the entirety of the data provided from these available assays was used to determine the potential for hazard posed by PFHxS on the hematopoietic system.

Hematocrit (Hct), hemoglobin (Hb), and red blood cell (RBC) count

The hematocrit assay measures the amount (i.e., as a percent of blood volume) of red blood cells (RBCs) in the blood. This measurement can provide insight on oxygen delivery capacity. All three studies measured PFHxS effects on hematocrit. Two out of the three observed effects related to PFHxS exposure 3M (2000a) observed a significant decrease (5%–6%) in hematocrit in male and female Crl:Cd Br rats following 28 days of daily oral exposure to 10 mg/kg-day PFHxS (the only

[25]The bone marrow is the site of blood stem cell formation. Blood stem cells transform into a variety of blood cells with distinct functions such as white cells (immune function); red blood cells (oxygen carrying) and platelet cells (clotting and injury repair) (Manz et al., 2004).

tested dose). In the multigenerational study, Butenhoff et al. (2009) also observed a significant (between 6% and 8%) decrease in hematocrit in male SD rats exposed to PFHxS at ≥3 mg/kg-day for 44 days in F0 rats; however, females were unaffected. Further, changes in hematocrit were not observed by NTP (2018a) in male or female SD rats exposed for 28 days to doses of PFHxS up to 10 or 50 mg/kg-day, respectively.

Hemoglobin is an oxygen-carrying protein found in red blood cells. Its function is to deliver oxygen from red blood cells to organs and tissues and to transport carbon dioxide from these back to the lungs. All three studies measured hemoglobin in response to PFHxS exposure (NTP, 2018a; Butenhoff et al., 2009; 3M, 2000a). Similar to the results for hematocrit, Butenhoff et al. (2009) observed a significant decrease (between 5% and 7%) in hemoglobin in male, but not female, rats orally exposed to ≥1 mg/kg-day PFHxS after 44 days of exposure, while 3M (2000a) observed a significant decrease (4%–7%) in hemoglobin in male and female rats at the only dose, 10 mg/kg-day, at day 28. Changes in hemoglobin were not observed by NTP (2018a) in either male or female SD rats exposed to a similar dose range of PFHxS for 28 days.

Red blood cells carry oxygen, and their abundance can affect how much oxygen is received by tissues and organs. RBC count provides a screening tool to assist in diagnosing or monitoring conditions such as anemia. All studies measured RBC counts in response to PFHxS exposure, with similar findings as for Hct and Hb, specifically: decreased RBC counts (between 7% and 8%) at ≥3 mg/kg-day in male, but not female, rats exposed to PFHxS for at least 42 days (Butenhoff et al., 2009); decreased RBC counts (between 6% and 7%) in male and female rats exposed to 10 mg/kg-day PFHxS for 28 days (3M, 2000a); and, in the second 28-day study, no changes in RBC counts in male or female rats at up to 10 mg/kg-day (males) or 50 mg/kg-day (females) PFHxS (NTP, 2018a).

Reticulocytes count

Reticulocytes are RBC precursors produced in the bone marrow and released into the bloodstream where they develop into mature RBCs. Reticulocyte counts can provide information about the health of the bone marrow and its ability to produce RBCs. Only the NTP study measured reticulocyte counts., A significant decrease (10% –27%) in number of reticulocytes was observed in SD male rats at ≥1.25 mg/kg-day and a significant increase (40%) in reticulocyte counts in female rats at 3.12 mg/kg-day, but not higher or lower doses (NTP, 2018a). The other two studies (Butenhoff et al., 2009; 3M, 2000a) did not evaluate reticulocytes, preventing interpretation as to whether a compensatory response of the bone marrow to the observed effects on red blood cell parameters might exist.

Platelet count

Platelets are cell fragments found within the blood that are critical for clot formation when blood vessels are damaged. Together with prothrombin time, platelet counts provide information on coagulation potential. Two studies, 3M (2000a) and NTP (2018a), measured PFHxS effects on platelet counts. 3M (2000a) observed a significant decrease (11%–26%) in total platelet numbers in male and female rats exposed to 10 mg/kg-day PFHxS for 28 days. NTP (2018a) did not report any changes in platelet counts in male or female rats exposed to PFHxS for 28 days at up to 10 mg/kg-day (males) or up to 50 mg/kg-day (females).

Prothrombin time

Prothrombin time is an assay measuring the amount of time it takes blood to clot. Two studies, Butenhoff et al. (2009) and 3M (2000a), measured PFHxS effects on prothrombin time. Butenhoff et al. (2009) observed a significant increase (between 3%–6%) in prothrombin time in male, but not female, rats at 0.3, 3 and 10 mg/kg-day (doses tested: 0.3, 1, 3, and 10 mg/kg-day). Under similar study conditions, the single dose (10 mg/kg-day) 28-day study by 3M (2000a) observed that prothrombin time significantly decreased (between 5%–6%) in female rats and male rats in response to 10 mg/kg-day PFHxS. Figure 3-76 below summarizes the study design and results for each hematology parameter described in these three studies.

Figure 3-76. Hematopoietic effects of PFHxS exposure in animals. For additional details see HAWC link.

Evidence Integration

The currently available evidence is inadequate to assess whether PFHxS exposure may cause hematopoietic effects in humans. The evidence informing the potential for PFHxS exposure to cause hematopoietic effects is limited to hematology measures in three *high* confidence studies in rats, with exposure durations of 28–44 days, and which together are considered to provide *slight* evidence (see Table 3-37). Two of the three studies were consistent to some degree, demonstrating a pattern of changes in male rats. Specifically, male rats exposed to PFHxS at doses ranging from 0 to 10 mg/kg-day for 28–44 days exhibited decreases in multiple RBC parameters (i.e., Hct, Hb, and RBCs). However, there were inconsistencies, such as reported decreases in platelets counts in one

28-day study (3M, 2000a), which were not observed in a separate 28-day study with similar study design (NTP, 2018a). Prothrombin time was reported to increase in male rats as a result of PFHxS exposure in one study (Butenhoff et al., 2009) and decrease in male and female rats in another (3M, 2000a). Butenhoff et al. (2009) did not measure hematological parameters in female rats). There was unexplained inconsistency across studies. The two 28-day studies (NTP, 2018a; 3M, 2000a) reported opposite findings, despite similar study designs and rat strains (the Crl:CD Br rats used by 3M (2000a) are a Sprague Dawley strain). Specifically, NTP (2018a) did not observe consistent effects on these same parameters (i.e., Hct, Hb, RBCs, and platelets were unchanged; reticulocytes were decreased) in male animals exposed to doses of PFHxS ranging from 0.625 to 10 mg/kg-day. Thus, there is no clear explanation (e.g., study methods; doses; exposure duration; species, strain, or sex) for this inconsistency.

As noted above, the observations in male rats across RBC parameters and other measures reported in 3M (2000a) and Butenhoff et al. (2009) appear somewhat coherent. RBCs play an important role in hemostasis, as increased Hct has been shown to increase blood viscosity (reviewed in Litvinov and Weisel (2017)). Additionally, RBCs interact with platelets and modulate their reactivity through cell signaling molecules or through direct adhesive RBC-platelet interactions (reviewed in Litvinov and Weisel (2017)). Therefore, if RBC counts, along with Hb and Hct measures are decreased following PFHxS exposure, then it is reasonable that an increase in prothrombin time would be observed.

The observed effects in the study by Butenhoff et al. (2009) were dose dependent, with effects generally observed at or greater than 3 mg/kg-day, although some changes at lower doses were also noted. The duration dependence of these effects could not be determined; the 28-day study by 3M (2000a) that reported similar findings to those observed by Butenhoff et al. (2009) only tested 10 mg/kg-day and the PFHxS-related effects on RBC parameters were no longer observed at or after recovery day 14. Further the magnitude of effects across the various hematological endpoints measured (ranging from about 4% to 8%) is small and their biological significance is questionable. The animal evidence is considered *slight* due to the questionable biological significance and unexplained inconsistencies in the reported PFHxS effects on hematology among the available studies.

The currently available **evidence is inadequate to assess** whether PFHxS may cause adverse hematopoietic effects in humans given sufficient exposure conditions[26]. This conclusion is based on the three available animal studies that assessed PFHxS doses ranging from 0 to 10 mg/kg-day in male rats.

[26] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-37. Evidence profile table for PFHxS hematopoietic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Hematopoietic Human Studies Section)					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	**Inferences across evidence streams**
No informative studies (1 *uninformative*)	No informative studies identified			⊙⊙⊙ *Indeterminate*	⊕⊙⊙ **Evidence is inadequate** *Primary basis*: Despite coherent decreases in multiple RBC parameters in two studies in male rats, there were unexplained inconsistencies across studies and an unclear biological significance of effect magnitude for most endpoints *Human relevance*: Without evidence to the contrary, effects in rodent models are considered relevant to humans. *Cross-stream coherence*: NA; human evidence indeterminate *Susceptible Populations and lifestages*: NA
Evidence from in vivo animal studies (see Hematopoietic Animal Studies Section)					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
3 *high* confidence studies in rats	• All *high* confidence studies	• *Unexplained inconsistencies* across sexes and studies. • Unclear biological significance of effect magnitude for most endpoints (~4%–8%)	2 of the 3 studies reported male rats exposed for 28–44 d exhibited small decreases in multiple, coherent RBC parameters (i.e., Hct, Hb, and RBCs), as well as decreases in prothrombin time. However, these effects were observed in both sexes in one study, only males in a second study, and results were null in the third.	⊕⊙⊙ *Slight*	

3.2.8. Female Reproductive Effects

Human Studies

Studies of possible female reproductive effects of PFHxS are available for fecundity (i.e., time to pregnancy), reproductive hormones, pubertal development, gynecological conditions (endometriosis and polycystic ovary syndrome [PCOS]), ovarian reserve (including POI), menstrual cycle characteristics, and developmental measures (anogenital distance). While the evidence for each of these outcomes is synthesized separately, many of them are closely interconnected, with almost all of the outcomes having the potential to influence fecundity, as well as each other. For example, fecundity may be reduced by gynecological conditions and diminished ovarian reserve. Both of these may influence or be influenced by reproductive hormones levels, as are menstrual cycle characteristics, timing of pubertal development, and anogenital distance. The direction of association across these related outcomes is not always straightforward, which complicates considerations of coherence across outcomes. For example, low levels of anti-Mullerian hormone (discussed with ovarian reserve) may indicate difficulty getting pregnant (i.e., decreased fecundity) but high levels may be associated with PCOS, which may also decrease fecundity. In addition, preterm birth and spontaneous abortion could be driven by either female reproductive or developmental toxicity. These latter two outcomes are reviewed in the developmental section of this assessment but are also included in the consideration of coherence across outcomes for female reproductive effects.

In total, 35 epidemiology studies are available for these outcomes. The study evaluations are summarized below for each outcome or group of outcomes.

<u>Fecundity (time to pregnancy)</u>

Fecundity is the biological capacity to reproduce. Time to pregnancy, defined as the number of calendar months or menstrual cycles from the time of cessation of contraception to detection of pregnancy, is the primary outcome measure used to study fecundity. Many of the other outcomes described in this section contribute to fecundity. There are nine epidemiology studies that report on the association between PFHxS exposure and fecundity and related outcomes. A summary of the study evaluations is presented in Figure 3-77, and additional details can be obtained from HAWC. One study (Cariou et al., 2015) was considered *uninformative* due to lack of consideration of any potential confounders and excluded from further analysis. Of the remaining studies, two were preconception cohorts and considered *medium* confidence (Crawford et al., 2017; Vestergaard et al., 2012), and four were pregnancy cohorts and considered *low* confidence (Bach et al., 2018; Bach et al., 2015; Vélez et al., 2015; Jørgensen et al., 2014). The pregnancy cohorts were rated lower due to potential selection bias from excluding women who were unable to conceive. Two studies examined related outcomes in women undergoing treatment for infertility. Wang et al. (2021a) describes a cohort of women undergoing *in vitro* fertilization (IVF)-embryo transfer and reports rates of human chorionic gonadotropin (hCG) negativity following treatment; this study was rated *medium* confidence. Kim et al. (2020b) is a cross-sectional study of fertilization rate in women who underwent fully stimulated assisted reproductive treatment at an IVF clinic; this study was rated *low* confidence primarily due to concerns for residual confounding.

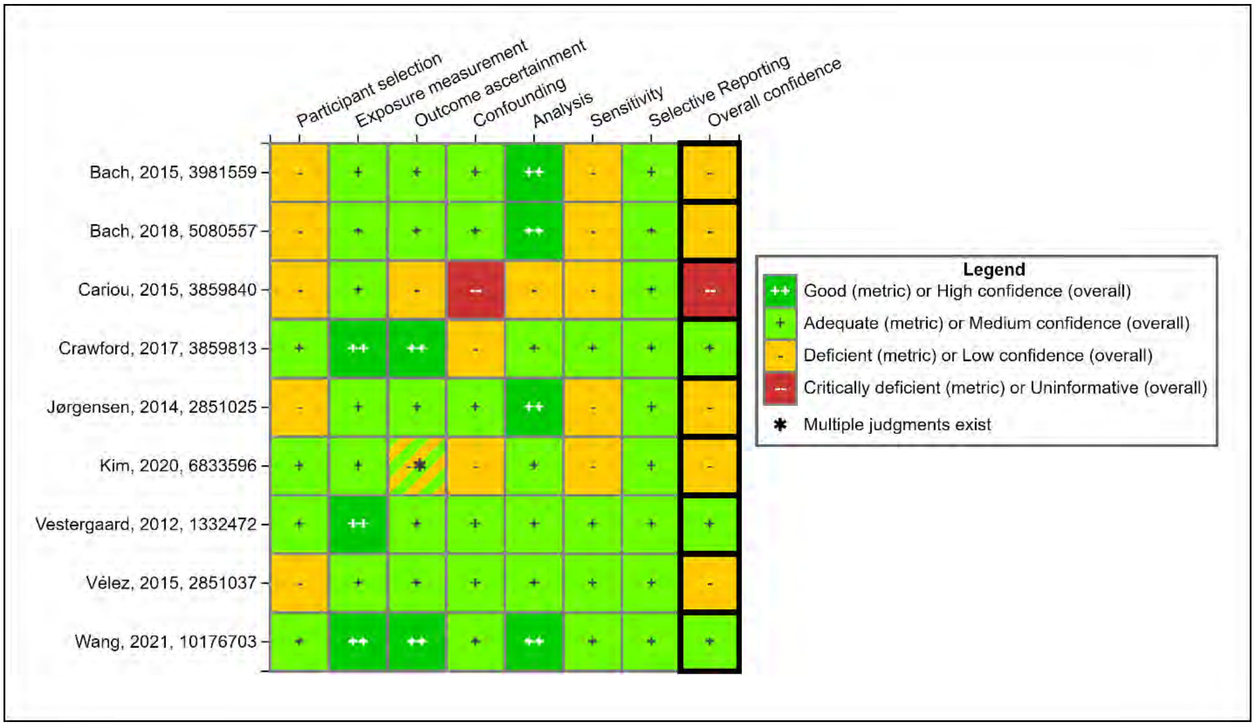

Figure 3-77. Summary of study evaluation for epidemiology studies of fecundity. For additional details see HAWC link.

The results for the association between PFHxS exposure and fecundity are presented in Table 3-38. A fecundability ratio less than 1 indicates a decrease in fecundity/increase in time to pregnancy. Of the seven studies, two *low* confidence studies (Bach et al., 2018; Vélez et al., 2015) reported a statistically significant decrease in fecundity/increase in time to pregnancy with increased exposure (only in parous women in Bach et al. (2018)). The remaining studies reported no decrease in fecundity. In addition to the time to pregnancy results, three studies (Bach et al., 2015; Vélez et al., 2015; Vestergaard et al., 2012) also analyzed infertility as an outcome. Only the *low* confidence study by Vélez et al. (2015) reported an increase in infertility with increased exposure (OR:1.27 (95% CI:1.09,1.48). Neither study of IVF outcomes (fertilization rate, hCG negativity) reported an association between PFHxS exposure and reduced fertility.

There is unexplained inconsistency in the evidence for this association. A decrease in fecundity with higher exposure was observed in two *low* confidence studies, but not the other four studies, which included the two *medium* confidence studies. The primary limitation in both Bach et al. (2018) and Vélez et al. (2015) was the potential for selection bias resulting from enrollment of participants during pregnancy. This approach would exclude women who were ultimately unable to conceive. If there is a true association between PFHxS and fecundity, this would be a bias against the most exposed women, which would likely result in an underestimate of the association. However, if there is no association, selection would not be related to exposure, so is unlikely to cause bias. Thus, the observed associations should not be dismissed as due to selection bias. On the other hand, as suggested by the authors, the lack of association in nulliparous women in Bach et al. (2018) suggests the possibility of confounding by factors related to previous pregnancies in the results of parous women, which could also exist in Vélez et al. (2015), where the population was only 29% nulliparous. Overall, there is considerable uncertainty in the strength of this inconsistently observed association.

Table 3-38. Summary of results for epidemiology studies of fecundity

Reference, confidence	Population	Exposure median (IQR)	Comparison for effect estimate	Fecundability ratio (95% CI)
Bach et al. (2015), low	Aarhus pregnancy cohort (2008–2013), Denmark; 1,372 nulliparous women	0.5 (0.4–0.6)	0.1 ng/mL increase	1.00 (0.99,1.01)
			Quartiles vs. Q1	Q2: 1.05 (0.89,1.24) Q3: 1.06 (0.89,1.25) Q4: 1.12 (0.94,1.32)
Bach et al. (2018), low	Danish National Birth Cohort sub-sample (1996–2002), Denmark Nulliparous women (n = 638)	0.9 (0.7–1.2)	Quartiles vs. Q1	Q2: 1.03 (0.81–1.32) Q3: 1.05 (0.83–1.35) Q4: 0.92 (0.72–1.18)
	Parous women (n = 613)			Q2: 0.74 (0.55–1.01) Q3: 0.79 (0.59–1.04) Q4: 0.60 (0.45–0.80)*
Vélez et al. (2015), low	MIREC pregnancy cohort (2008–2011), Canada; 1,625 women (29% nulliparous)	1	SD increase	0.91 (0.86,0.97)*
Vestergaard et al. (2012), medium	Preconception cohort (1992–1995), Denmark; 222 nulliparous women	1.2 (0.9–1.8)[a]	log-unit increase	1.33 (1.01,1.75)
			Above median vs. below	1.29 (0.90,1.83)
Crawford et al. (2017), medium	Time to Conceive cohort (2008–2009), U.S.; 99 women (40% nulliparous)	1.6 (GM)	dichotomous cutoff 75th percentile	Cycle-specific model 1.40 (0.79,2.49) d-specific model 0.96 (0.31,1.71)
Jørgensen et al. (2014), low	INUENDO pregnancy cohort (2002–2004), Greenland, Poland, Ukraine; 938 women	1.9	ln-unit increase	Pooled 0.97 (0.85,1.11)
	Greenland (n = 448, 31% nulliparous)	2.0	Tertiles vs. T1	T2: 1.05 (0.79,1.38) T3: 0.90 (0.68,1.19)
	Poland (n = 203, 92% nulliparous)	2.4		T2: 0.86 (0.57,1.30) T3: 0.94 (0.62,1.42)
	Ukraine (n = 287, 79% nulliparous)	1.6		T2: 0.85 (0.59,1.23) T3: 1.11 (0.78,1.58)

*$p < 0.05$.
[a]In participants with pregnancy.

Reproductive hormones in females

Reproductive hormones and related proteins examined in the evaluated studies include testosterone, estradiol, insulin like growth factor 1 (IGF-1), follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, as well as sex hormone-binding globulin (SHBG), all measured in blood, or in one study, saliva. Reproductive hormone levels are associated with all of the other female reproductive outcomes discussed in this section, but the relationships are often complex.

Key issues for the evaluation of studies of reproductive hormones were sample collection and processing. For testosterone, LH, FSH, and prolactin, due to diurnal variation, blood sample collection should occur at the same time of day for all participants, and if not, time of collection must be accounted for in the analysis. If there is no consideration of time of collection, the study is classified as deficient for outcome ascertainment and *low* confidence overall for these hormones as this is expected to result in nondifferential outcome misclassification. This applied to eight studies (Timmermann et al., 2022; Aycan, 2019; Elavarasi et al., 2019; Heffernan et al., 2018; Lopez-Espinosa et al., 2016; Lewis et al., 2015; Osterman et al., 2008; Martin, 1978). Lastly, the etiologic timing of PFHxS exposure relevant for influencing reproductive hormones is unclear and likely dependent on several factors, and thus all exposure windows with available data were considered, including cross-sectional since circulating hormone levels can be rapidly upregulated or downregulated in response to a change in exposure.

Fifteen studies (reported in 16 publications) examine potential associations between PFHxS exposure and reproductive hormones. One study was deemed *uninformative* due to multiple serious deficiencies in the participant selection, confounding, and analysis domains (McCoy et al., 2017). Most studies examined only testosterone and estradiol and measured exposure and outcome concurrently, though some studies measured additional hormones and/or measured exposure prospectively (prenatal exposure in Maisonet et al. (2015),Jensen et al. (2020b), and Timmermann et al. (2022), early pregnancy for outcomes in late pregnancy (Yang et al., 2022b), and pre-menopause in Harlow et al. (2021)). Eight studies (Timmermann et al., 2022; Yang et al., 2022b; Harlow et al., 2021; Wang et al., 2021b; Heffernan et al., 2018; Zhang et al., 2018b; Barrett et al., 2015; Lewis et al., 2015) examined associations in adults, three studies (Zhou et al., 2016; Lewis et al., 2015; Maisonet et al., 2015) in adolescents, one study (Lopez-Espinosa et al., 2016) in children, and three studies (Jensen et al., 2020b; Liu et al., 2020b; Yao et al., 2019) in infants. The study evaluations are summarized in Figure 3-78. Six studies were considered *medium* confidence and seven were *low* confidence. However, of the *medium* confidence studies, two did not consider time of day of sample collection for hormones and were thus *low* confidence for testosterone (Yao et al., 2019; Lopez-Espinosa et al., 2016). Notably, two studies (Heffernan et al., 2018; Zhang et al., 2018b) included participants with gynecological conditions (polycystic ovarian syndrome [PCOS] and premature ovarian insufficiency (POI), respectively). These conditions are associated with changes in reproductive hormone levels, and thus stratified results were used. These studies may also be affected by reverse causality, as menstrual cyclicity is associated with both hormone levels and these conditions, and menstrual cycle length/regularity may influence PFAS excretion (discussed further below, see Menstrual cycle characteristics below).

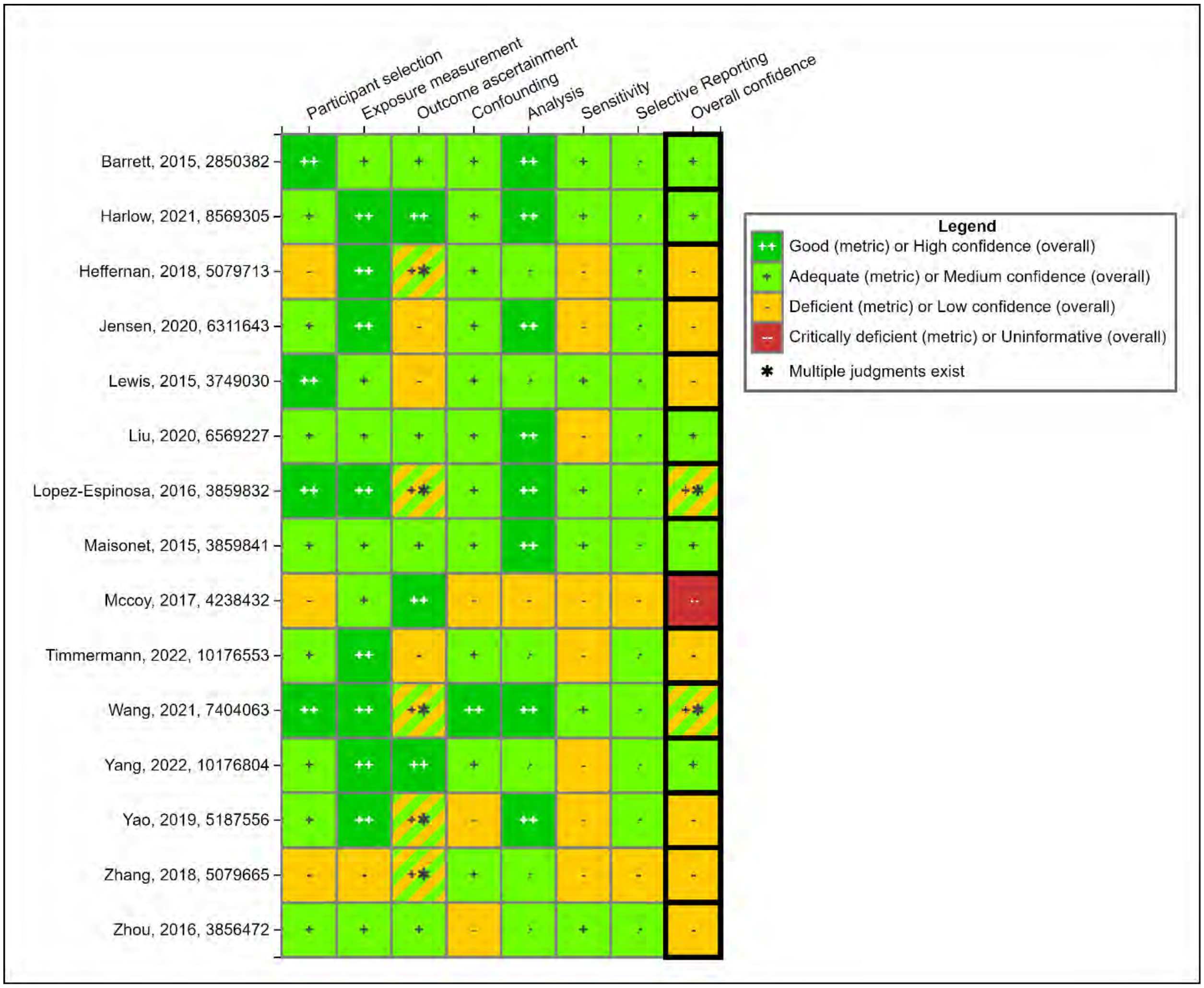

Figure 3-78. Summary of study evaluations for epidemiology studies of female reproductive hormones. For additional details see HAWC link. Multiple publications of the same study: **Yao et al. (2019)** also includes **Yao et al. (2021)**.

Estradiol

Nine studies examined estradiol levels in association with PFHxS. In six studies of adults, one *low* confidence study reported lower estradiol with higher exposure in women with premature ovarian insufficiency (POI) (β: −0.19 (95% CI: −0.37, −0.02)) but no change in women without POI (Zhang et al., 2018b). Conversely, one *low* confidence study reported higher estradiol with higher exposure in adult women without PCOS (β: 223, SE 255), although this was not statistically significant, and no change was observed in women with PCOS (Heffernan et al., 2018). In both of these studies, the results in controls (without POI or PCOS) are more straightforward to interpret since the presence of these conditions may influence hormones levels and as discussed below, PFAS levels. The remaining studies of adults, all *medium* confidence, including one in healthy non-pregnant women (Barrett et al., 2015), one in pregnant women (Yang et al., 2022b), one in premenopausal (or transitioning to menopause) women (Harlow et al., 2021), and one in postmenopausal women (Wang et al., 2021b), reported no association. In younger populations, a single *low* confidence study of adolescents reported no association (Zhou et al., 2016), while a single *low* confidence study of children (Lopez-Espinosa et al., 2016) reported higher ln-estradiol levels with higher PFHxS (2.1% difference (95% CI: −2.2, 6.5)). Lastly, in one *medium* confidence study of infants (Yao et al., 2019), there was higher estradiol with higher PFHxS (β: 0.30 (95% CI: 0.27, 0.37)). Overall, there are three studies reporting higher estradiol (one statistically significant) in at least one subpopulation, one study reporting lower estradiol, and five studies reporting no association with PFHxS exposure. There was no apparent pattern of association by study confidence or study sensitivity ratings/exposure levels and contrast, and thus these inconsistent results are difficult to interpret.

Testosterone

As described above, most studies were *low* confidence for testosterone. In adult women, there were five studies available, all *low* confidence except Harlow et al. (2021). Two of these reported nonstatistically significant inverse associations between testosterone and PFHxS exposure. Lewis et al. (2015) reported results stratified by age group and observed stronger associations in lower ages (β (95% CI) for 20–<40: −3.3 (−8.7, 2.5), 40–<60: −2.4 (−8.7, 4.3), 60–80: −0.2 (−8.3, 8.7). Zhang et al. (2018b), also reported an inverse association in controls without POI (β −0.11, 95% CI: −0.27, 0.05). In contrast, Heffernan et al. (2018) reported a statistically significant positive association in controls without PCOS (β 0.50, SE 0.17). Studies in pre- and post-menopausal women reported no association (Harlow et al., 2021; Wang et al., 2021b). In adolescents, three studies were available. Maisonet et al. (2015), a *medium* confidence study, reported higher testosterone levels in 15-year-old girls with the increasing tertiles of PFHxS exposure, although there was no apparent exposure-response gradient across the narrow tertiles (1.3–1.9 ng/mL (β: 0.18 (95% CI: 0.00,0.37), and >1.9ng/mL (β: 0.18 (95% CI: 0.00, 0.35) compared with ≤1.2ng/mL PFHxS). Lewis et al. (2015) reported an inverse association (β −5.3, 95% CI: −11.6, 1.5) (with median exposure of 0.8 ng/mL) while Zhou et al. (2016) reported no association (with mean PFHxS exposure of 1.2 ng/mL). One *low* confidence study in children reported no association with testosterone (Lopez-Espinosa et al., 2016) with median exposure of 7 ng/mL, and one *low* confidence study in infants (Yao et al., 2019) reported an inverse association (β = −0.16 (95% CI: −0.36, 0.04) with median exposure of 0.3 ng/mL.

Overall, there are three of ten studies reporting inverse associations between testosterone and PFHxS exposure, including two of five studies in adults, one of three studies in adolescents, zero of one study in children, and one of one study in infants. In addition, one study in adults reported a positive association. There was no apparent pattern of association by exposure levels. The study with the highest exposure levels and greatest contrast (Lopez-Espinosa et al., 2016) reported no association, while inverse associations were observed in studies with narrow contrast (Yao et al., 2019; Zhang et al., 2018b), although not statistically significant.

Other hormones and related molecules

For other hormones and related molecules, Lopez-Espinosa et al. (2016) examined associations between PFHxS and IGF-1, reporting inverse, although nonmonotonic in categorical analyses, associations. Sex hormone-binding globulin (SHBG) was not associated with PFHxS levels in four studies (Harlow et al., 2021; Wang et al., 2021b; Heffernan et al., 2018; Maisonet et al., 2015). Barrett et al. (2015) observed no evidence of association with luteal phase progesterone in saliva in normally cycling women, while in infants, Liu et al. (2020b) reported a small but not statistically significant positive association (2.8% increase) with progesterone. Zhang et al. (2018b) reported positive associations with FSH (β 0.16, 95% CI: 0.04, 0.28) and prolactin (β 0.11, 95% CI: −0.01, 0.22) in women with premature ovarian sufficiency, but no association in controls, while Harlow et al. (2021) reported an inverse association with FSH only in nulliparous women (-4.62, 95% CI; -8.60, -0.47). In Jensen et al. (2020b), there were positive associations ($p > 0.05$) with LH, androstenedione, and DHEAS in infant girls. Lastly, Timmermann et al. (2022) reported a statistically non-significant inverse association with prolactin in pregnant women at gestational week 10 (3.1% decrease) but no difference at gestational week 28.

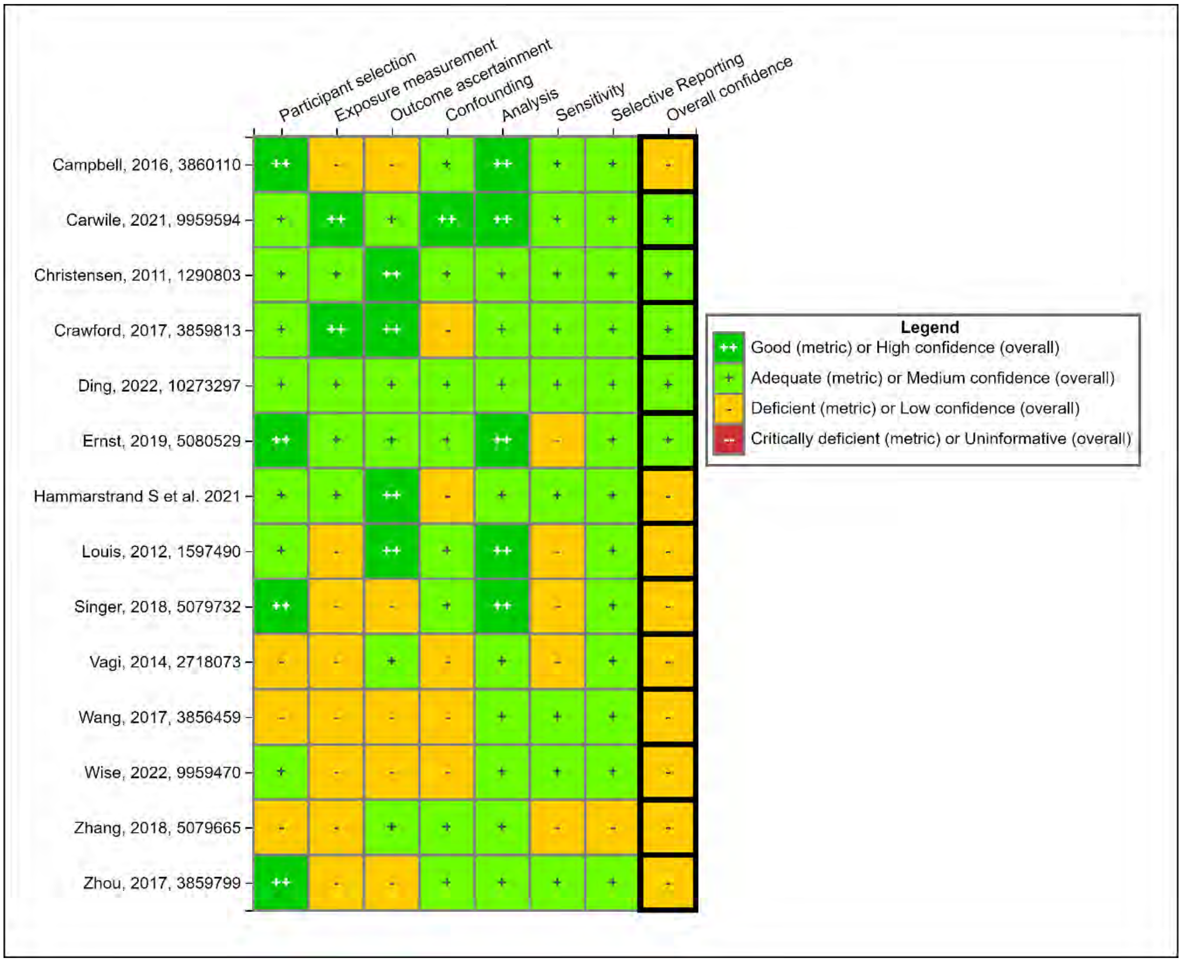

Figure 3-79. Summary of study evaluation for epidemiology studies of other female reproductive effects (menstrual cycle characteristics, gynecological conditions, ovarian reserve, and pubertal development). For additional details see HAWC link.

Menstrual cycle characteristics

Three epidemiology studies report on the association between PFHxS exposure and menstrual cycle characteristics. One was a pregnancy cohort in Norway (Singer et al., 2018), one was a cross-sectional study of participants in a preconception cohort in China (Zhou et al., 2017a), and one was a cross-sectional study of reproductive aged Black women in the U.S. (Wise et al., 2022). For this outcome, there is potential for reverse causation because menstruation is one of the mechanisms by which PFAS are removed from the body. It is expected that a longer cycle would result in less clearance of PFAS, and therefore higher PFAS in the body, possibly resulting in inflated effect estimates. Thus, all three studies were considered *low* confidence (see Figure 3-58). There were also concerns for potential outcome misclassification due to self-report, since the questionnaires used were not validated. Zhou et al. (2017a) reported an increase in odds of irregular and long cycle (OR (95% CI) for continuous exposure = 1.80 (1.17,2.77) and 1.73

(1.13,2.65), respectively), and a decrease in the odds of menorrhagia (OR = 0.14 (0.06,0.36). Singer et al. (2018) also reported higher PFHxS levels in participants with irregular (4% change, 95% CI: −3, 11) and long cycles (5% change, 95% CI; −4, 14), although neither was statistically significant. Wise et al. (2022) reported lower intensity of menstrual bleed with higher exposure, but no difference in bleed length in days. These associations with irregular and long cycles in two studies and lower bleeding in one study is consistent with either a true association or reverse causation due to less PFAS excretion through menstruation compared to women with regular cycles, and it is difficult to interpret with currently available evidence.

Gynecological conditions

Four epidemiology studies report on the association between PFHxS exposure and endometriosis. Three of the studies were cross-sectional, which decreases confidence for this chronic outcome due to the inability to establish temporality (Buck Louis et al., 2018; Wang et al., 2017; Campbell et al., 2016). There is potential for reverse causality as described above since endometriosis can influence the menstrual cycle, and this could be toward a protective direction given that endometriosis can be associated with heavier and more frequent bleeding. Because of this issue, these studies were classified *low* confidence, although the study by Buck Louis et al. (2018) is considered stronger in other study design aspects than the remaining two studies; this study included two groups of women, one group scheduled for surgery (laparoscopy or laparotomy), and one group identified through a population database who underwent pelvic MRI to identify endometriosis (Buck Louis et al., 2018) (see Figure 3-79). The remaining two studies were deficient for outcome ascertainment, specifically due to self-report of endometriosis diagnosis (Campbell et al., 2016) and case definition including only endometriosis-related infertility among surgically confirmed cases (Wang et al., 2017). Both of these methods are likely to include asymptomatic cases among the controls. In addition, one study that reported results only on a mixture of PFAS was determined to meet the PECO criteria due to very high exposure to PFHxS in participants. Hammarstrand et al. (2021) examines a population in Ronneby, Sweden with high PFAS contamination in drinking water. This study estimated exposure using residence location linked to data on the municipal water supply (validated against serum measurements in a subsample) and was thus not able to develop individual PFAS estimates. PFHxS and PFOS were predominant in this population (subsample mean serum levels in participants living in the area at the time of high contamination were 243 and 279, respectively, compared to 15 for PFOA), so any effect observed can likely be largely attributed to those PFAS, but it is not possible to separate their effects, and thus the study is considered *low* confidence.

Two of the *low* confidence studies, including the Buck Louis et al. (2018) study, reported slightly increased odds of endometriosis with higher exposure, although the estimates were imprecise (Buck Louis et al. (2018): operative sample OR: 1.14 (95% CI: 0.58,2.24); population sample OR: 1.52 (95% CI: 0.40,5.80); Campbell et al. (2016) OR (95%) versus T1: T2: 0.66

(0.37,1.19), T3: 0.47 (0.25,0.87)). Hammarstrand et al. (2021) found no association with endometriosis despite the very high exposure to PFHxS and PFOS.

In addition, two studies examined PCOS and PFHxS exposure, including the study in Ronneby, Sweden (Hammarstrand et al., 2021) described above and a case-control study in the U.S. (Vagi et al., 2014). Vagi et al. (2014) suffers from potential for reverse causality due to association with menstruation, similar to the studies of endometriosis. Because PCOS is associated with irregular menstruation and thus less frequent bleeding, it is possible that effect estimates will be inflated. This study is *low* confidence for this reason and concerns with participant selection and confounding. There was no association between PFHxS and PCOS, but due to the study limitations, this is difficult to interpret. Hammarstrand et al. (2021) reported higher odds of PCOS in participants with the highest exposure (HR: 2.18, 95% CI: 1.43, 3.34), but this is also difficult to interpret due to the co-exposure with PFOS.

Ovarian reserve

Three studies examined the association between PFHxS exposure and ovarian reserve, an indication of a woman's egg count or remaining reproductive potential. The available studies were two *medium* confidence studies, a cohort (Crawford et al., 2017) and a nested case-control study (Donley et al., 2019), examining anti-Mullerian hormone (AMH), and a *low* confidence case-control study examining POI (Zhang et al., 2018b). AMH is commonly used as an endocrine marker for age-related decline of ovarian reserve in healthy women, with reduced AMH an indication of small primordial follicle pool, as well as predicting poor oocyte yield for in vitro fertilization (Grynnerup et al., 2012). However, a single measurement in healthy women may not be informative in predicting fecundity (ACOG, 2019) and, as mentioned above, elevated levels of AMH are associated with PCOS, so these results should be interpreted with caution. In contrast to AMH, POI is a more specific outcome (defined as an elevated FSH level greater than 25 IU/L on two occasions more than four weeks apart and oligo/amenorrhea for at least four months in Zhang et al. (2018b), but because this definition is closely tied to menstruation, there are concerns for reverse causality as with the previous outcomes, which would be expected to be biased away from the null. In Zhang et al. (2018b), there were higher odds of POI with higher exposure, with an exposure-response gradient across tertiles (OR (95% CI) versus tertile 1: T2: 2.04 (1.03, 4.04), T3: 6.63 (3.22, 13.65)). In Crawford et al. (2017), there was an inverse association between AMH and PFHxS, consistent with decreased ovarian reserve, although this was not statistically significant (β: −0.12, $p = 0.4$). No association was observed with AMH in Donley et al. (2019), despite similar exposure contrast (median 1.6 ng/mL) in the two AMH studies and lower exposure levels in Zhang et al. (2018b). The results of Zhang et al. (2018b) and Crawford et al. (2017) are coherent with each other as well as with the positive association with FSH observed in women with POI in Zhang et al. (2018b), although no association was observed in control women without POI (discussed with reproductive hormones). Overall, due to the study limitations and small number of studies, there is still considerable uncertainty.

Pubertal development

Three *medium* confidence studies, including birth cohorts in Denmark (Ernst et al., 2019) and the U.S. (Carwile et al., 2021) and a case-control study nested in a birth cohort in the United Kingdom (Christensen et al., 2011), and *low* confidence cross-sectional study in the U.S. (Wise et al., 2022) examined timing of pubertal development with prenatal PFHxS exposure. Ernst et al. (2019) and Carwile et al. (2021) reported results for several pubertal outcome measures, while Christensen et al. (2011) and Wise et al. (2022) focused on age at menarche. In Ernst et al. (2019), with median exposure of 1.1 ng/mL (10th–90th percentile: 0.6–1.7), the participants in the third tertile of exposure had earlier age of breast development, axillary hair, and menarche, although none were statistically significant. Looking at a combined puberty indicator outcome, there was lower age at puberty in the third tertile (age difference −2.22 months; 95% CI: −8.37, 3.93). Carwile et al. (2021), with median exposure of 1.9 ng/mL, reported no association with pubertal development score or peak height velocity (i.e., the age at which a child experiences the largest increase in height, a proxy for pubertal timing). In Christensen et al. (2011), with median exposure of 1.5 ng/mL (IQR 0.5–0.8), there was not a clear association, as there were higher odds of earlier age at menarche when PFHxS was analyzed as dichotomous based on above/below the median (OR 1.11; 95% CI: 0.76, 1.64) but lower odds when analyzed as continuous (OR 0.89; 95% CI: 0.65, 1.22), neither statistically significant. Lastly, the *low* confidence study found no association with age at menarch (Wise et al., 2022). Overall, there is considerable uncertainty for this outcome given the inconsistency in three *medium* confidence studies and imprecision of the effect estimates.

Menopause

One *medium* confidence study, a cohort of midlife women in the U.S., examined timing of menopause (Ding et al., 2022). The effect estimate is in the direction of earlier onset of natural menopause, though not statistically significant. (relative survival: 0.90, 95% CI: 0.76, 1.05 for total effect (including author-proposed mediation by FSH)).

Animal Studies

The database of animal toxicity studies for PFHxS-induced female reproductive effects consists of five oral exposure studies that include two short-term studies in Harlan Sprague Dawley or Crl:CD BR rats exposed for 28 days (NTP, 2018a; 3M, 2000b), two reproductive/developmental toxicity studies in Crl:CD (SD) rats or Crl:CD1 (ICR) mice with exposures starting during premating through postnatal days (PND) 22–35 (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003) and a developmental toxicity study in Wistar rats with exposure during gestion and lactation (gestational days [GD] 7 to PND 22) (Ramhøj et al., 2018). The studies evaluated several endpoints relevant to the assessment of female reproductive toxicity, namely mating and fertility, estrous cycle, hormone levels, histopathology, organ weight and markers of sexual differentiation and maturation (U.S. EPA, 1996). Other developmental outcomes reported in the Ramhøj et al. (2018) study are described in the synthesis of developmental effects (see Section 3.2.3).

Mating and fertility

Mating and fertility measures (i.e., fertility index, mating index and pre-coital interval) were evaluated across two *high* confidence studies with no outstanding issues regarding risk of bias or sensitivity (see Figure 3-80). The studies exposed F0 female SD rats or CD-1 mice to doses ranging from 3 to 10 mg/kg-day during premating, gestation, and lactation (PND 22) (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). No treatment-related effects were noted in mating and fertility indices, including length of pre-coital interval in female parental animals.

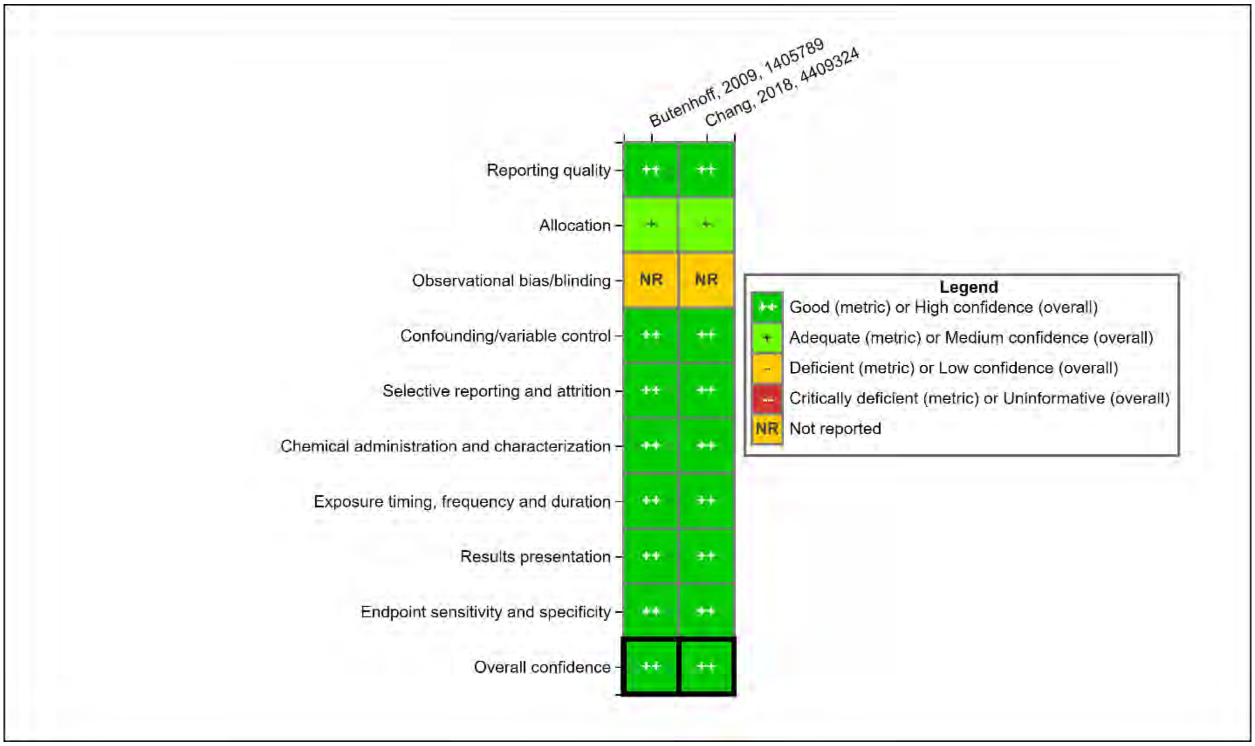

Figure 3-80. PFHxS mating and fertility animal study evaluation heatmap. For additional details see HAWC link.

Estrous cycle characteristics

Effects on the estrous cycle were measured in four studies: a short-term study in rats exposed for 28 days (NTP, 2018a) and two reproductive-developmental toxicity studies in F0 rats or mice exposed during premating, gestation, and lactation (PND 22) (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003), and one sub-chronic study that exposed ICR mice for 42 days (Yin et al., 2021) (see Figure 3-81). Two of the studies were considered *high* confidence (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003) and two were considered *medium* confidence because of uncertainties surrounding presentation of results and selection of animals for outcome assessment (Yin et al., 2021; Chang et al., 2018) (see Figure 3-81). Yin et al. (2021) reported decreased increased estrous cycle duration in treated animals, but the remaining studies which evaluated this outcome report that PFHxS exposure had no effects in the number of cycles, cycle length, or time in each estrous stage (proestrus, estrus, metestrus, and diestrus) of female rats or mice exposed to doses of 0.3–50 mg/kg-day and 0.3–3 mg/kg-day, respectively (Chang et al., 2018; NTP, 2018a; Butenhoff et al., 2009; 3M, 2003).

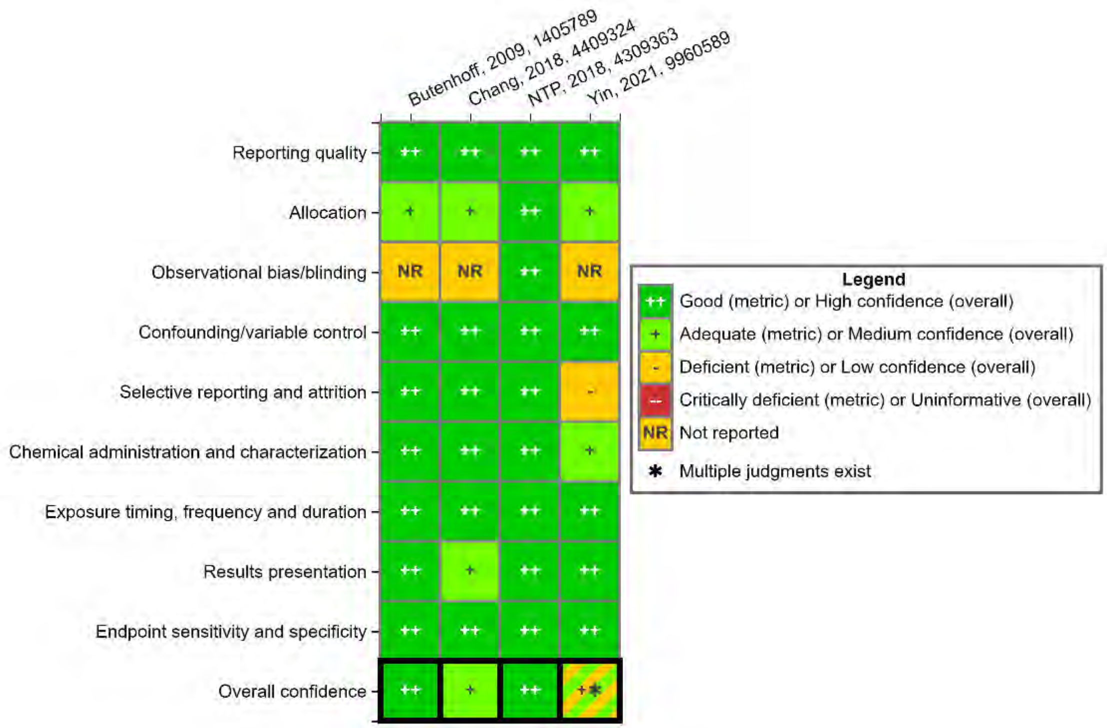

Figure 3-81. PFHxS estrous cycle animal study evaluation heatmap. For additional details see HAWC link.

Hormone levels

The available studies have measured reproductive hormones including testosterone, follicle stimulating hormone (FSH), Luteinizing hormone (LH), and estrogen. Serum testosterone levels were measured in female rats in a single short-term *high* confidence study with no notable concerns in any of the study evaluation domains (NTP, 2018a) (see Figure 3-82). Female rats were exposed to 0, 3.12, 6.25, 12.5, 25, and 50 mg/kg-day PFHxS for 28 days. Serum testosterone levels were slightly increased in PFHxS-exposed rats at all doses (9%–29% compared with controls) but the changes were not statistically significant compared with controls and did not display a dose-response gradient. A *medium* confidence study using ICR mice reported that exposure to 5 mg/kg-day PFHxS decreased serum FSH, LH, and estrogen (Yin et al., 2021). These observations suggest that PFHxS exposure may alter reproductive hormones in exposed female animals, however several issues were identified with the Yin et al. (2021) study including lack of randomization and selective reporting. Therefore, additional studies are needed.

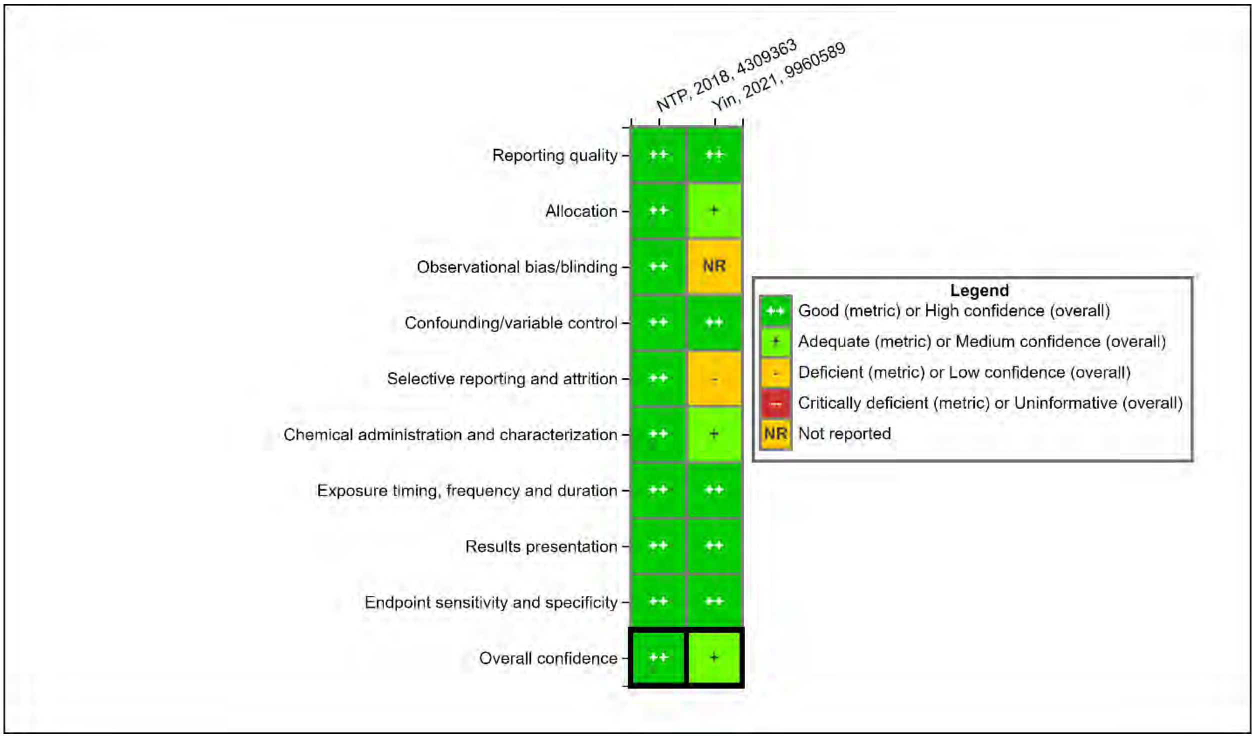

Figure 3-82. PFHxS hormone levels animal study evaluation heatmap. For additional details see HAWC link.

Histopathology

Histopathology of female reproductive organs including the ovary, uterus, vagina, and clitoral and mammary glands were examined across four studies. Two short-term studies in rats exposed for 28 days (NTP, 2018a; 3M, 2000b) and two reproductive-developmental toxicity studies in F0 rats or mice exposed from 14 days of premating to PND 22 (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). Three of the studies were considered *high* confidence (NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000b) and one was rated as *medium* confidence due to deficiencies in the presentation of histopathological findings (data were only reported qualitatively) (Chang et al., 2018) (see Figure 3-83).

Bilateral dilation of the uterus (minimal to mild severity) was reported in rats in the control (1/10 rats) and PFHxS exposure groups (1/1, 1/1, 3/3, and 1/10 rats at 3.12, 6.25, 12.5, and 50 mg/kg-day, respectively) in the NTP (2018a) study. Although lesions were observed in 100% of the animals evaluated in the 12.5 mg/kg-day dose group, the incidence rates were identical for the control and high dose groups (10%) and a limited number of animals were examined in the other exposure groups; therefore, the biological interpretation of these findings is unclear. Butenhoff et al. (2009) and 3M (2003) also observed uterine lesions in rats (mild-moderate distention and microphage infiltration of mostly moderate severity) but the incidence rates were not significantly different between control and PFHxS exposure (10 mg/kg-day). Two medium *confidence* mouse studies report conflicting evidence. Chang et al. (2018) reported no lesions in the uterus of CD-1 mice exposed to 10 mg/kg-day PFHxS for 42 days (Chang et al., 2018). However, a similar study also using CD-1 mice exposed to 5 mg/kg-day PFHxS for 42 days reported decreased number of secondary follicles and corpora lutea, but no effect on primordial or primary follicles (Yin et al., 2021). A single case of minimal focal necrosis was reported in the mammary gland of rats (1/10) at a dose of 10 mg/kg-day (Butenhoff et al., 2009; 3M, 2003) but no lesions were observed in the mammary gland of rats exposed to doses ranging from 3.12–50 mg/kg-day in a different study (NTP, 2018a). Histological examination of the ovaries (including primordial follicle counts), clitoral gland and vagina showed no treatment-related effects in exposed rats or mice (Chang et al., 2018; NTP, 2018a; Butenhoff et al., 2009; 3M, 2003, 2000b).

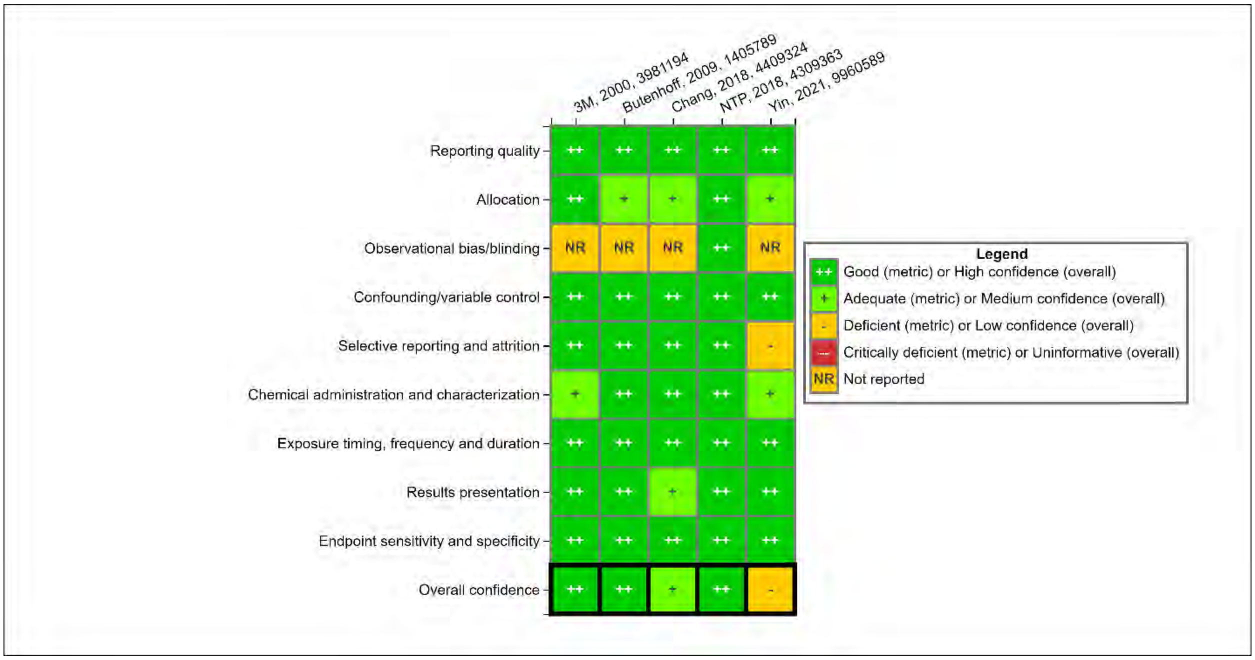

Figure 3-83. PFHxS female reproductive histopathology animal study evaluation heatmap. For additional details see HAWC link.

Organ weight

There are six available animal toxicity studies that evaluated effects on reproductive organ weights in females (i.e., ovary and uterus). One study exposed CD-1 mice for 42 days (Yin et al., 2021), two studies exposed SD rats for 28 days (NTP, 2018a; 3M, 2000b) and three reproductive-developmental toxicity studies examining effects in F0 rats and mice exposed during premating and/or gestation and lactation (PND 22) (Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003) and in F1 mice exposed in utero, via lactation and directly from PND 22 to PND 35 (Chang et al., 2018). Overall study confidence was *medium* in the Chang et al. (2018) study due to incomplete reporting of organ weight data (quantitative results were not provided) (see Figure 3-84). The study by Yin et al. (2021) was also considered *medium* confidence due to concerns related to animal selection for outcome assessment. There were no major concerns with respect to risk of bias or sensitivity in the other studies deemed as *high* confidence (NTP, 2018a; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003, 2000b). Yin et al. (2021) reported decreased absolute (but not relative) ovary weight in animals exposed to 50 mg/kg-day for 42 days. However, in all other available studies PFHxS exposure did not significantly impact ovarian and uterine weights (both absolute and relative) in animals at doses ranging from 0.05–50 mg/kg-day in any of the studies (Chang et al., 2018; NTP, 2018a; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003, 2000b).

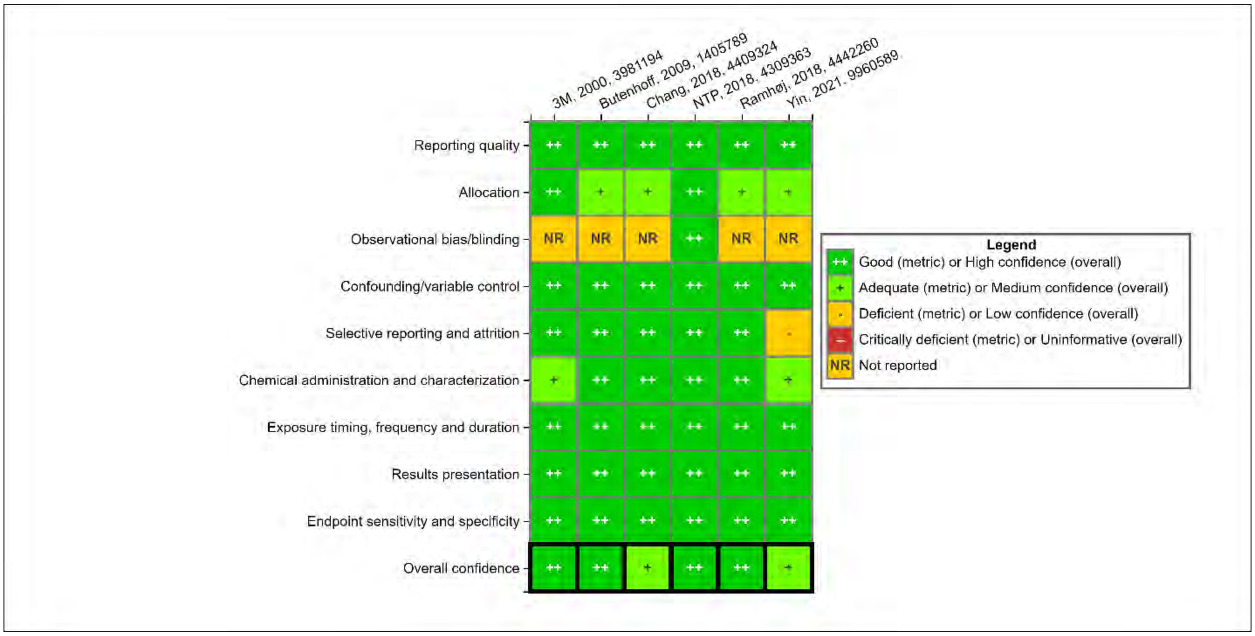

Figure 3-84. PFHxS female reproductive organ weight animal study evaluation heatmap. For additional details see HAWC link.

Landmarks of female reproductive system development and maturation

Markers of sexual differentiation and maturation, namely anogenital distance (AGD)[27] and onset of puberty (vaginal patency), were evaluated in F1 offspring in two reproductive-developmental toxicity studies of *medium* confidence in rats exposed during gestion to PND 22 (Ramhøj et al., 2018) or in mice exposed in utero, via lactation and directly from PND 22 to PND 35 (Chang et al., 2018). Key issues related to animal allocation and presentation of results for AGD (no adjustment for body weight [28]) reduced confidence in one study (Ramhøj et al., 2018) (see Figure 3-85). Ambiguity surrounding the reporting of sample size raised potential concerns in the second study (Chang et al., 2018).

[27]AGD is a phenotypical marker of androgen levels during gestational development (Thankamony et al., 2016). Increased AGD in considered indicative of an adverse response in the developing female reproductive system (U.S. EPA, 1996).
[28]Relative AGD adjusted to the cube root of body weight is the preferred measurement for this endpoint (Daston and Kimmel, 1998).

Statistically significant reductions in relative AGD (adjusted to body weight) evaluated on PND 1 were noted in F1 mice exposed to 1 mg/kg-day (5% compared with controls) but the effects were not seen at other dose levels (0.3 and 3 mg/kg-day) (Chang et al., 2018). Furthermore, absolute AGD was unaffected by treatment in F1 mice or rats up to doses of 45 mg/kg-day (Chang et al., 2018; Ramhøj et al., 2018). Similarly, PFHxS had no effect on the onset of puberty (vaginal patency) in F1 mice exposed to doses of 0.3–3 mg/kg-day.

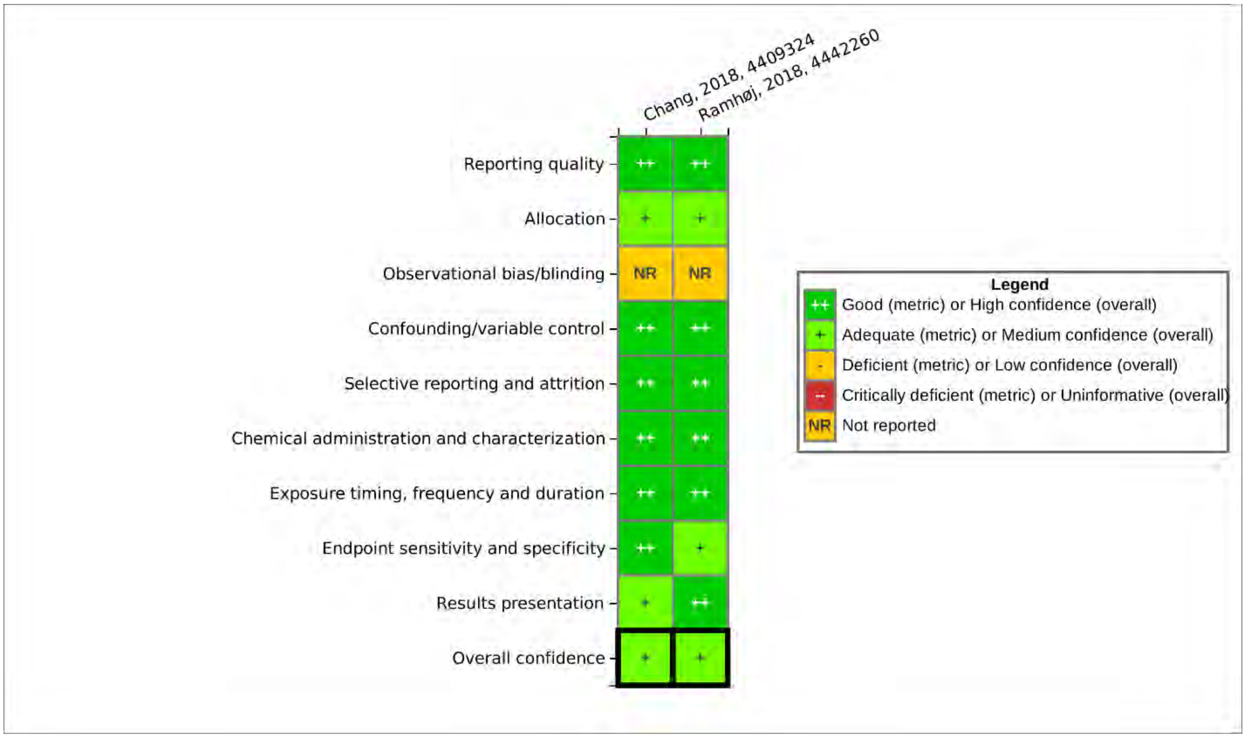

Figure 3-85. PFHxS female reproductive sexual differentiation and maturation animal study evaluation heatmap. For additional details see HAWC link.

Evidence Integration

The available studies provide **inadequate evidence** to determine whether PFHxS exposure has the potential to affect female reproduction in humans. This conclusion is based on studies in both humans and animals (see Table 3-39).

The available evidence on PFHxS-induced female reproductive effects in human studies is considered *indeterminate*. Outcomes evaluated in human studies include fecundity, reproductive hormones, pubertal development, menstrual cycle characteristics, gynecological conditions, and ovarian reserve. Associations were observed with many of these outcomes in some studies, but there was considerable inconsistency across studies within outcomes and uncertainty due to considerations such as reverse causality and confounding (e.g., parity for fecundity) that reduced study confidence. Looking across outcomes, there is some coherence. The observed increase in estradiol and FSH and decrease in testosterone in some studies (one study for FSH) is coherent with risk factors for endometriosis, which in turn is coherent with reduced ovarian reserve and fecundity. Similarly, the decrease in anogenital distance in one study of newborn girls (see Developmental Effects Section) is coherent with the decrease is testosterone levels in some of the studies, including the single study in infants. These connections between the outcomes increase the strength of the evidence, but because of the limitations described above, there is too much uncertainty in the association to draw a stronger judgment than *indeterminate*.

The available animal evidence on PFHxS-induced female reproductive effects is also considered *indeterminate*. One medium confidence, study using mice reported PFHxS-induced alterations in estrus cycle, histopathology, ovary weight, and reproductive hormone levels (Yin et al., 2021). In all other *medium* and *high* confidence studies there were no clear exposure-related effects were observed in reproductive organ weights, estrous cycle characteristics, histopathology, reproductive hormones levels, and functional measures of mating and fertility. In addition to the inconsistencies between the Yin, 2021, 9960589@@author-year and the other available studies there are no subchronic or chronic exposure studies available, which also limits the interpretation of the current findings.

Table 3-39. Evidence profile table for PFHxS exposure and female reproductive effects

<table>
<tr><th colspan="5">Evidence stream summary and interpretation</th><th>Evidence integration summary judgment</th></tr>
<tr><td colspan="5">Evidence from studies of exposed humans</td><td rowspan="7">⊙⊙⊙
Evidence inadequate

Primary Basis:
Evidence is inconsistent across studies or largely null.

Human relevance: Without evidence to the contrary, effects in rodent models are considered relevant to humans.

Cross-stream coherence: N/A, evidence indeterminate for both human and animal studies.

Susceptible populations and lifestages:
None identified.</td></tr>
<tr><th>Studies and confidence</th><th>Factors that increase certainty</th><th>Factors that decrease certainty</th><th>Summary and key findings</th><th>Evidence stream judgment</th></tr>
<tr><td>Fecundity
3 medium and 5 low confidence studies</td><td>• No factors noted</td><td>• Unexplained inconsistency
• High risk of bias</td><td>Decreased fecundity/longer time to pregnancy in 2 low confidence studies, but no effect in medium confidence studies.</td><td rowspan="5">⊙⊙⊙
Indeterminate

Associations between exposure and female reproductive outcomes observed in studies of multiple outcomes. Inconsistency across studies and concerns for reverse causality and other bias hinder interpretation.</td></tr>
<tr><td>Reproductive hormones
7 medium and 7 low confidence studies</td><td>• No factors noted</td><td>• Unexplained inconsistency
• High risk of bias – Most testosterone studies were low confidence</td><td>3 of 9 studies report higher estradiol. 3 of 9 studies report lower testosterone.</td></tr>
<tr><td>Pubertal development
3 medium and 2 low confidence studies</td><td>• No factors noted</td><td>• Unexplained inconsistency</td><td>Earlier age of puberty (not statistically significant) in one study, but no clear association in other studies</td></tr>
<tr><td>Menstrual cycle
3 low confidence studies</td><td>• Consistency</td><td>• Low confidence studies-potential reverse causality</td><td>Higher odds of irregular and long cycle in 2 studies, lower odds of menorrhagia in 1 study, and less intense bleeding in one study</td></tr>
<tr><td>Gynecological conditions
5 low confidence studies</td><td>• No factors noted</td><td>• Unexplained inconsistency
• All low confidence studies-potential reverse causality
• Imprecision of effect estimate</td><td>Higher odds of endometriosis in 2 of 4 studies. Lower odds of endometriosis-related infertility in one study. 1 of 2 studies reported higher likelihood of PCOS, but there is potential for confounding by PFOS.</td></tr>
</table>

Evidence stream summary and interpretation					Evidence integration summary judgment
Ovarian reserve 2 *medium* and 1 *low* confidence studies	• *Coherence* in associations between POI and AMH in one study	• Potential for reverse causality • *Unexplained inconsistency* across studies of AMH	Higher odds of premature ovarian insufficiency (POI) and lower levels of anti-Mullerian hormones (AMH) (in 1/2 studies)		
Evidence from in vivo animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Mating and fertility 2 *high* confidence studies in adult rats and mice: • 14-d (×2)	• No factors noted	• No factors noted	No observed effects on mating or fertility index	⊙⊙⊙ *Indeterminate* [Note: although no notable findings, no long-term studies were available]	
Estrous cycle 2 *high* confidence studies in adult rats: • 28-d • 14-d premating to PND22 2 *medium* confidence study in adult mice: • 14-d premating to PND22 • 42-d		• Unexplained inconsistency across studies	Altered cycle duration reported in one medium confidence study		
Hormone levels 1 high confidence study in adult rats • 28-d		• Lack of expected dose response	Slight increase in testosterone, decreased estrogen, LH, and FSH		

Evidence stream summary and interpretation					Evidence integration summary judgment
• 1 *medium* confidence study in adult mice. • 42-d					
Histopathology 3 *high* confidence studies in adult rats • 28-d (×2) • 14-d premating to PND22 1 *medium* confidence study • 14-d premating to PND22 1 low confidence study • 42-d		• Unexplained inconsistency across studies	Decreased number of secondary follicles and corpora lutea in 1 *low* confidence study		
Organ weights 1 *high* confidence study in adult rats • 28-d (×2) • 14-d premating to PND22 3 *medium* confidence studies in rats and mice • GD7–PND22 • 14-d premating to PND22		• Unexplained inconsistency across studies	Decreased ovary weight reported in 1 *medium* confidence study		

Evidence stream summary and interpretation					Evidence integration summary judgment
• 42-d					
Developmental effects 2 *medium* confidence studies in rats and mice • GD7–PND22 • GD0–PND22			No observed effects on female reproductive organ development		

3.2.9. Male Reproductive Effects

Human Studies

Twelve epidemiology studies (reported in 15 publications) examined the association between PFHxS exposure and male reproductive effects. The outcomes included in these studies were semen parameters, reproductive hormones, timing of pubertal development, and anogenital distance. These studies are described below.

<u>Semen parameters</u>

Semen concentration and sperm motility and morphology were considered the core endpoints for the assessment of semen parameters. Other outcomes, such as specific sperm morphology and motility defects, were not consistently reported across studies and were considered secondary; these outcomes are most useful to probe into associations observed in the core endpoints. Key issues for the assessment of semen parameters involve sample collection and sample analysis. Samples should be collected after an abstinence period of 2–7 days, and analysis should take place within 2 hours of collection and follow guidelines established by the World Health Organization (WHO, 2010). While exposure would ideally be measured during the period of spermatogenesis rather than concurrent with the outcome, a cross-sectional design is considered adequate because the period of spermatogenesis is fairly short (<3 months) relative to the half-life of PFHxS (years), and there is no concern for reverse causality with this outcome.

Five epidemiology studies (reported in seven publications) examined the association between PFHxS exposure and semen quality. The evaluations for these studies are summarized in Figure 3-65, and additional details can be obtained from HAWC. Three studies were *medium* confidence: one was a cross-sectional analysis of male partners in a pregnancy cohort (Toft et al., 2012) and two were cross-sectional studies of healthy young men (Petersen et al., 2022; Joensen et al., 2013). The remaining two studies were *low* confidence due to multiple identified deficiencies and were cross-sectional studies of men seeking infertility assessment (Huang et al., 2019b; Song et al., 2018). All the studies analyzed PFHxS in serum using appropriate methods and thus exposure misclassification is expected to be minimal.

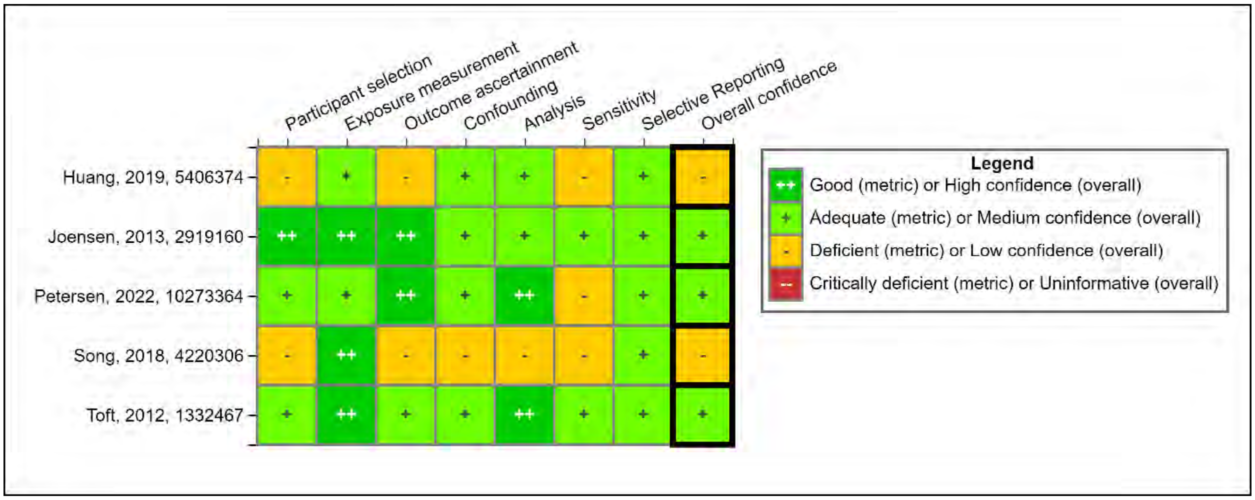

Figure 3-86. Semen parameters epidemiology study evaluation heatmap. For additional details see HAWC link.

The results for the association between PFHxS exposure and semen quality in *medium* confidence studies are presented in Table 3-34. The studies analyzed the outcomes differently, so the effect estimates are not directly comparable, but a negative effect estimate indicates a reduction in sperm quality with higher exposure. There was a statistically significant and dose-dependent decrease in normal sperm morphology in one *medium* confidence study (Toft et al., 2012) and an imprecise and non-dose-dependent decrease (>10% change) in concentration in the same study (Toft et al., 2012). A *low* confidence study (Huang et al., 2019b) reported a higher concentration ($p < 0.05$) and motility ($p > 0.05$) with PFHxS exposure. No association was reported in the other *medium* (Petersen et al., 2022; Joensen et al., 2013) or *low* (Song et al., 2018) studies. Other publications of the same study described in Toft et al. (2012) reported no clear association between PFHxS exposure and sperm DNA damage (Leter et al., 2014; Specht et al., 2012), indicating that PFHxS-induced DNA damage is unlikely to explain the decreases in the percent of sperm with normal morphology (and the slightly decreased sperm numbers) observed in Toft et al. (2012). Exposure levels were slightly higher in Toft et al. (2012) than Joensen et al. (2013), which could explain the differing results, but this cannot be confirmed with the currently available evidence.

Table 3-40. Associations between PFHxS and semen sperm parameters in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Concentration	Motility[a] (% progressively motile)	Morphology[a] (% normal)
Petersen et al. (2022)	Cross-sectional study of young men (2017–2019), Denmark; 1,041 men (18–20 yrs)	0.3 (P5–P95: 0.2–0.6)	% Change vs. T1	T2: 0 (-12, 13) T3: 2 (-10, 16)	T2: -7 (-12, -1) T3: -2 (-8, 4)	T2: 1 (-10, 12) T3: 6 (-5, 18)
Joensen et al. (2013)	Cross-sectional study of men evaluated for military service (2008–2009), Denmark; 247 men (18–22 yrs)	0.7 (0.5–0.9)	β (95% CI) for 1-unit increase	Cubic root transformed 0.05 (−0.12,0.22)	% Immotile Square transformed -2.82 (−232,227)	Square root transformed 0.12 (−0.02,0.26)
Toft et al. (2012)	INUENDO cohort cross-sectional analysis (2002–2004), Greenland, Ukraine, Poland; 588 men	1.1 (P33–P66: 0.7–1.5)	% Change vs. T1	(mill/ mL) T2: −12 (−52,28) T3: −11 (−57,35)	T2: 11 (−12,35) T3: 10 (−18,37)	T2: −27 (−58,3) T3: −35 (−70,-1)*

*$p < 0.05$, CD: critically deficient, T: tertile.
[a]Percent motile in population was 37% in Petersen et al. (2022), 58% in Joensen et al. (2013), and 56%–64% in Toft et al. (2012), varying by country. Percent normal morphology in population was 6% in Petersen et al. (2022), 7% in Joensen et al. (2013) and 6%–7% in Toft et al. (2012).

Reproductive hormones in males

Testosterone and estradiol were considered the primary endpoints for male reproductive hormones, although findings for LH, FSH, and SHBG were also reviewed where available. Key issues for the evaluation of these studies were sample collection and processing. For testosterone, LH, and FSH, blood sample collection should be performed in the morning due to diurnal variation, and if not possible, time of collection must be accounted for in the analysis. If there is no consideration of time of collection, the study is classified as deficient for outcome ascertainment and *low* confidence overall for these hormones.

Nine studies (reported in ten publications) examined the associations between PFHxS and male reproductive hormones. Most studies examined only testosterone and estradiol. All the studies measured exposure and outcome concurrently which was considered appropriate since levels of these hormones are capable of being rapidly upregulated or downregulated and they are not expected to directly bind to or otherwise interact with circulating PFAS. Four studies (Petersen et al., 2022; Lewis et al., 2015; Joensen et al., 2013; Specht et al., 2012) examined associations in adults, two studies in adolescents (Zhou et al., 2016; Lewis et al., 2015), one study in children (Lopez-Espinosa et al., 2016), and three studies in infants (Jensen et al., 2020b; Liu et al., 2020b; Yao et al., 2019). The study evaluations are summarized in Figure 3-66. Four studies were rated *medium* in overall study confidence (Petersen et al., 2022; Liu et al., 2020b; Lopez-Espinosa et al., 2016; Joensen et al., 2013), and five were *low* confidence (Jensen et al., 2020b; Yao et al., 2019; Zhou et al., 2016; Lewis et al., 2015; Specht et al., 2012). However, of the *medium* confidence studies, one did not consider timing of sample collection and was thus *low* confidence for testosterone (Lopez-Espinosa et al., 2016).

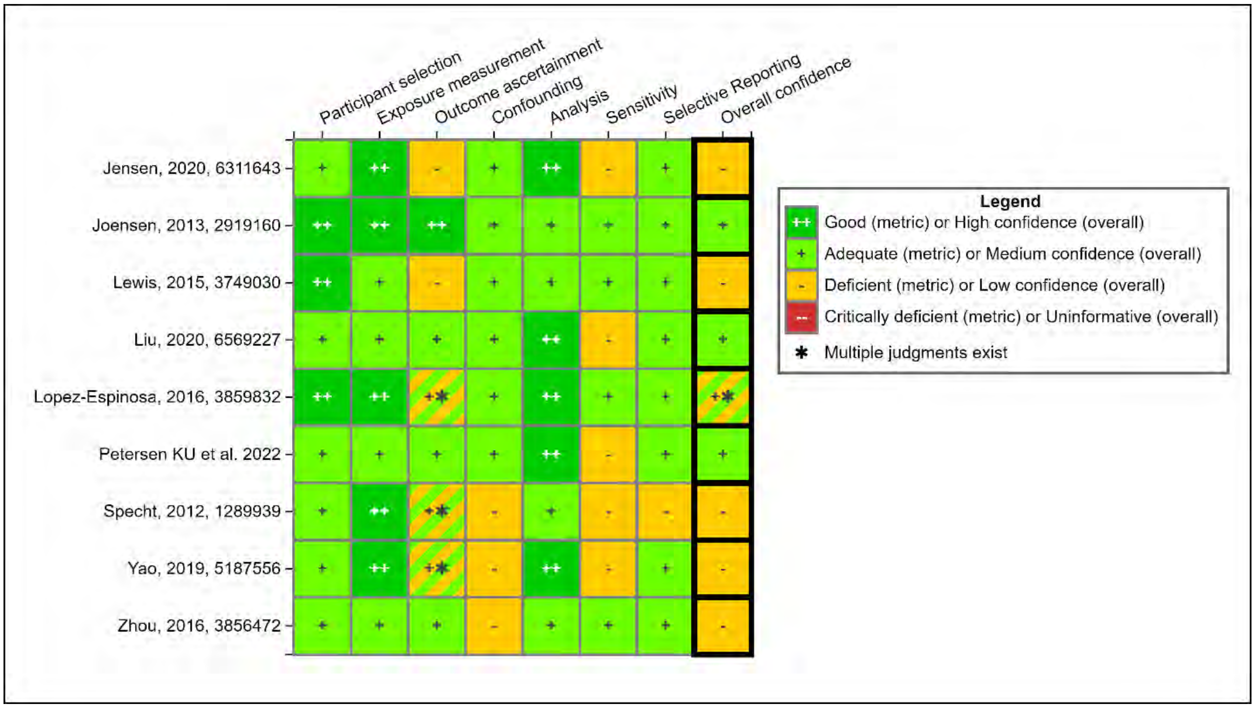

Figure 3-87. Summary of study evaluation for epidemiology studies of male reproductive hormones. For additional details see HAWC link.

Testosterone

As described above, most studies were *low* confidence for testosterone. In adult men, four studies were available and two were *low* confidence. In the two *medium* confidence studies, both populations of young men in Sweden (Joensen et al., 2013) and Denmark (Petersen et al., 2022), no association was reported between PFHxS exposure and testosterone levels, at mean concentrations of 0.7 and 0.3, respectively. Non-statistically significant inverse associations were observed in one *low* confidence study of adults (Lewis et al., 2015), and only in age groups 20 to <40 and 40 to 60 (β (95% CI); for 20 to 40: −1.2 (−4.7, 2.4), for 40 to 60: −3.6 (−8.2, 1.2), and 60 to 80: 3.3 (−3.8, 10.8). The other *low* confidence study did not report quantitative results but stated that associations were not consistent across countries in the study (Specht et al., 2012). For adolescents, one *low* confidence study (Lewis et al., 2015) reported a non-statistically significant positive association (β 2.4, 95% CI: −9.1, 15.2), and the other reported no association (Zhou et al., 2016). A study in children (Lopez-Espinosa et al., 2016) reported a non-statistically significant inverse association (β −2.7, 95%CI: −6.4, 1.2), while two studies in infants (Jensen et al., 2020b; Yao et al., 2019) reported no association. Overall, there is inconsistent evidence of an association between PFHxS exposure and testosterone. Some *low* confidence studies report inverse associations, but the *medium* confidence studies reported no association. It is possible that this is due to differences in PFHxS levels, as the *medium* confidence studies had exposure levels lower than the studies that observed an association (median blood concentrations 0.3–0.7 ng/mL versus 1.3–1.8 ng/mL in Lewis et al. (2015) and 8 ng/mL in Lopez-Espinosa et al. (2016)), but given the concerns for outcome misclassification in the *low* confidence studies, the results are difficult to interpret.

Estradiol

Six studies examined associations between PFHxS exposure and estradiol in male subjects. Among the three *medium* confidence studies (Petersen et al., 2022; Lopez-Espinosa et al., 2016; Joensen et al., 2013) reported no association between increasing PFHxS exposure and estradiol. Results across the *low* confidence studies are mixed, as Zhou et al. (2016) reported higher estradiol levels with higher PFHxS exposure, while Specht et al. (2012) reported that estradiol levels were not consistently associated with PFHxS across countries with no data shown and Yao et al. (2019) reported no association.

Other reproductive hormones

For other reproductive hormones, SHBG was not associated with PFHxS levels in Specht et al. (2012), Joensen et al. (2013), or Petersen et al. (2022). FSH and LH were not associated with PFHxS in Joensen et al. (2013) or Petersen et al. (2022) and associations were not consistent across regions in Specht et al. (2012). In Jensen et al. (2020b), positive but nonstatistically significant associations were reported with LH, dehyroepiandosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), androstenedione, and 17-hydroxyprogesterone (17-OHP). Liu et al. (2020b) reported a small but not statistically significant positive association (2.7% increase) with progesterone in infants.

Overall, there is little evidence of an association between PFHxS exposure and male reproductive hormones, but there are limitations in the available evidence that hinder interpretation of the null findings.

Pubertal development

Two *medium* confidence studies, birth cohorts in Denmark (Ernst et al., 2019) and the U.S. (Carwile et al., 2021), examined timing of pubertal development with PFHxS exposure. Ernst et al. (2019) used maternal exposure (median 1.1 ng/mL, 10th–90th percentile: 0.6–1.7) while Carwile et al. (2021) used childhood exposure at around 8 years of age. One study reported that the participants in the third tertile of exposure had earlier genital development, pubic hair, axillary hair, acne, voice break, and first ejaculation, with axillary hair acne, and voice break being statistically significant. Looking at a combined puberty indicator outcome, there was lower age of puberty in the third tertile (age difference −6.89 (95% CI: −12.57, −1.20)) (Ernst et al., 2019). The second study reported no association between PFHxS exposure and a pubertal development score or age at peak height velocity (Carwile et al., 2021).

Summary of human studies on male reproductive effects

Overall, there is some limited evidence of an association between PFHxS exposure and sperm motility, timing of pubertal development, and anogenital distance, but there is considerable uncertainty in the available data due to lack of consistency across the studies on each outcome and lack of coherence with reproductive hormones.

Animal Studies

The database of animal toxicity studies on PFHxS-induced male reproductive effects consists of five oral exposure studies that include two short-term studies in Harlan Sprague Dawley rats exposed for 28 days (NTP, 2018c; 3M, 2000b), two multigeneration reproduction studies in Crl:CD (SD) rats or Crl:CD1 (ICR) mice with exposures starting during 2–week premating through postnatal days (PND) 22–35 (Chang et al., 2018; Butenhoff et al., 2009) and a single-generation reproduction study in Wistar rats with exposure during gestation and lactation (gestational days [GD] 7 to PND 22) (Ramhøj et al., 2018). The studies evaluated several endpoints relevant to the assessment of male reproductive toxicity, namely mating and fertility, sperm measures, hormone levels, histopathology, organ weights, and morphological markers of sexual differentiation and maturation (U.S. EPA, 1996).

Sperm parameters

Sperm measures (count, motility, morphology, concentration, and production rate) were evaluated in three *low* confidence studies that exposed animals for 28 or 44 days (see Figure 3-88). In SD rats, exposure to PFHxS for 28 days did not impact sperm count, spermatid count, or sperm motility. Additionally, Butenhoff et al. (2009), 3M (2003) and Chang et al. (2018) did not observe PFHxS-induced alterations in sperm motility, morphology, or concentration after exposing SD rats or CD-1 mice for 44 and 42 days respectively. Overall, these results suggest that PFHxS exposure does not affect sperm measures. However, these findings should be interpreted with caution as the available studies were of *low* confidence due to experimental design features that may have resulted in reduced sensitivity and a potential bias toward the null[29].

[29]In rodent models such as the rat it takes approximately eight weeks for spermatogonia to develop to spermatozoa (Foster and Gray, 2013). Damage to the spermatogonial cells would not be detected in ejaculate or cauda epididymis samples from animals exposed for periods that are shorter than eight weeks (U.S. EPA, 1996).

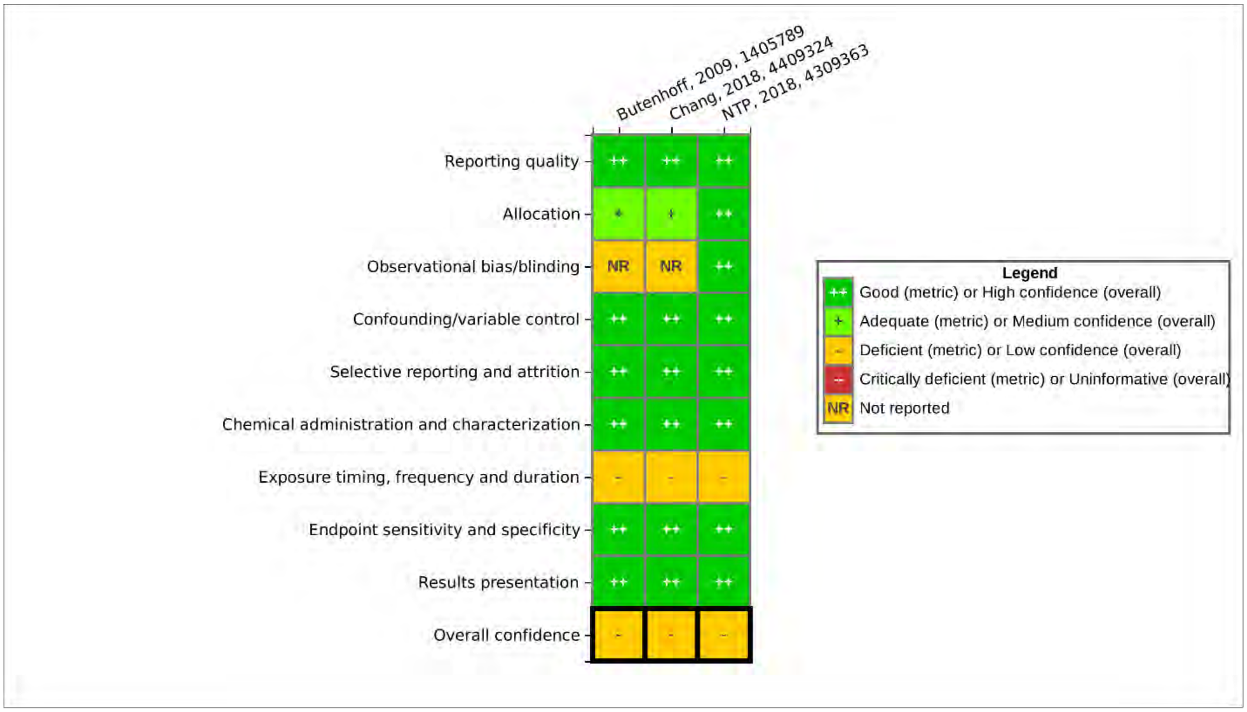

Figure 3-88. Male reproductive animal study evaluation heatmap – sperm measures. For additional details see HAWC link.

Histopathology

Histopathology of male reproductive organs was evaluated in two *high* confidence studies and one *medium* confidence study (see Figure 3-89). In SD rats, exposure to PFHxS for 28 to 44 days at doses ranging from 0.3 to 10 mg/kg-day did not affect the histopathology of the testes, preputial glands, epididymis, or seminal vesicles (NTP, 2018c; Butenhoff et al., 2009; 3M, 2003, 2000b).

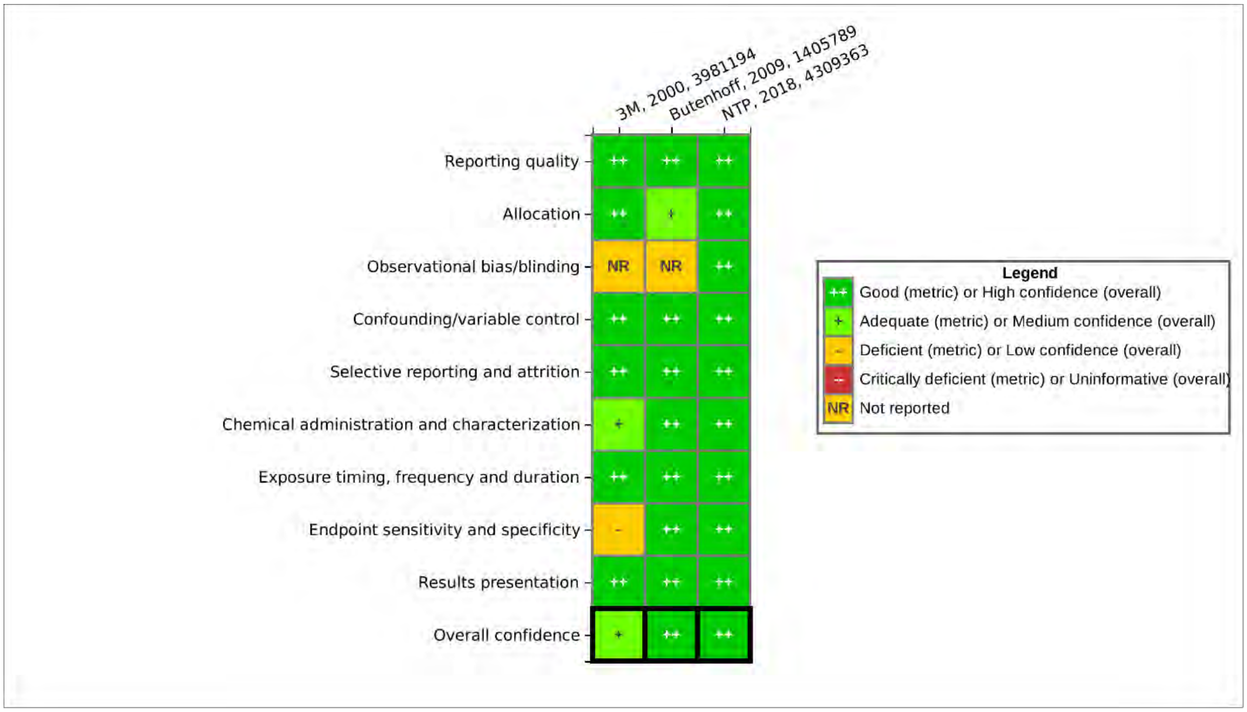

Figure 3-89. Male reproductive histopathology animal study evaluation heatmap. For additional details see HAWC link.

Hormone levels

The effects of PFHxS exposure on reproductive hormones was evaluated in one *high* confidence study using SD rats (see Figure 3-90). Exposure to PFHxS for 28 days at doses ranging from 0.625 to 10 mg/kg-day did not affect serum testosterone levels (NTP, 2018c).

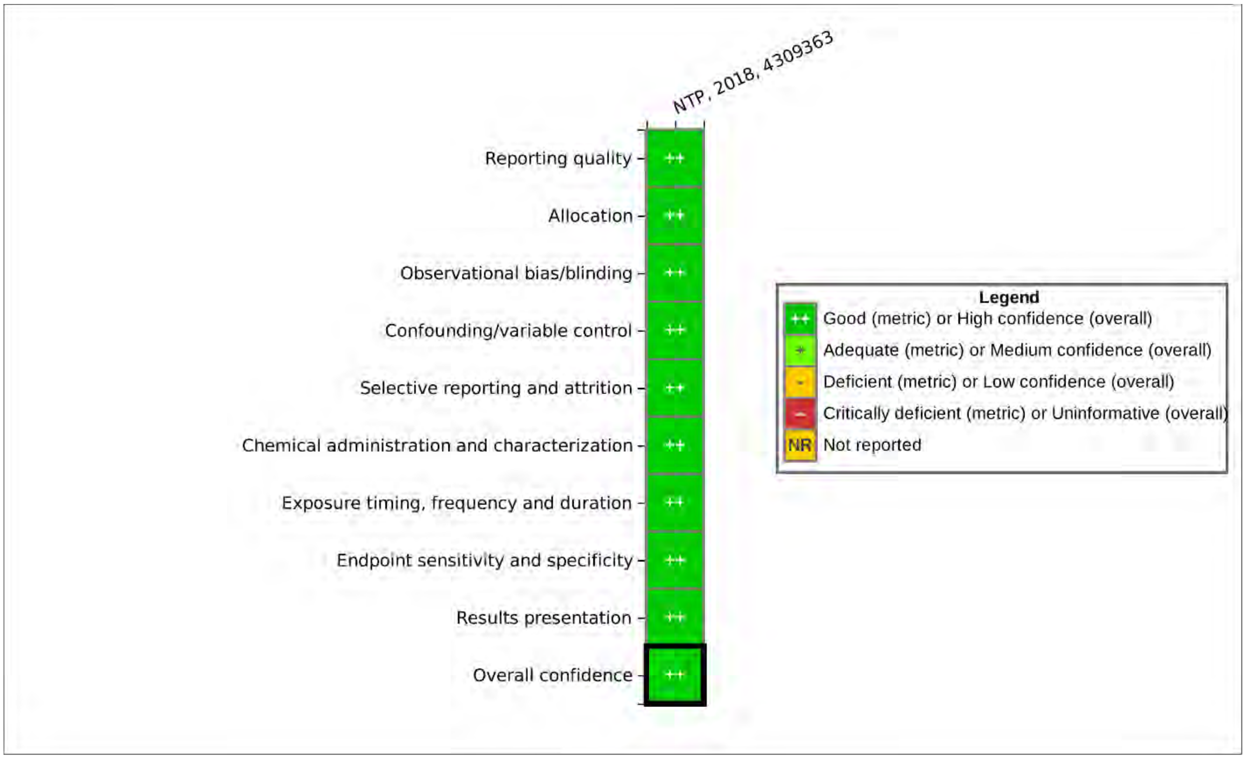

Figure 3-90. Male reproductive animal study evaluation heatmap – reproductive hormones. For additional details see HAWC link.

Organ weights

Potential PFHxS-induced effects on male reproductive organ weights were evaluated in three *high* confidence studies using SD rats (NTP, 2018c; Butenhoff et al., 2009; 3M, 2003, 2000b) and one *medium* confidence study using Wistar rats (Ramhøj et al., 2018) (see Figure 3-91). In SD rats, exposure to PFHxS for 28 to 44 days at doses ranging from 0.3 to 10 mg/kg-day did not affect the weights of the testis, epididymis, or seminal vesicle (NTP, 2018c; Butenhoff et al., 2009; 3M, 2003, 2000b). Furthermore, gestational plus lactational exposure to PFHxS (0.05 to 25 mg/kg-day) also did not affect organ weights for epididymis, ventral prostrates, seminal vesicles, levator ani, or testes in Wistar rats (Ramhøj et al., 2018).

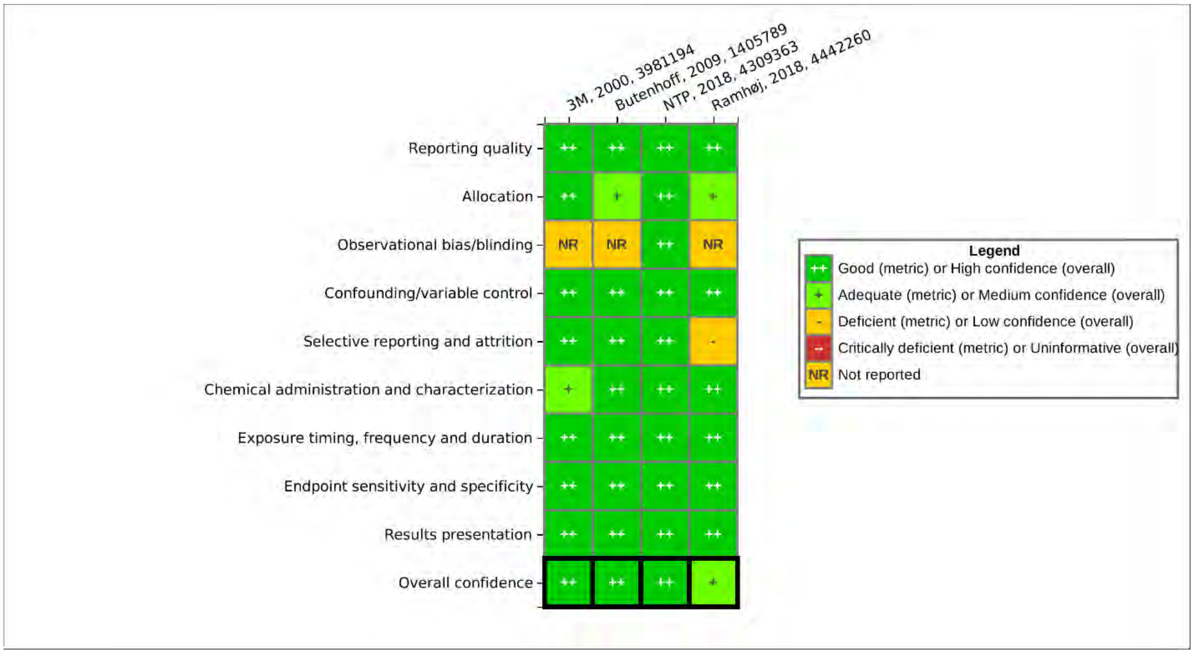

Figure 3-91. Male reproductive animal study evaluation heatmap – reproductive organ weights. For additional details see HAWC link.

Landmarks of male reproductive system development and maturation

One *medium* confidence gestational exposure study evaluated PFHxS-induced effects on androgen sensitive developmental landmarks in F1 Wistar rats (Ramhøj et al., 2018). Gestational plus lactational exposure to PFHxS at doses ranging from 0.05 to 45 mg/kg-day did not affect anogenital distance or nipple retention in Wistar rats. The developmental effects and pregnancy outcomes of PFHxS exposure are summarized in Section 3.2.3.

Functional measures

Functional measures were evaluated in *medium* and *high* confidence studies using mice or rats (see Figure 3-92). PFHxS exposure for 14 days before mating at doses ranging from 0.3 to 10 mg/kg-day did not have a significant impact on mating or fertility indices in rats or mice (Chang et al., 2018; Ramhøj et al., 2018; Butenhoff et al., 2009; 3M, 2003).

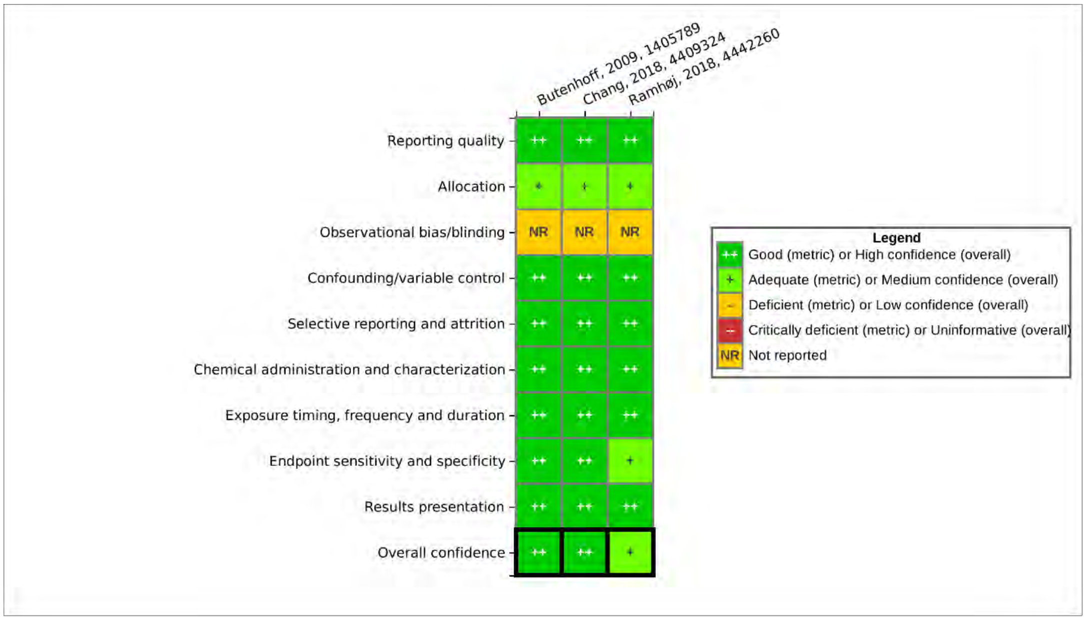

Figure 3-92. Male reproductive animal study evaluation heatmap – developmental effects and functional measures. For additional details see HAWC link.

Evidence Integration

The available studies provide **inadequate evidence** to determine whether PFHxS exposure has the potential to affect male reproduction in humans. This conclusion is based on studies in both humans and animals (see Table 3-41).

The available evidence on PFHxS-induced male reproductive effects in human studies is considered *indeterminate*. Outcomes evaluated in human studies include semen parameters, male reproductive hormones, and onset of puberty. No associations were observed for reproductive hormone measures. Exposure-related alterations in sperm morphology and age of puberty were reported. However, considerable uncertainties were also identified that reduce the strength of evidence (see Table 3-41).

The available evidence on PFHxS-induced male reproductive effects in animal toxicity studies is also considered *indeterminate*. Experimental studies using different laboratory rodent species measured parameters considered indicative of potential adverse responses, including reproductive organ weights, sperm measures, histopathology, reproductive hormones, and developmental and functional measures. No significant exposure-related effects were observed for the measured reproductive parameters in the available studies. While a judgment of *compelling evidence of no effect* was considered for characterizing the animal evidence, significant uncertainties in the animal study database prevent judgments about PFHxS exposure and male reproductive toxicity from being drawn. Specifically, the short exposure duration in the available studies is considered inadequate for the evaluation of sperm measures, only a single study evaluated androgen levels, and other reproductive hormones were not studied.

Table 3-41. Evidence profile table for PFHxS exposure and male reproductive effects

Evidence stream summary and interpretation					**Evidence integration summary judgment**
Evidence from studies of exposed humans					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	⊙⊙⊙ ***Evidence inadequate*** *Primary Basis*: Evidence is inconsistent across studies or largely null. *Human relevance*: Without evidence to the contrary, effects in rodent models are considered relevant to humans. The rodent and human male reproductive systems share many conserved features. *Cross-stream coherence*: N/A, human and animal evidence indeterminate *Susceptible populations and lifestages*: N/A **evidence inadequate** to draw inferences
Sperm parameters 3 *medium* and 2 *low* confidence studies	• No factors noted	• *Unexplained inconsistency* across studies • *Imprecision* – for sperm concentration	Decreased normal morphology and concentration in one *medium* confidence study.	⊙⊙⊙ *Indeterminate* Some evidence of association with sperm motility, and pubertal development. Significant uncertainty due to lack of consistency and coherence	
Reproductive hormones 4 *medium* and 5 *low* confidence studies		• *Unexplained inconsistency* across studies • *Low* confidence studies	Inverse association with testosterone and estradiol in some *low* confidence studies, but *medium* confidence studies were null. No association with LH or FSH levels.		
Pubertal development 2 *medium* confidence study		• No factors noted	Significant association between exposure and lower puberty age in 1 of 2 studies.		
Evidence from in vivo animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	

Evidence stream summary and interpretation					Evidence integration summary judgment
Sperm parameters 3 *low* confidence studies in adult rats and mice: • 28-d • 44-d • 42-d		• All *low* confidence studies – Low sensitivity	No observed effects on sperm measures in *low* confidence, insensitive studies	⊙⊙⊙ *Indeterminate* Certainty in the consistently null findings was reduced due to notable data gaps.	
Histopathology 2 *high* confidence studies in adult rats: • 28-d • 44-d 1 *medium* confidence study in adult rats • 42-d	• *High* or *medium* confidence in studies, with sensitive outcome measures and low risk of bias.	• No factors noted	No observed effects on histopathological outcomes		
Hormone levels 1 *high* confidence study in adult rats • 28-d			No observed effects on testosterone levels		
Organ weights 3 *high* confidence studies in adult rats • 28-d (×2) • 44-d 1 *medium* confidence study in rats • GD7–PND22			No observed effects on reproductive organ weights		

Evidence stream summary and interpretation					Evidence integration summary judgment
Developmental effects 1 *high* confidence study in rats • GD7–PND22			No observed effects on male reproductive organ development		
Functional measures 2 *high* confidence studies in rats and mice • 14-d (×2)			No observed effects on mating or fertility index		

3.2.10. Renal Effects

Human Studies

Seventeen studies (reported in 27 publications) investigate the relationship between PFHxS exposure and markers of renal function, specifically measures of glomerular filtration rate (GFR) and uric acid (UA). Three studies (Zhang et al., 2019b; Seo et al., 2018; Rotander et al., 2015b) were considered *uninformative* due to critical deficiencies in confounding (see Figure 3-93). The remaining 14 studies were primarily cross-sectional analyses and were classified as *low* confidence primarily due to concerns for reverse causality without other major methodological limitations. In essence, as described in Watkins et al. (2013), decreased renal function (as measured by decreased GFR or other measures) could plausibly lead to higher levels of PFAS, including PFHxS, in the blood. This hypothesis is supported by data presented by Watkins et al. (2013), although there is some uncertainty in the conclusions due to the use of modeled exposure data as a negative control and the potential for the causal effect to occur in addition to reverse causality. The results least likely to be affected by reverse causality were analyses in four studies (four publications) designed to assess reverse causality (e.g., stratification by glomerular filtration stage or modeling with PFHxS as the dependent variable) (Lin et al., 2021; Moon, 2021); Jain (2019); (Zeng et al., 2019c; Conway et al., 2018) and two studies with prospective designs (Lin et al., 2021); Blake et al. (2018). Of these, Lin et al. (2021) had the benefit of both prospective data analysis and additional analyses and was thus rated as *medium* confidence. Across studies, because of the potential for reverse causation, there is considerable uncertainty in interpreting the results of the available studies. However, the informative studies were otherwise well conducted and had adequate or good ratings for all domains other than exposure measurement.

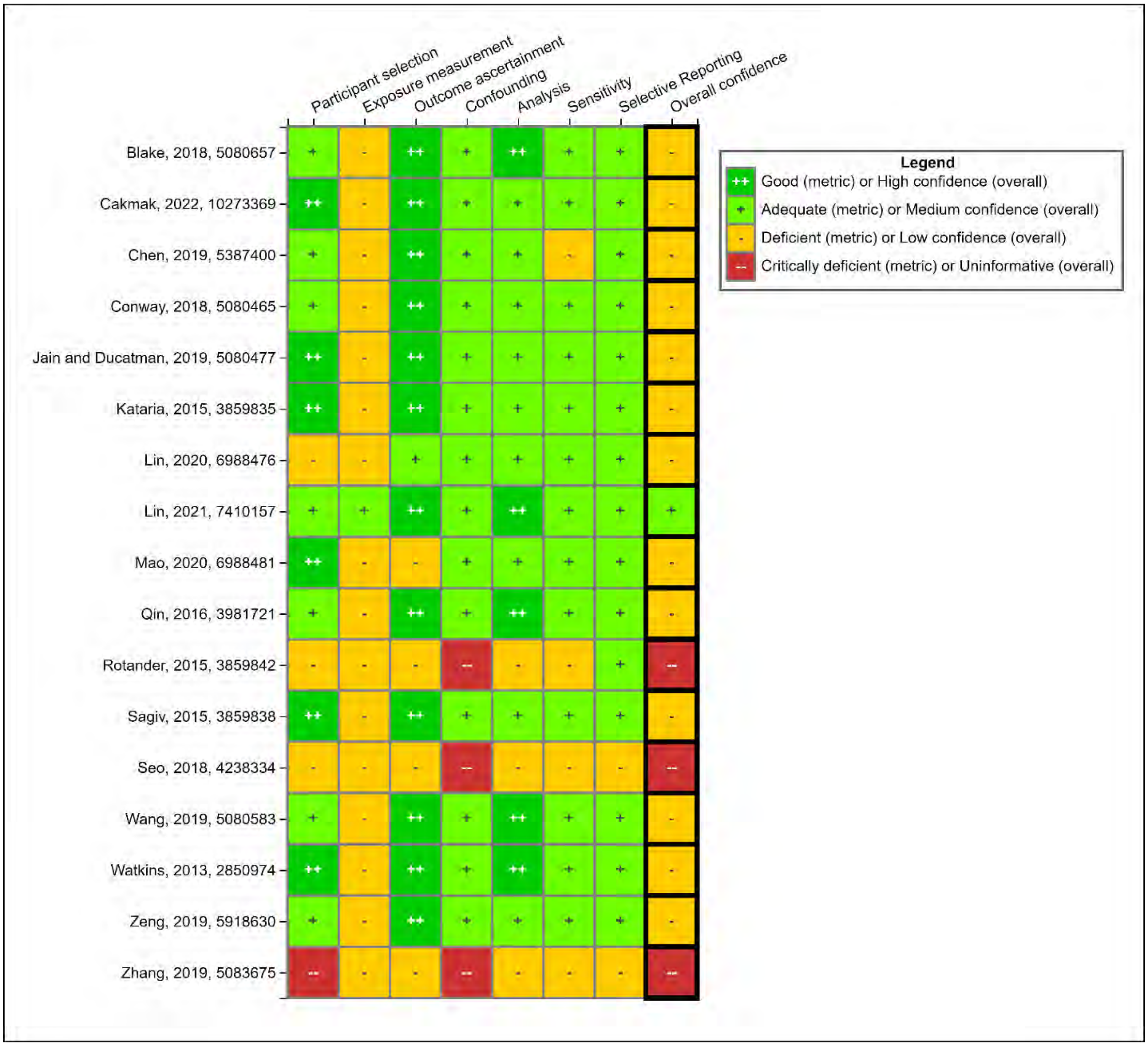

Figure 3-93. Renal effects human study evaluation heatmap. For additional details see HAWC link. Multiple publications of the same study: **Jain and Ducatman (2019b)** also includes **Jain and Ducatman (2019a)**, **Jain (2019)**, **Jain (2013)**, **Jain (2021b)**, **Jain (2020)**, **Jain (2021a)**, **Moon (2021)**, and **Scinicariello et al. (2020b)**.

Across the 14 available studies, there is an indication of impaired renal function (i.e., lower GFR, higher UA, creatinine, or disease) in nine (Cakmak et al., 2022; Lin et al., 2021; Lin et al., 2020c; Mao et al., 2020; Blake et al., 2018; Qin et al., 2016; Sagiv et al., 2015; Watkins et al., 2013), including multiple NHANES publications (Moon, 2021; Scinicariello et al., 2020b; Jain and Ducatman, 2019b), but there are some inconsistencies (see Table 3-42). In adults, Blake et al. (2018), Sagiv et al. (2015), Moon (2021), Lin et al. (2021) reported lower GFR with higher exposure, all statistically significant, though the association in Lin et al. (2021) was observed only in participants with hypertension (the direction was in the opposite direction for participants without hypertension). A different analysis of NHANES data overlapping with Moon (2021), Jain and Ducatman (2019b), reported an inverted U-shape response with GFR (higher exposure levels in the second and third tertiles than first and fourth, also observed in analyses stratified by sex). In contrast to the majority of studies, Conway et al. (2018) and Wang et al. (2019a) reported higher GFR with higher exposure (not statistically significant). Looking at hyperuricemia, Scinicariello et al. (2020b) reported higher odds (unstratified by sex) with an exposure-response gradient observed across quartiles. Zeng et al. (2019c) reported higher odds in women but not men, while Lin et al. (2020c) reported higher uric acid in the fourth quartile in men but not women. A positive association with creatinine was observed in Cakmak et al. (2022) and with kidney stones in (Mao et al., 2020). However, no association was observed with chronic kidney disease in the only study that reported it (Wang et al., 2019c). In children and adolescents, Watkins et al. (2013) reported lower GFR with higher exposure and Qin et al. (2016) reported higher UA, while Kataria et al. (2015) also reported the inverted U-shape with GFR.

Overall, there are generally consistent associations between impaired renal function and PFHxS exposure but the potential for reverse causation is an important source of uncertainty. However, in the studies with less potential for reverse causation, there is an indication that this bias is unlikely to fully explain the observed associations. Significant associations were observed in both studies with prospective exposure measurement (Lin et al., 2021; Blake et al., 2018), though only in participants with hypertension in Lin et al. (2021). While prospective measurement does not eliminate the possibility of reverse causation due to ongoing exposure prior to study enrollment, the effect is likely lower. Further, Lin et al. (2021) performed a secondary analysis using baseline GFR as the independent variable and repeated measures of PAS as the dependent variable and found that PFAS levels did not differ significantly by baseline GFR. A similar analysis without repeated measures in Moon (2021) also indicated that reverse causation was not likely to explain the results.

Table 3-42. Associations between PFHxS exposure and renal function

Reference, confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Glomerular filtration rate (GFR) *Decrease* indicates impaired renal function				
Wang et al. (2019a), *Low*	Cross-sectional study (2015–2016); China; 1,612 adults	0.7 (0.01,2.7)	Mean change (95% CI) in eGFR per ln-unit change	0.24 (−0.02, 0.50)
Watkins et al. (2013), *Low*	Cross-sectional study of 9,660 children in U.S. exposed to high PFOA	IQR 1.3	Mean change (95% CI) per IQR increase exp	-1.0 (−1.5, -0.4)*
Jain and Ducatman (2019b), *Low*	Cross-sectional study (NHANES) (2007–2014); U.S.; 6,836 adults	1.4	Adjusted geometric means (95% CI) by glomerular function stage (GF-1 is normal or high filtration; GF-3B/4 is moderately to severely decreased)	All participants GF-1: 1.20 (1.14–1.27) GF-2: 1.73 (1.61–1.86) GF-3: 1.83 (1.63–2.05) GF-3B/4: 1.01 (0.78–1.31)
Moon (2021), *Low*	Cross-sectional study (NHANES) (2003–2018); U.S.; 14,373 adults	1.5 (0.8-2.6)	β (p-value) for ln-unit increase	-1.52 (-2.10, -0.94)*
Kataria et al. (2015), *Low*	Cross-sectional study of 1,960 adolescents in U.S.	2	β (95 CI) for quartiles vs. Q1	Q2: 1.4 (−3.6,6.3) Q3: 1.9 (−3.4,7.1) Q4: −0.3 (−4.4,3.8)
Sagiv et al. (2015), *Low*	Cross-sectional study of 1,645 pregnant women in U.S.	2.4 (1.6–3.8)	% change GFR	-4.3 (−5.3, -3.3)*
			Geometric means (IQR) of exp by quartile	Q1: 3.0 (1.9,4.3) Q2: 2.7 (1.7,4.1) Q3: 2.3 (1.5,3.2) Q4: 2.2 (1.5,3.5)*
Lin et al. (2021), *Medium*	Cohort study within placebo and lifestyle intervention arms of a diabetes prevention randomized controlled trial of 875 adults in the U.S.	2.4 (1.6–3.8)	β (95 CI) for doubling of baseline exposure	0.21 (-0.79, 1.21) With hypertension -2.35 (-4.46, -0.25)* Without hypertension 1.24 (0.09, 2.39)*
Blake et al. (2018), *Low*	Prospective cohort of residents near a uranium processing site (1990–2008); U.S.; 210 adults	2.7 (1.7–4.1)	Percent change (95% CI) in eGFR per IQR change	-2.06 (−3.53, −0.59)*
Conway et al. (2018), *Low*	Cross-sectional study of 53,650 adults in U.S. exposed to high PFOA	3.0 (1.9–4.8)	OR (95% CI) for 1-unit increase	GF-1: 2.07 (1.69–2.55) GF-2: 2.29 (1.86–2.81) GF-3A: 2.37 (1.87–2.84) GF-3B: 2.30 (1.83–2.90) GF-4/5: 1.0 (ref)
Uric acid (UA) *Increase* indicates impaired renal function				

Reference, confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Zeng et al. (2019c), *Low*	Cross-sectional study of 1,612 adults in China	0.7 (0.01–2.7)	Mean difference per log-unit increase	0.01 (-0.15, 0.03) GF-1: -0.01 (-0.06, 0.04) GF-2: -0.00 (-0.03, 0.03) GF-3: 0.05 (-0.04, 0.15) GF-4: -0.04 (-0.23, 0.15)
			OR (95% CI) for hyperuricemia for log-unit increase	1.01 (0.97, 1.06) Women: 1.18 (1.01, 1.37)* Men: 0.99 (0.95, 1.04)
Chen et al. (2019a), *Low*	Cross-sectional study of 122 adults in China	GM 0.8, range 0.3–2.4	β (95% CI) for ln-unit increase	-4.42 (−24.23, 15.38)
Qin et al. (2016), *Low*	Cross-sectional study of 225 children in Taiwan	1.3 (0.6–2.8)	β (95 CI) for ln-unit increase	0.14 (0.02,0.26)*
			OR (95% CI) for quartile increase exp and high UA	1.4 (0.9,2.1)
Jain and Ducatman (2019a), *Low*	Cross-sectional study (NHANES) (2007–2014); U.S.; 6,836 adults	1.4	β (*p*-value) for 1-unit increase	In GF-1 participants Women: 0.023 (<0.01)* Men: 0.015 (0.06)
Scinicariello et al. (2020b), *Low*	Cross-sectional study (NHANES) (2009–2014); U.S.; 4,917 adults	1.4 (GM)	β (95% CI) in serum uric acid for quartiles vs Q1	Q2: 0.14 (0.02, 0.26)* Q3: 0.22 (0.08, 0.36)* Q4: 0.33 (0.19, 0.47)*
			OR (95% CI) in hyperuricemia for quartiles vs Q1	Q2: 1.15 (0.89, 1.50)* Q3: 1.33 (0.95, 1.86)* Q4: 1.51 (1.12, 2.03)*
Kataria et al. (2015), *Low*	Cross-sectional study of 1,960 adolescents in the U.S.	2	β (95% CI) for quartiles vs. Q1	Q2: 0.04 (−0.1,0.2) Q3: 0.05 (−0.1,0.2) Q4: −0.05 (−0.2,0.1)
Lin et al. (2020c), *Low*	Cross-sectional study (2016–2017); Taiwan; 397 older adults (55–75 yrs)	2.7 (1.9-3.7)	β (95% CI) in serum uric acid for quartiles vs Q1	Q2: 0.01 (-0.32, 0.33) Q3: -0.1 (-0.44, 0.23) Q4: 0.39 (0.05, 0.72)* Women: Q2: 0 (-0.36, 0.35) Q3: -0.1 (-0.46, 0.26) Q3: 0.05 (-0.31, 0.42) Men: Q2: -0.31 (-0.97, 0.35) Q3: 0.3 (-0.37, 0.96) Q4: 0.89 (0.22, 1.56)*
Creatinine Increase indicates impaired renal function				
Cakmak et al. (2022), *Low*	Cross-sectional study (2007–2017); Canada; 6,045 adults	1.5 (GM)	% change per 1 mean increase in PFDA	1.0 (0.1, 1.8)*
Chronic kidney disease				
Wang et al. (2019b), *Low*	Cross-sectional study (2015–2016); China; 1,612 adults	0.7 (0.01–2.7)	OR (95% CI) for chronic kidney disease per ln-unit change in PFDA	1.01 (0.94, 1.07)

Reference, confidence	Study population	Median exposure level (IQR) in ng/mL	Form and units of effect estimate	Effect estimate
Kidney stones				
Mao et al. (2020), *Low*	Cross-sectional study (NHANES) (2007–2016); U.S.; 8,453 adults	1.5 (0.8–2.5)	OR (95% CI) for kidney stone history for tertiles vs T1	T2: 1.24 (1.03, 1.51)* T3: 1.35 (1.10, 1.68)*

*$p < 0.05$.

Animal Studies

There are two 28-day oral gavage exposure studies in Sprague Dawley rats (NTP, 2018b; 3M, 2000a) and two 42–44 day exposure oral gavage studies in CD-1 mice (Chang et al., 2018) and Sprague Dawley rats (Butenhoff et al., 2009; 3M, 2003) that measure effects relevant to the assessment of the urinary system after repeated oral dose exposure to PFHxS. The studies report on clinical chemistry (serum) biomarkers of effect, histopathology, and organ weights. Overall study confidence was *high* for most endpoints evaluated in these studies with the exception of organ weights and serum markers in Chang et al. (2018), which had incomplete reporting of null data (results were only discussed qualitatively) resulting in a *medium* confidence rating (see Figure 3-94).

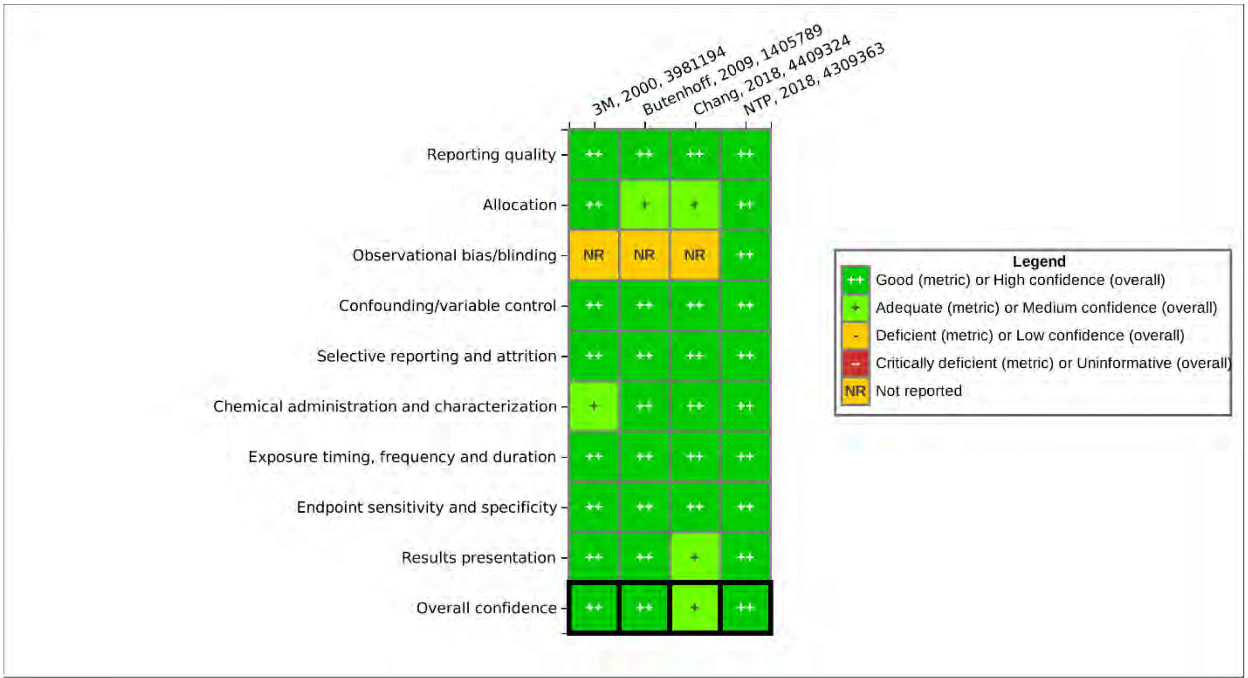

Figure 3-94. Renal effects – animal study evaluation heatmap. For additional details see HAWC link.

Clinical chemistry

Serum biomarkers of renal injury (including blood urea nitrogen [BUN], creatinine, creatinine kinase, and total protein) were measured in Sprague Dawley rats after short-term (28 day) exposure (NTP, 2018b; 3M, 2000a), and two 42- or 44-day exposure studies using CD-1 mice and Sprague Dawley rats (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003). In the F0 generation male Sprague Dawley rats, 44 days of exposure to PFHxS at the highest tested dose, 10 mg/kg-day, resulted in a 31% increase in BUN when compared with controls (Butenhoff et al., 2009; 3M, 2003). However, no effects were observed for creatinine, creatinine kinase, or total protein in male animals and female animals from the same study (Butenhoff et al., 2009; 3M, 2003); a similar study using CD-1 mice reported no effects on creatinine, urea nitrogen, and electrolytes in F0 generation male and female animals exposed to same levels of PFHxS (10 mg/kg-day) for 44 days; and two 28-day study using SD rats reported no exposure-related effects in creatinine, creatinine kinase, blood urea nitrogen (BUN), or total protein after PFHxS exposure at doses ranging from 0.6 to 10 mg/kg-day (NTP, 2018b; 3M, 2000a). BUN is considered a late biomarker of renal injury not normally affected until at least half of the kidney mass is compromised (Khan et al., 2018). The biological significance of the PFHxS-induced BUN increase observed in the NTP study is not clear as BUN was not affected in similar studies, and other clinical indicators of kidney damage were not altered in the available studies.

Histopathology

Renal histopathology was evaluated across two 28-day gavage studies (NTP, 2018a; 3M, 2000a) and one 42- to 44–day exposure toxicity study (Butenhoff et al., 2009; 3M, 2003). All studies used Sprague Dawley rats. Exposure to PFHxS for 28 to 44 days at doses ranging from 0.3 to 10 mg/kg-day did not have any notable treatment-related impacts on kidney histopathology. One 28-day short-term study also evaluated the urinary bladder and reported no effects (NTP, 2018a). In this study, chronic progressive nephropathy[30] graded as minimal occurred in the kidneys of all exposed animals, including controls.

Organ weight

Absolute and relative (to body weight) kidney weights were measured in the two 28-day gavage studies using Sprague Dawley rats (NTP, 2018a; 3M, 2000a) and the two 42- to 44-day exposure studies using Sprague Dawley rats (Butenhoff et al., 2009; 3M, 2003) or CD-1 mice (Chang et al., 2018). Exposure to 10 mg/kg-day PFHxS for 28 days increased relative kidney weights in male Sprague Dawley rats (NTP, 2018a). This response was not observed in female animals (NTP, 2018a) and none of the remaining studies exposing rats or mice to similar doses and durations (ranging from 28 to 44 days) did not observe significant PFHxS-induced changes in relative or absolute kidney weights (Chang et al., 2018; Butenhoff et al., 2009; 3M, 2003, 2000a).

Evidence Integration

The available ***evidence suggests*** but is not sufficient to infer that exposure to PFHxS might cause renal system effects in humans given sufficient exposure conditions[31] (see Table 3-43).

The available evidence on PFHxS-induced renal effects in humans is considered *slight.* The evidence for potential renal system effects in humans is based on reported associations between PFHxS exposure and impaired renal function in nine out of 14 informative epidemiological studies including several statistically significant findings. There is considerable uncertainty remaining due to the potential for reverse causation, but study analyses examining this bias indicate that it is unlikely to fully explain the observed associations.

The available evidence on PFHxS-induced renal effects in animal toxicity studies is also considered *indeterminate*. The experimental animal evidence informing potential renal system effects is limited to two 28-day gavage studies in Sprague Dawley rats (NTP, 2018a; 3M, 2000a), and two 42- to 44-day exposure studies using Sprague Dawley rats (Butenhoff et al., 2009; 3M, 2003) or CD-1 mice (Chang et al., 2018). The studies were generally well conducted (confidence ratings were *high*/*medium*) and reported on relevant measurements, including serum biomarkers

[30]Chronic progressive nephropathy is a commonly observed spontaneous lesion frequently observed in 2 to 13-week studies using SD rats (Khan et al., 2018).

[31]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

of renal injury (i.e., BUN, creatinine, and creatinine kinase), kidney and urinary bladder histopathology and kidney weights. Although a few significant findings were observed, PFHxS exposure generally did not affect the renal system in the available studies. However, the absence of long-term studies limits the evaluation of potential renal system toxicity in animals following PFHxS exposure, hence a conclusion of *compelling evidence of no effect* was not considered appropriate.

Table 3-43. Evidence profile table for PFHxS urinary system effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					**Evidence suggests** ⊙⊙⊙ *Primary Basis:* Generally consistent evidence across studies in humans. *Human relevance*: N/A *Cross-stream coherence:* N/A. Evidence in animals is indeterminate.
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Renal Functions 1 *medium* and 13 *low* confidence studies	• Consistency • Precision	• Primarily *low* confidence studies – potential reverse causality	9 of 14 studies reported associations between PFHxS exposure and impaired renal function. Reverse causality is an important source of uncertainty.	⊕⊙⊙ *Slight*	
Evidence from in vivo animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Serum Biomarkers of Renal Injury, Histopathology, Organ Weights 3 *high* confidence studies in adult rats: • 28-d (×2) • 44-d 1 *medium* confidence study using mice • 44-d	• All *high* or *medium* confidence studies	• Unexplained inconsistency	• Increased BUN reported in one study, bit no effects in remaining studies and no response in other markers of renal disease. • No PFHxS-induced effects on histopathological outcomes. • No observed PFHxS-induced effects on kidney weights	⊙⊙⊙ *Indeterminate*	

3.2.11. Other Noncancer Health Effects

Human Studies

No epidemiological studies in the database were identified to inform health effects other than those discussed in prior sections.

Animal Studies

Several other health effects were examined in experimental animals; however, there were very little data to inform whether PFHxS exposure might have the potential to cause these effects. Specifically, the *high* confidence, 28-day rat study conducted by NTP (2018c) investigated the potential for PFHxS exposure to cause effects on the alimentary system (including the esophagus, large, small intestine, pancreas, salivary glands, and stomach), musculoskeletal system, and respiratory system. For each of these systems, there were no clear PFHxS exposure-related effects in male or female animals, with the exception of an observation of minimal[32] olfactory epithelium degeneration and minimal hyperplasia along with minimal suppurative inflammation in females, but not males, in the highest exposure group (8/10 rats in 50 mg/kg-day exposure group). Overall, the sparsity of evidence on these outcomes prevents any interpretation from being drawn.

Evidence Integration

The currently available **evidence is inadequate** to assess whether PFHxS may cause other noncancer health effects in humans, including those related to the alimentary system, musculoskeletal system, and respiratory system. In general, the data available for these health outcomes were largely null and/or absent (i.e., *indeterminate* evidence from human and animal studies) and considerable data gaps remain for these health effects.

3.3. CARCINOGENICITY

3.3.1. Cancer

The systematic review identified twelve epidemiologic studies that evaluated the risks of cancer associated with exposures to PFHxS (Li et al., 2022a; Velarde et al., 2022; Liu et al., 2021b; Omoike et al., 2021; Lin et al., 2020a; Tsai et al., 2020; Ghisari et al., 2017; Wielsøe et al., 2017; Christensen et al., 2016; Bonefeld-Jørgensen et al., 2014; Hardell et al., 2014; Yeung et al., 2013). Six cancer studies by (Li et al., 2022a; Velarde et al., 2022; Omoike et al., 2021; Lin et al., 2020a; Wielsøe et al., 2017; Christensen et al., 2016) were evaluated as '*Uninformative*.' One study (Yeung et al., 2013) was screened as related to hepatocellular carcinoma cancer, but actually examined the serum and liver concentrations of PFAS, including PFHxS, among patients who had liver

[32]Minimal refers to average histological severity grade as follows: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked) as determined by NTP (2018c).

transplants—some of whom had hepatocellular carcinoma cancer; this study did not assess cancer risk and was not evaluated for study quality.

No animal in vivo, mutagenicity or genotoxicity studies were identified in the database.

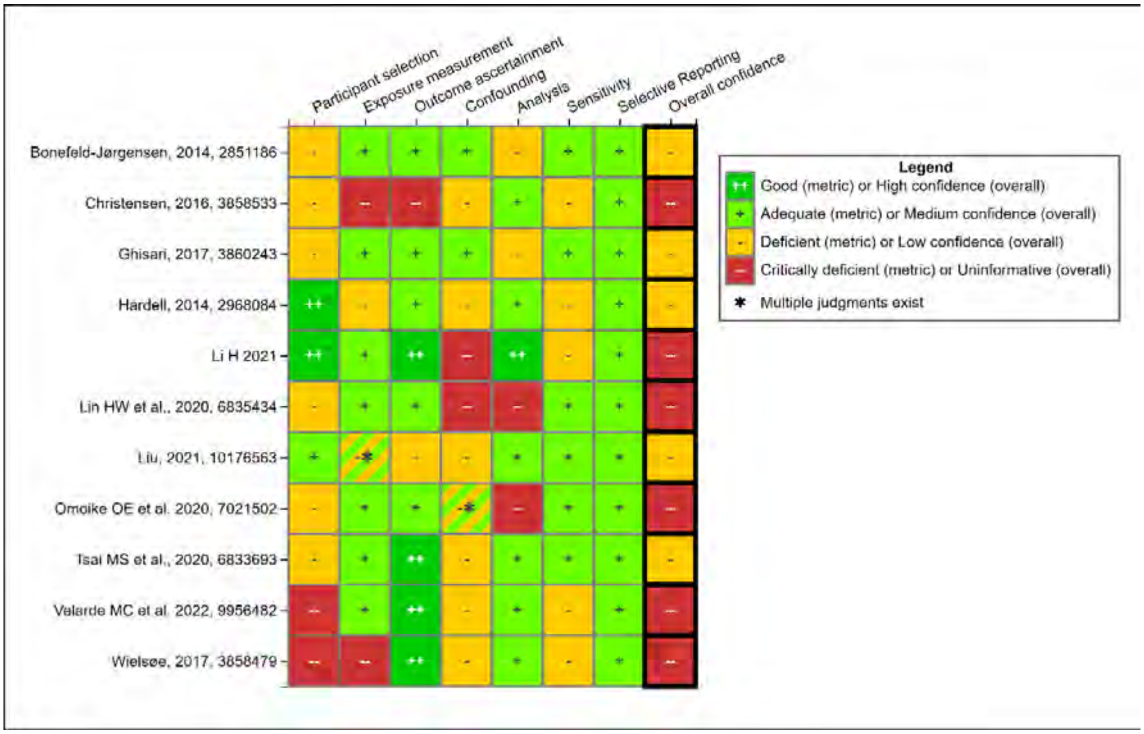

Figure 3-95. Study evaluation results for epidemiology studies of PFHxS and cancer. For additional details see HAWC link.

Human Studies

The study of prostate cancer (Hardell et al., 2014) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant time period, potential for confounding, insufficiencies in the analysis, and concerns about sensitivity (see Figure 3-95). Hardell et al. (2014) reported a non-significantly increased risk of prostate cancer among men with PFHxS concentrations in blood that were above the median value; and a higher, borderline significant, risk of prostate cancer among men with PFHxS concentration greater than the 75th percentile. Hardell et al. (2014) also reported that men with PFHxS concentrations above the median and with a first-degree relative with prostate cancer were at significantly increased risk. The study of thyroid cancer (Liu et al., 2021b) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant time period, deficiencies regarding the outcome definition, and potential for confounding, (see Figure 3-95). Liu et al. (2021b) reported significantly decreased risk of thyroid cancer associated with increasing quartiles of PFHxS. The first study of breast cancer (Bonefeld-Jørgensen et al., 2014) was *low* confidence due to concerns about participant selection and potential selection bias as there was: (1) no explanation of why 29% of cases were withdrawn from the National Patient Registry, (2) no comparisons of the subjects' details between the withdrawn cases and the originally selected cases, and (3) no consideration of how the originally matched controls might no longer match the final set of cases. Bonefeld-Jørgensen et al. (2014) studied the effect of PFHxS on the risks of breast cancer in Danish women using a case-control study, and initially found a significantly decreased risk of breast cancer with increases in continuously measured PFHxS, although in subsequent analyses, excluding 72 breast cancer cases (29% of the cases) which were withdrawn from the National Patient Registry, the effects changed slightly and lost statistical significance. The second of breast cancer Ghisari et al. (2017) was *low* confidence because it was based on the same case-control as Bonefeld-Jørgensen et al. (2014) and had the same deficiencies. Ghisari et al. (2017) investigated genetic polymorphisms as potential effect modifiers of the risk of PFAS on breast cancer. They reported that none of the genetic polymorphisms evaluated was an effect modifier, but that some genotypes (CYP1B1 Val/Val, COMT Val/Val, CYP17 A1/A1 and CYP19 CT) were associated with significantly decreased risks of breast cancer associated with increased PFHxS exposure. The third study of breast cancer (Tsai et al., 2020) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant time period, potential for confounding, and concerns about low sensitivity (see Figure 3-95). Tsai et al. (2020) reported significantly increased risk of breast cancer per ln-transformed unit increase in PFHxS concentration in blood among women <= 50 years of age who were estrogen receptor positive; and non-significantly decreased risk of breast cancer per ln-transformed unit increase in PFHxS concentration in women <= 50 years of age and estrogen receptor negative and in all women > 50 years of age. In summary, the available epidemiologic evidence on PFHxS and the risk of cancer is limited and generally *uninformative*.

Animal Studies

No studies were identified in the evidence base evaluating the carcinogenicity of PFHxS in animals.

Evidence Integration Summary

The available evidence for any effect of PFHxS on the risk of developing or dying from cancer is scant, inconsistent, and limited to *low* confidence studies. Thus, the available human evidence on breast, thyroid or prostate cancer is considered *indeterminate* and, overall, based on EPA guidelines (U.S. EPA, 2005), there is ***inadequate information to assess carcinogenic potential.***

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

As described in detail in Section 3, the currently available evidence indicates that exposure to perfluorohexane sulfuric acid [PFHxS] and its related salts likely results in thyroid (see Section 3.2.1) and immune (see Section 3.2.2) effects in humans given sufficient PFHxS exposure conditions. These judgments are based primarily on data from epidemiologic studies for immune effects and on short-term (28-day exposure), and reproductive (gestational and postnatal exposure) oral exposure studies in rodents for thyroid effects. Further characterizations of the exposure conditions relating to these two identified hazards are provided in Section 5.

The hazard identification judgment that the **evidence indicates** PFHxS exposure is likely to cause thyroid toxicity, specifically decreased thyroid hormones, in humans given sufficient PFHxS exposure conditions, is based primarily on a short-term study and two multigenerational studies in rats reporting a consistent and coherent pattern of hormonal changes at PFHxS exposure levels ≥2.5 mg/kg-day. A consistent dose-dependent decrease of T4, and to a lesser extent T3, in adult and juvenile rats, with a magnitude of effect (up to 70%) in the absence of effects in TSH was observed (with males being more sensitive). In addition, one multigenerational study reported increased incidence of minimal thyroid hypertrophy and moderate hyperplasia in male rats after PFHxS exposure. Due to the similarities in thyroid hormone production between rodents and humans, the effects in rodents were considered relevant to humans. A detailed discussion of thyroid effects is included in Section 3.2.1.

The hazard identification judgment that the **evidence indicates** PFHxS exposure is likely to cause immunotoxicity in humans given sufficient exposure conditions is based on generally consistent evidence of reduced antibody response to vaccination at median blood concentrations of 0.2–0.6 ng/mL in children. The direction of association was generally consistent across studies and timing of exposure and outcome measures, although not all the results were statistically significant. Further, three studies reported higher odds of infectious disease with higher PFHxS exposure, including total infectious disease, lower respiratory infection, throat infection, pseudocroup, and gastroenteritis. Lastly, there was some evidence of hypersensitivity, based primarily on a single well-conducted study of asthma, although findings were inconsistent across studies. A detailed discussion of immune effects is included in Section 3.2.2.

The ***evidence suggests*** but is not sufficient to infer that, given sufficient exposure conditions, PFHxS exposure may result in adverse health effects on the hepatic, cardiometabolic, and neurodevelopmental systems, along with developmental effects. These judgments highlight the notable data gaps and uncertainties identified in the available epidemiological and experimental animal PFHxS studies (see Section 3.2.3, Section 3.2.4, Section 3.2.5, and Section 3.2.6). The uncertainties in the above-mentioned hazards were considered too large for developing toxicity values (see Section 5). However, to convey some sense of the magnitude of a potential estimate for developmental effects, calculations based on this suggestive evidence are provided for comparison purposes. The objective was to inform the database uncertainty factor (UF) for quantitative estimates of thyroid and immune effects.

For all other health effects described in Section 3 (i.e., renal, male, and female reproductive, cardiometabolic, hematopoietic, and other noncancer effects) the **evidence is inadequate** to assess whether PFHxS exposure might cause effects in humans. No quantitative estimates were attempted for these health effects.

The potential for multi-organ effects of PFHxS exposure exists. As an example, the reported hypertrophy and hyperplasia in the follicular epithelium cells of the thyroid and in the centrilobular hepatocytes in the F0 male rats exposed to 10 mg/kg-day PFHxS (Butenhoff et al., 2009) may be related effects. It has been shown that exposure to compounds that cause microsomal enzyme induction in the liver can result in a compensatory hypertrophy and hyperplasia of the thyroid due to increased plasma turnover of T4 and TSH (Butenhoff et al., 2009; Sanders et al., 1988). However, as discussed in Section 3.2.1, the authors did not measure thyroid hormones as part of their study design and therefore the reported observation that thyroid hypertrophy and hyperplasia are compensatory mechanisms due to turnover of T4 and TSH is speculative. In addition, decreases in T3 and T4 observed in adult and juvenile animals exposed to PFHxS could be linked to metabolic effects as well as neurodevelopmental effects such as cognitive decline in children discussed in detail Section 3.2.1). Lastly, the decreased immune response observed in children exposed to PFHxS could lead to increased risk of infection as well as cancer (Germolec et al., 2022), although neither of these latter effects were well-studied in the available PFHxS evidence base.

Table 4-1. Hazard conclusions across published EPA PFAS human health assessments

Health outcome	PFAS assessments[a,b,c]							
	PFHxS	PFDA	PFHxA	PFBA	PFBS[d]	Gen X chemicals[d]	PFOA[d]	PFOS[d]
Endocrine/ Thyroid	+	-	+	+	+	ND	Human: + Animal: +/–	Human: +/– Animal: +/–
Hepatic/Liver	+/-	+	+	+	–	+	Human: + Animal: +	Human: – Animal: +
Developmental	+/-	+	+	+	+	+/–	Human: + Animal: +	Human: + Animal: +
Reproductive	-	-	–	–	–	+/–	Human: – Animal: +/–	ND
Immunotoxicity	+	-	–	–	–	+/–	Human: + Animal: +	Human: +/– Animal: +
Renal	-	-	–	–	+	+/–	Human: +/– Animal: +/–	ND
Hematopoietic/ Hematological	-	-	+	–	ND	+/–	ND	ND
Ocular	-	-	ND	–	ND	ND	ND	ND
Serum Lipids	-	+/-	ND	ND	–	ND	Human: + Animal: +	Human: +
Hyperglycemia	-	-	ND	ND	ND	ND	Human: – Animal: –	Animal: +/–
Nervous System	-	+/-	–	ND	ND	ND	Human: – Animal: –	Animal: +/–
Cardiovascular	-	+/-	ND	ND	–	ND	ND	ND
Cancer	-	-	–	–	–	+/–	+/–	+/–

[a]Assessments used multiple approaches for summarizing their noncancer hazard conclusion scales; for comparison purposes, the conclusions are presented as follows: '+' = evidence demonstrates or evidence indicates (e.g., PFHxA), or evidence supports (e.g., PFBS); '+/–' = suggestive evidence, '–' = inadequate evidence (e.g., PFHxA) or equivocal evidence (e.g., PFBS); '–/–' = sufficient evidence to conclude no hazard (no assessment drew this conclusion); ND = no data available for this outcome for this PFAS.

[b]The assessments all followed the EPA carcinogenicity guidelines (U.S. EPA, 2005) a similar presentation to that used to summarize the noncancer judgments is applied for the cancer hazard conclusions, as follows: '+' = carcinogenic to humans or likely to carcinogenic to humans;'+/–' = suggestive evidence of

carcinogenic potential; '−' = inadequate information to assess carcinogenic potential; '−/−' = not likely to be carcinogenic to humans(no assessment drew this conclusion); ND = no carcinogenicity data available for this PFAS.

[c]The hazard conclusions for the various EPA PFAS assessments presented in this table were not considered during evidence integration and thus did not inform the evidence integration conclusions presented in the PFHxA assessment.

[d]The U.S. EPA PFOA (U.S. EPA, 2016b) and PFOS (U.S. EPA, 2016a) assessments did not use structured language to summarize the noncancer hazard conclusions. The presentation in this table was inferred from the hazard summaries found in the respective assessments; however, this is for comparison purposes only and should not be taken as representative of the conclusions from these assessments. Those interested in the specific noncancer hazard conclusions for PFOA and PFOS must consult the source assessments. Note that new assessments for PFOA and PFOS are currently being finalized to support a National Primary Drinking Water Regulation; note that hazard conclusions in these updated assessments will differ from those presented in this table as the new assessments use structured language to summarize the noncancer hazard conclusions. For access to the more recent draft assessment materials please follow this link.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

The evidence currently available to make a judgment as to whether PFHxS exposure might affect the development of any specific cancers is scant, inconsistent, and limited to *low* confidence studies. Consistent with EPA guidance (U.S. EPA, 2005) to apply a standard descriptor as part of the hazard narrative and to express a conclusion regarding the weight of evidence for the carcinogenic hazard potential, a descriptor of ***inadequate information to assess carcinogenic potential*** is applied for PFHxS.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES

Understanding of potential areas of susceptibility to the identified human health hazards of PFHxS can help to inform expectations of variability in responses across individuals, as well as uncertainties and confidence in candidate toxicity values (see Section 5.2). The available human and animal evidence indicate that early lifestages represent a susceptible population for the adverse effects of PFHxS exposure. *High* confidence experimental studies report alterations in thyroid function, including reduced serum T4 and T3, after gestational and early postnatal PFHxS exposures in rats (see Section 3.2.1). In addition, *medium* confidence epidemiological studies report that exposure to PFHxS was associated with decreased immune response after routine vaccinations against tetanus and diphtheria vaccines in children at ages 5 and 7 (see Section 3.2.2). Although there are considerable uncertainties in the developmental epidemiological database (e.g., potential impact on PFHxS biomarkers due to pregnancy hemodynamics), consistent and coherent epidemiological findings on fetal growth restriction including several *medium* and *high* confidence developmental epidemiological studies also provide support for examination of critical in utero exposure windows (see Section 3.2.3).

The significant difference in clearance between male and female rats (7.2 vs. 84.1 mL/kg-day, respectively; see Section 3.1.4 for details) implies a sex-dependent susceptibility in that species: for given dose, blood and tissue levels are predicted and were observed to be significantly higher in male rats than female rats. While clearance levels in male and female mice were quite similar to each other (3.9 and 3.2 mL/kg-day), the markedly lower clearance in female mice compared to female rats predicts a strong species difference for susceptibility to developmental effects. Results for adult humans are consistently much lower than observed in either mice or rats (0.02-0.07 mL/kg-day), which is predicted to result in a strong species difference in susceptibility. But only one of the human studies observed a clear sex difference, with that in younger women being about 50% higher than men and older women (Zhang et al. (2013b); see Table 3-4). Additional clearance due to menstrual fluid loss could significantly reduce internal doses in women of childbearing age. The rate of menstrual fluid clearance estimated by Verner and Longnecker (2015) (0.033 mL/kg-day) is only slightly lower than (80% of) the geometric mean clearance for fecal and urinary elimination (0.041 mL/kg-day), so blood levels in a 30-year-old woman might be 55% of those in a 30-year-old man exposed to the same dose (Jain and Ducatman, 2022). In addition, serial blood measurement of PFHxS in pregnant women show that the decrease in clearance due to the lack of menstruation during pregnancy does not result in an increase in internal dose (Oh et al., 2022). This implies that other pharmacokinetic changes during pregnancy mediate the decreased clearance during that time and that the clearance for women of reproductive age (prior to pregnancy) is also appropriate for evaluating maternal dosimetry for developmental endpoints in humans. Animal-to-human extrapolations do account for the species- and sex-specific clearance observed among mice and rats, so in that regard PK-related susceptibility is addressed.

Given the effects seen in the developing individuals (i.e., altered thyroid and immune functions), prenatal and early postnatal lifestages represent a potentially sensitive population for the effects of PFHxS exposure. No evidence was available to inform other factors that could inform the potential for susceptibility to PFHxS exposure including demographics, genetic variability, health status, behaviors or practices or social determinants. The potential impact of these other susceptibility factors remains unknown.

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

The available evidence indicates that oral exposure to perfluorohexane sulfuric acid [PFHxS] and its related salts is likely to cause adverse immune effects in humans on the basis of the evidence presented in human studies and adverse thyroid effects on the basis of the evidence presented in animal toxicity studies. The dose levels associated with these two identified hazards were considered for the derivation of reference doses (RfDs) as presented below. The available **evidence suggests** but is not sufficient to infer that PFHxS exposure may result in developmental, neurodevelopmental, cardiometabolic, and hepatic effects. Given the uncertainty in these latter conclusions, ultimately no toxicity values were derived for these health effects. A dose-response assessment is typically not performed for health effect judgments of "**evidence suggests,**" although when the database contains at least one well-conducted study, quantitative analyses may still be useful for some purposes, such as providing a sense of the magnitude and uncertainty of estimates for health effects of concern, ranking potential hazards, informing responses in potentially susceptible populations and lifestages, or setting research priorities (U.S. EPA, 2020, 2005). The available evidence on PFHxS-induced developmental effects includes *high* confidence epidemiological studies in which the observed outcome (low birth weight) occurs during a susceptible lifestage and is associated with increased lifetime risk for developing a variety of adverse health conditions such as type 2 diabetes, cardiovascular disease, neurodevelopmental disorders, and renal disease (Tian et al., 2019a; Reyes and Mañalich, 2005; Hack et al., 1995). Thus, for comparison purposes during toxicity value derivation for immune and thyroid effects, a point of departure (POD) was estimated for developmental effects (see Section 5.2.1). For all other health effects (i.e., female, and male reproductive, hematopoietic, and renal) the currently available evidence is inadequate to assess whether PFHxS exposure might be capable of causing these potential health effects; therefore, these endpoints were not considered for the derivation of toxicity values.

There are no available studies to inform the potential for PFHxS to cause adverse health effects via inhalation exposure precluding the derivation of reference concentration (RfC) (see Section 5.2.3). Likewise, evidence pertaining to the evaluation of carcinogenicity was considered inadequate to assess carcinogenic potential of PFHxS in humans, precluding the derivation of cancer toxicity values via any exposure route (see Section 5.3).

5.2. NONCANCER TOXICITY VALUES

Noncancer toxicity values, including reference doses (RfDs) for oral exposure and reference concentrations (RfCs) for inhalation exposure, are estimates of an exposure for a given duration to the human population (including susceptible subgroups and/or life stages) that are likely to be without an appreciable risk of adverse health effects over a lifetime. The RfD derived in Section 5.2.1 corresponds to chronic, lifetime exposure and is the primary focus of this document. In addition, a less-than-lifetime, subchronic toxicity value (referred to as a “subchronic RfD”), which corresponds to exposure durations ranging from a month to 10% of the life span in humans, is derived in Section 5.2.2. Subchronic toxicity values may be useful for certain decision-making contexts (e.g., site-specific risk assessments with less-than-lifetime exposures). Both RfD and subchronic RfD derivations include organ-/system-specific RfDs (osRfDs) associated with health effect-specific PODs considered for use in deriving the RfD (or subchronic RfD). As with the subchronic RfD, osRfDs can be useful for certain decision-making contexts (e.g., cumulative risk assessment). Subsequent decisions related to dosimetric extrapolation, application of uncertainty factors, and confidence in toxicity values are discussed below. No information exists to inform the potential toxicity of inhaled PFHxS or derive an RfC; this decision is discussed in Section 5.2.3.

5.2.1. Oral Reference Dose (RfD) Derivation

Study/Endpoint Selection

Data sufficient to support dose-response analyses and POD calculations for oral exposure to PFHxS or its salts were available for both identified human health hazards: thyroid and immune effects. As mentioned above, although a definitive health hazard was not identified, a POD was also calculated for developmental effects because the evidence base for developmental effects caused by PFHxS includes well-conducted epidemiological studies. In addition, derivation of a POD for developmental outcomes was considered informative of the potential magnitude of effects relevant to susceptible populations and lifestages and thus might inform toxicity value derivation for thyroid or immune effects.

Rationales for study selection, details of the POD calculations, and toxicity value estimation, as well as determination of confidence in the derived toxicity values, are detailed in this section. The general considerations used to prioritize studies for estimating PODs for potential use in derivation of toxicity values are described in the IRIS PFAS Protocol (see Appendix A). Well-conducted (i.e., *high* or *medium* confidence) human studies that were deemed influential to the hazard conclusions were prioritized for POD derivation and compared with PODs derived from well-conducted animal studies when possible. Such human studies were available for developmental and immunotoxicity effects.

A summary of endpoints and rationales considered for toxicity value derivation is presented below.

Thyroid effects

Human studies provide conflicting evidence as to the potential effects of PFHxS on thyroid outcomes (e.g., thyroid hormone levels). While a few studies did suggest an association between increasing PFHxS exposure levels and decreased circulating thyroid hormones (i.e., T4) or subclinical thyroid disease, these associations were not consistent across studies (see Section 3.2.1 for details). Overall, the available human evidence on PFHxS effects on the thyroid was considered *indeterminate*, and thus these studies were not considered for use in deriving toxicity values.

The database of animal studies examining PFHxS-induced thyroid effects includes two short-term studies in rats and mice (Chang et al., 2018; NTP, 2018a) and two multigenerational reproductive studies (one study, two publications: Ramhøj et al. (2018) and Ramhøj et al. (2020); (Butenhoff et al., 2009)). Of these, a study in ICR mice (Chang et al., 2018) was judged as *low* confidence and thus was not considered for POD derivation, leaving three *high* confidence studies in SD rats (NTP, 2018a; Butenhoff et al., 2009) or Wistar rats (Ramhøj et al., 2020; Ramhøj et al., 2018).

NTP (2018a) examined effects on serum concentrations of total and free T4 in adult rats, while Ramhøj et al. (2018) evaluated effects of PFHxS on free T4 serum levels in exposed dams and their offspring (exposed during gestation and lactation) through PND 22. NTP (2018a) observed a statistically significant, dose-dependent decrease ($p < 0.01$) of free and total T4 levels starting at the lowest experimental dose (0.625 mg/kg-day) in male rats (up to 60% in free T4 and 78% decrease in total T4). In female rats, T4 levels were significantly decreased beginning at higher doses (12.5 mg/kg-day and above), with 38% decrease in free T4 and 33% decreases in total T4 at the highest dose (50 mg/kg-day) ($p < 0.01$). Ramhøj et al. (2018) reported similar findings to those reported by NTP (2018a) in Wistar rat dams, with statistically significant, dose-dependent decreases in serum-free T4 at 5 mg/kg-day and above in dams at PND 22 after exposure from GD 7 through PND16 or 17 (Ramhøj et al., 2018). In addition, Ramhøj et al. (2018) also reported statistically significant ($p < 0.001$) decreases in free T4 in the F1 offspring born from these PFHxS-exposed dams, with free T4 decreases at ≥5.0 mg/kg-day at both the end of exposure, PND16 or 17 (26%–32% decrease), and when pups were euthanized at PND22 (26%–71% decrease). Total T4 assay measurements are more reliable that those provided by the assays available to measure free T4 in rodents as these are insufficiently sensitive to measure the very small quantity of unbound (ie 'free') T4 in circulation and therefore less reliable than total T4 measurements (personal communication with Mary Gilbert, EPA, ORD). For this reason, total, but not free, T4 was moved forward for POD and candidate value derivation.

Two studies measured T3 in serum (Ramhøj et al., 2020; NTP, 2018a). NTP (2018a) observed a statistically significant and dose-dependent decrease ($p < 0.05$) in serum T3 levels in male, but not female, SD rats at ≥0.625 mg/kg-day ($p < 0.01$). Ramhøj et al. (2020) analyzed samples taken in Ramhøj et al. (2018) and observed a significant decrease in serum T3 in Wistar rat dams at the highest tested dose: 19% decrease at 25 mg/kg-day ($p < 0.001$) measured on PND 22 after exposure from GD 7 through postnatal day 16 or 17. Overall, for TH changes, findings for both T4 and T3 in nonpregnant adult females were relatively insensitive as compared with adult males and thus set aside from further consideration.

Butenhoff et al. (2009) reported increased incidences of hypertrophy/hyperplasia in the thyroid. In this 44-day exposure study, Butenhoff et al. (2009) observed increased incidences of hypertrophy (characterized as "minimal") of thyroid follicular epithelial cells in adult male rats that were exposed to 0.3 mg/kg-day PFHxS and an increase in "moderate" hypertrophy at the 10 mg/kg-day PFHxS dose for up to 44 days. Hypertrophy was not observed in control animals. Decreased thyroid hormone levels are judged relevant to human health, given the many similarities in the production, regulation, and functioning of thyroid hormones between rodents and humans (Vansell, 2022; Stagnaro-Green and Rovet, 2016; Dong et al., 2015; Navarro et al., 2014; Rovet, 2014; Berbel et al., 2010; Morreale de Escobar et al., 2008; Cuevas et al., 2005; Rovet, 2005; Zoeller and Rovet, 2004; Hood and Klaassen, 2000; Hood et al., 1999a; Hood et al., 1999b). In addition, rodents are known to be more sensitive to increases in thyroid follicular hypertrophy and hyperplasia than humans, and thus the observed changes in thyroid hormone levels (which are not known to suffer from this same limitation) were preferentially advanced over these histopathological changes for deriving points of departure and the increases in thyroid hypertrophy/hyperplasia were not considered further (see Table 5-1).

Table 5-1. Endpoints considered for dose-response modeling and derivation of points of departure for thyroid effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD Derivation	Notes
Decreased Total T4	NTP (2018a), high confidence	Gavage, 28 d	Rat/SD/Male	Yes	Dose-dependent effects were observed across sexes, but responses were much more sensitive in males, even after considering sex-dependent PK differences.
	Ramhøj et al. (2018), high confidence	Exposure in utero and lactation GD7–PND16 or 17; measurements taken at PND 16/17	Rat/ Wistar /F1 Combined[a]	Yes	Dose-dependent effects in combined serum from (male plus female) offspring were consistent across timepoints. Responses in dams were much less sensitive.
		Exposure in utero and lactation GD7–PND16/17 measurements taken at PND 22	Rat/ Wistar /F1 Combined[a]	Yes	
		Gavage	Rat/ Wistar /P0 Female	No	

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD Derivation	Notes
		GD7–PND16; Free T4 measured at GD15			
		Gavage GD7–PND 16; Free T4 measured at PND 22	Rat/ Wistar /P0 Female	No	
Decreased T3	NTP (2018a), high confidence	Gavage, 28 d	Rat/SD/Male	Yes	Dose-dependent effects were only observed in male rats.
	Ramhøj et al. (2020), high confidence	Gavage GD7–PND16/17; T3 measured at PND 22	Rat/ Wistar /P0 Female	No	Decrease was only observed in exposed dams and F1 pups at the highest dose. Responses in dams were much less sensitive.
		In utero and lactation GD7–PND16/17 measurements taken at PND 16/17	Rat/ Wistar /F1 Combined[a]	Yes	
Thyroid histopathology	Butenhoff et al. (2009)	44 d	Rat/SD/P0 Male	No	Concern for potential reduced human relevance as compared with TH measures.

[a]Ramhøj et al. (2018) reported as combined male and female fetal and juvenile rats; individual female pup data not reported. TH= Thyroid hormone.

Immune effects

Consistent findings of reduced antibody responses from human epidemiological studies provide *moderate* human evidence of immunosuppression with PFHxS exposure. This conclusion is based primarily on two *medium* confidence studies (reported in three publications) in children (Grandjean et al., 2017b; Grandjean et al., 2017a; Grandjean et al., 2012), supported by additional studies in children and adults (Kielsen et al., 2016; Stein et al., 2016b; Stein et al., 2016a; Granum et al., 2013). Although there may be some residual uncertainty regarding the potential for confounding by other PFAS, including PFOA and PFOS, the evidence overall supports a concern for immunosuppression in PFHxS-exposed humans.

The two *medium* confidence studies of antibody response following vaccination are birth cohorts of similar populations in the Faroe Islands (see Table 5-2) (Grandjean et al., 2017b; Grandjean et al., 2017a; Grandjean et al., 2012). Across these studies, PFHxS exposure was measured during gestation, and at 18 months and 5, 7, and 13 years, and measures of antibody levels were taken at 5, 7, and 13 years for both diphtheria and tetanus. Inverse associations, indicating immunosuppression, were generally observed between PFHxS exposure and antibody levels across different combinations of timing of exposure and outcome measures, and similar findings were reported for other long-chain PFAS. However, there are a minority of combinations for which positive associations (higher antibody levels with higher PFHxS exposure) were observed (not statistically significant). This heterogeneity in results does not have a clear biologic explanation and the relevant etiologic window of exposure for this outcome is not known, although (Grandjean et al., 2017b) noted that associations were generally weaker for two early life windows of PFHxS when exposures were measured at 18 months (as compared to PFHxS exposures measured prenatally or in early infancy) antibodies were measured at age 5 years, and for PFHxS exposures measured at 5 years of age and antibodies measured at age 5 years. Still, given the inverse associations observed for most of the exposure-outcome combinations and the low risk of bias in these studies (sensitivity was the primary concern), they are considered appropriate candidates for POD derivation. In Budtz-Jørgensen and Grandjean (2018), the study authors performed benchmark dose modeling for a subset of the data presented in these papers, specifically antibody levels at age 7 and PFHxS concentrations at age 5, and antibody levels at age 5 (prebooster) and perinatal PFHxS concentrations. The authors selected these combinations due to the strong inverse associations and because they are reasonably representative of the study results across exposure/outcome combinations, so after review of the BMD methods, their exposure-response results were used to inform the benchmark dose analyses. EPA selected a different BMR in deriving the BMDs and BMDLs (see Appendix E, Section 1 for more details).

Table 5-2. Endpoints considered for dose-response modeling and derivation of points of departure for immune (decreased serum antibody) effects in humans

Study reference and confidence	Antibody type; Measurement timing	POD derivation	Notes
Antibody concentrations for diphtheria and tetanus	Grandjean et al. (2012) and Grandjean et al. (2017a); Grandjean et al. (2017b); medium confidence	No	Effect was generally coherent with epidemiological evidence for other antibody effects. However, while these results contribute to understanding the hazard for PFHxS, the analytic models in these specific publications used log-transformed exposure and log-transformed outcome variables and such log-log models cannot be used for BMD calculations and thus PODs were not derived.
Budtz-Jørgensen and Grandjean (2018) using data from Grandjean et al. (2017b); (Grandjean et al., 2017a); Grandjean et al. (2012)	Decreased serum anti-tetanus antibody concentration in children at age 7 yrs and PFHxS measured at age 5 yrs	Yes	Both vaccine antibody types and the two exposure and outcome measurement timing combinations were generally coherent with the broader epidemiological evidence for antibody effects. Results were based on analytic models using log-transformed outcome and untransformed exposure which were suitable for BMD calculations and POD derivations (see Appendix D1 for more details on BMD modeling results).
	Decreased serum anti-diphtheria antibody concentration in children at age 7 yrs and PFHxS measured at age 5 yrs	Yes	

Study reference and confidence	Antibody type; Measurement timing	POD derivation	Notes
medium confidence	Decreased serum anti-tetanus antibody concentration in children at age 5 yrs and PFHxS measured perinatally	Yes	
	Decreased serum anti-diphtheria antibody concentration in children at age 5 yrs and PFHxS measured perinatally	Yes	

Developmental effects

Although the human evidence on developmental effects was highly uncertain and ultimately judged as *slight* (see Section 3.2.3), the database includes several well-conducted *medium* and *high* confidence epidemiological studies reporting birth weight deficits of varying magnitude in male or female neonates or both. A meta-analysis of the available studies showed a small but statistically significant decrease in birth weight per each ln-unit increase in PFHxS exposure (see Section 3.2.3; and Appendix C). However, in contrast to previous meta-analyses for PFOS and PFOA (Dzierlenga et al. (2020) and Steenland et al. (2018)), differences in detected deficits based on sample timing were evident for early sampled studies as well as *high* and *medium*/*high* confidence studies combined. Notably large effects were seen for postpartum measures, but this stratum was based on considerably fewer studies. This suggests that studies based on post-partum samples may be most prone to potential bias from pregnancy hemodynamics, but the meta-analytical data are indicative of complex patterns of influence due to pregnancy hemodynamic that are not completely understood. Nevertheless, the apparent influence of pregnancy hemodynamics introduces considerable uncertainty in the interpretation of these associations of evidence of PFHxS-induced developmental effects and was a major contributing factor in the overall evidence integration judgment for this health effect (see Section 3.2.3). Despite these important concerns regarding sample timing, as noted above, derivation of a POD(s) for developmental outcomes was considered potentially informative to toxicity value derivation for thyroid or immune effects.

For developmental effects, 22 epidemiology studies evaluated associations between PFHxS exposure and fetal growth restriction, seven of which were considered *high* confidence. Three of these *high* confidence studies measured maternal blood levels of PFHxS in the first trimester (Buck Louis et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a). One study each sampled in the second (Shoaff et al., 2018) third trimester (Valvi et al., 2017), while two studies collected samples across multiple trimesters (Starling et al., 2017; Bach et al., 2016).

Five of the seven *high* confidence studies reported adverse associations between birth weight and PFHxS, with no evidence of adverse associations reported in Valvi et al. (2017) or Sagiv et al. (2018).

Thus, the five *high* confidence studies considered for illustrative use in dose-response analysis (see Table 5-3) were: Buck Louis et al. (2018); Shoaff et al. (2018), Starling et al. (2017), Manzano-Salgado et al. (2019), and Bach et al. (2016). These studies showed consistent results especially when re-expressed on the ln-unit scale for consistency (range: −12 to −22 grams per each ln-unit PFHxS increase).

As previously described, while no toxicity value for developmental effects will be derived due to the high uncertainty of any such value as compared with values based on thyroid or immune effects, the PODs for developmental effects are still useful for the purposes delineated above in Section 5.1.

Table 5-3. Mean birth weight deficit studies considered for dose-response modeling and derivation of points of departure for developmental effects in humans

Study reference and confidence	Population-overall population, sex-specific and all births vs. term births only	PFHxS biomarker sample timing	POD derivation	Notes
Buck Louis et al. (2018); high confidence	Overall population; term births	Trimester 1	Yes	Effect size was large in magnitude; study showed some association for other endpoints such as birth length deficits. Maternal samples were collected during trimester one (range: 10–13.9 wks) which should minimize the pregnancy hemodynamic impact.
Manzano-Salgado et al. (2019); high confidence	Overall population; all births	Trimester 1	Yes	Results based on continuous exposure increases were moderate in magnitude and consistent with larger birth weight deficits based on categorical data; study showed some coherence across other endpoints such as postnatal growth and other fetal growth indices. Maternal samples were collected during trimester one (mean = 12.3 wks) which should minimize the pregnancy hemodynamic impact. Multi-PFAS models were developed.
Shoaff et al. (2018); high confidence	Overall population; term births	Trimester 2	Yes	Effect size was moderate in magnitude; study showed some coherence across other endpoints such as postnatal growth. Although the mean reported sampling period was 18 wks, it was variable across study participants (range: 16–40 wks) which may make a subset of these data (i.e., those with later sampling) more prone to potential bias from pregnancy hemodynamic changes.

Study reference and confidence	Population-overall population, sex-specific and all births vs. term births only	PFHxS biomarker sample timing	POD derivation	Notes
Starling et al. (2017); high confidence	Overall population; term births	Trimesters 2–3	Yes	Effect size was moderate in magnitude. Multi-PFAS models were developed. Median of 27 gestational wks of sampling. Concerns regarding the influence of pregnancy hemodynamic changes are generally greater for any trimester three PFHxS measures, but authors statistically adjusted for sampling timing.
Bach et al. (2016); high confidence	Overall population; sex-specific; term births	Trimester 1–2	Yes[a]	Results based on continuous exposure increases were moderate in magnitude and consistent with larger deficits based on categorical data and across sexes; this study also showed some coherence across other endpoints such as head circumference. Maternal samples were largely collected during trimesters one and two (mode: 12 wks) which may minimize the pregnancy hemodynamic impact.
Valvi et al. (2017); high confidence	Sex-specific; all births	Trimester 3	No	Study reported increased birth weight (i.e., no adverse effects).
Sagiv et al. (2018); high confidence	Sex-specific; term births	Trimester 1	No	Study showed mixed results.

[a]Study reported sex-specific findings that boys have larger deficits compared with girls. The associations between exposure and birth weight were not consistent across quantiles of exposures in girls. Results based overall population were used for POD derivation since the general population was the target population.

Estimation or Selection of Points of Departure (PODs)

Benchmark dose modeling

Consistent with EPA's Benchmark Dose Technical Guidance Document (U.S. EPA, 2012), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR to represent a minimal, biologically significant level of change. The BMD Technical Guidance (U.S. EPA, 2012) sets up a hierarchy by which benchmark responses (BMRs) are selected. The first and preferred approach uses a biological or toxicological basis to define what minimal level of response or change is biologically significant. In the absence of information regarding the level of change that is considered biologically significant, a BMR of 1 SD from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data is used to estimate the BMD and BMDL. The BMRs selected for dose-response modeling of PFHxS-induced health effects are listed in Table 5-4 along with the rationale for their selection. Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix D. When dose-response modeling was not feasible, or adequate modeling results were not obtained, no-observed-adverse-effect level (NOAEL) or lowest observed adverse effect level (LOAEL) values were identified and used as the POD.

Table 5-4. Benchmark response levels selected for BMD modeling of PFHxS outcomes

Endpoint	BMR	Rationale
Thyroid effects		
Decreased serum-total T4	1 standard deviation	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance (U.S. EPA, 2012) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify the BMR.
Decreased serum-total T3		
Immune effects		
Decreased antibody concentrations for diphtheria and tetanus in children	½ standard deviation	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance (U.S. EPA, 2012) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify the BMR. Diphtheria and tetanus are serious and sometimes fatal infections. In addition, childhood represents a sensitive lifestage when immunosuppression during the developmental stage may impede children's ability to protect against a range of immune hazards. Given the potential severity of this outcome, a BMR of ½ SD was selected (see additional discussion in Appendix D, Section 1.1).
Developmental Effects		
Decreased birth weight in humans	5% extra risk of exceeding adversity cutoff (hybrid approach)	A 5% extra risk is commonly used for dichotomous developmental endpoints as recommended by *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). For birth weight, a public health definition of low birth weight exists, and the hybrid approach was used to estimate the dose at which the extra risk of falling below that cutoff equaled 5% (see Appendix D).

When modeling was feasible, the estimated BMDLs were used as PODs (see Table 5-5). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix D. For the modeling of immune effects, potential confounding by other PFOS and PFOA was considered in the POD derivation by comparing the effect estimates from the analyses in and BMDLs for PFHxS from single-PFAS models against those from multi-PFAS models controlling for PFOS and PFOA in analyses by Budtz-Jørgensen and Grandjean (2018) (see

Appendix D, Section 1 for details). When dose-response modeling was not feasible, or adequate modeling results were not obtained, NOAEL or LOAEL values were identified based on biological rationales when possible and used as the POD. The PODs (based on BMD modeling or NOAEL/LOAEL selection) for the endpoints advanced for dose-response analysis are presented in Table 5-5 alongside the corresponding POD_{HED}s derived based on the PK extrapolations as described in Section 3.1.6.

Table 5-5. Points of Departure (PODs) considered for the derivation of PFHxS candidate toxicity values

Endpoint	Study/confidence	Species/ Sex	POD type (% change if NOAEL or LOAEL)	Free Acid POD (mg/kg-d)[f]	DDEF[c]	Free Acid POD_{HED}[d] (mg/kg-d)
Thyroid						
Decreased Total T4	28-d study NTP (2018a), *high* confidence	SD rat, male	LOAEL[a] (−44%)	0.684	5.73×10^{-3}	3.92×10^{-3}
	Multigenerational Study Ramhøj et al. (2018), *high* confidence	Wistar rat, Combined F_1 (PND 16/17)	NOAEL[b] (+4%)	0.051	4.88×10^{-4}	2.49×10^{-5}
Decreased T3	Multigenerational Study Ramhøj et al. (2020), *high* confidence	Wistar rat, Combined F_1 (PND 16/17)	NOAEL[b](−7%)	5.5	4.88×10^{-4}	2.68×10^{-3}
	28-d study NTP (2018a), *high* confidence	SD rat, male	LOAEL[a] (−22%)	0.684	5.73×10^{-3}	3.92×10^{-3}
Endpoint	**Study/Confidence**	**Species/Sex**	**POD type (% change if NOAEL or LOAEL)**	**POD (mg/kg-d)**	**$POD_{internal}$ (mg/L)**	**POD_{HED}[d] (mg/kg-d)**
Immune (developmental)						
Decreased serum anti-tetanus antibody concentration in children at age 7 and PFHxS conc measured at age 5	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012), *medium* confidence	Human (children)/both	$BMDL_{½ SD}$	--[e]	2.82×10^{-4}	1.16×10^{-8}
Decreased serum anti-diphtheria antibody concentration in children at age 7 and PFHxS conc measured at age 5	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012), *medium* confidence	Human (children)/both	$BMDL_{½ SD}$	--[e]	3.00×10^{-4}	1.23×10^{-8}

Decreased serum anti-tetanus antibody concentration in children at age 5 and PFHxS conc measured perinatally	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012), *medium* confidence	Human (children)/both	$\mathrm{BMDL}_{½\,SD}$	--[e]	1.44×10^{-2}	5.90×10^{-7}
Decreased serum anti-diphtheria antibody concentration in children at age 5 and PFHxS conc measured perinatally	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012), *medium* confidence	Human (children)/both	$\mathrm{BMDL}_{½\,SD}$	--[e]	1.37×10^{-2}	1.01×10^{-6}
Developmental[g]						
Decreased birth weight	Bach et al. (2016), *high* confidence	Human (newborn)/Both	BMDL_{5ER} , Hybrid	--[e]	1.12×10^{-3}	8.29×10^{-8}
	Buck Louis et al. (2018), *high* confidence	Human (newborn)/Both	BMDL_{5ER} , Hybrid	--[e]	1.71×10^{-3}	1.27×10^{-7}
	Manzano-Salgado et al. (2019), *high* confidence	Human (newborn)/Both	BMDL_{5ER} , Hybrid	--[e]	1.33×10^{-3}	9.84×10^{-8}

[a]No models provided adequate fit; therefore, a freestanding LOAEL, no NOAEL was identified as there were statistically significant effects in the lowest dose.
[b]No models provided adequate fit; therefore, NOAEL approach was used.
[c]For thyroid effects, POD_{HED} = POD × DDEF, where the DDEF corresponding to the rat sex for the observation is taken from **Error! Reference source not found.**Table 3-7; the lower DDEF for female rats used for observations in combined sex groups.
[d]For immune and developmental effects observed at PND 16/17 in rats or associated with serum concentrations measured in children at age 5 POD_{HED} was calculated assuming steady-state serum concentrations using CL for human males and older women, since the endpoint is assumed to depend on serum concentrations in the offspring, for which the lower clearance (not including menstrual fluid loss) is relevant. For effects observed at birth or associated with perinatal maternal serum concentrations, CL for humans included menstrual fluid loss, since maternal serum concentrations throughout pregnancy are similar to or below pre-pregnancy concentrations, which result from the total clearance of the reproductive age woman.
[e] BMD modeling was done on serum concentrations and hence there was no POD based on external dose.
[f]POD for PFHxS free acid were calculated by taking the LOAEL or NOAEL and multiplying by the ratio of potassium salt/ molecular weight of the free acid.
[g] Although PODs were derived for five birth weight studies (see above), there was less uncertainty in three developmental epidemiological studies noted here with earlier maternal biomarker sampling (Manzano-Salgado et al., 2019; Buck Louis et al., 2018; Bach et al., 2016).

Derivation of Candidate Lifetime Toxicity Values for the Reference Dose (RfD)

As discussed, below the developmental period is recognized as a susceptible lifestage when exposure during a critical time window is more relevant to the induction of adverse effects than lifetime exposure. Thus, the derivation of a lifetime value for developmental thyroid and immune endpoints following PFHxS exposure is supported. Exposure during pregnancy was also considered a potentially susceptible lifestage. Consistent with EPA guidelines (U.S. EPA, 1994), the thyroid hormone PODs following 28-day PFHxS exposure in adult SD rats were not considered for derivation of candidate lifetime values given the high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting. However, these endpoints were considered for the derivation of the subchronic RfD (see Section 5.2.2). Overall, the developmental immune endpoints from epidemiological studies and thyroid endpoints, specifically decreases in T3 and total T4, from a multigenerational rodent study of PFHxS, were preferentially advanced for the derivation of candidate lifetime values.

For developmental immune effects, POD_{HED} values were derived for decreased serum antibody levels (for both diphtheria and tetanus) in children (male and female) at different timing of exposure and outcome measurement combinations, specifically antibody levels at age 7 and PFHxS concentrations at age 5, and antibody levels at age 5 and perinatal PFHxS concentrations (Budtz-Jørgensen and Grandjean, 2018) (see Table 5-5). The $BMDL_{½SD(HED)}$ of 1.16×10^{-8} mg/kg-day for decreased serum anti-tetanus antibody concentrations at age 7 and PFHxS measured at age 5 is selected for the derivation of osRfDs for immune effects. Confidence in the BMDL estimate was highest (*medium* confidence) for this endpoint in comparison with other exposure-outcome combinations evaluated by Grandjean et al. (2012) and Budtz-Jørgensen and Grandjean (2018) based on a better fit model for PFHxS in the single-PFAS model and less uncertainty with respect to potential confounding with other co-occurring PFAS (i.e., PFOS and PFOA) (see Appendix D, Section1.1 for more details). The $BMDL_{½SD(HED)}$ of 1.23×10^{-8} mg/kg-day for decreased serum anti-diphtheria antibody concentrations at age 7 and PFHxS measured at age 5 is also selected for the derivation of osRfDs for immune effects. Confidence in this BMDL estimate was somewhat lower (*medium/low* confidence) for this endpoint than for anti-tetanus antibody concentrations at age 7 (see Appendix D, Section1.1 for more details). Further, although both tetanus and diphtheria are rare in the United States, tetanus remains more of a concern primarily among older adults, who are unvaccinated or inadequately vaccinated and therefore are at higher risk of disease and mortality (Liang et al., 2018). The estimated $BMDL_{½SD}$ (2.82×10^{-4} mg/L) for this endpoint in the single-PFAS model is at about the 10th percentile of the observed distribution. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFHxS well (see Appendix D, Section 1.1 for more details). The fact that the derived POD_{HED} for immune effects on both tetanus and diphtheria antibody concentrations at the same ages are relatively close (1.16×10^{-8} mg/kg-day versus 1.23×10^{-8} mg/kg-day) lends support to the choice of the POD_{HED} of 1.16×10^{-8} mg/kg-day for decreased serum anti-tetanus antibody concentrations at age 7 and PFHxS measured at age 5 for the derivation of the osRfD.

For thyroid osRfD, POD_{HED} values were derived for decreased total thyroxine (T4) as well as decreased triiodothyronine (T3) in a multigenerational reproductive study, with exposure including all of gestation (Ramhøj et al., 2020; Ramhøj et al., 2018) and a 28-day comprehensive toxicity study in rats (NTP, 2018a) (see Table 5-5). The POD_{HED} of 2.49×10^{-5} for decreased total T4 in combined F_1 Wistar rats is selected for the derivation of osRfD for thyroid effects as it was the most sensitive and reliable measure of thyroid hormone function (see Table 5-5). As described previously, although candidate toxicity values were not derived for developmental effects (decreased birth weight), PODs for this outcome were derived as they were considered informative of the magnitude of effects relevant to susceptible lifestages and may help inform uncertainty factor selection for developmental immune effects and thyroid effects.

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFHxS. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated uncertainty factor (UF) to be applied to the candidate POD_{HED} values are listed in Table 5-6, below.

Table 5-6. Uncertainty factors for the development of the lifetime RfD for PFHxS

	Value	Justification
UF_A	1	A UF_A of 1 is applied to the POD derived from developmental immune effects as these responses were observed in epidemiological studies.
	3	For thyroid effects, a UF_A of 3 is applied to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral PFHxS exposure. Some aspects of the cross-species extrapolation of pharmacokinetic processes have been accounted for using a DDEF to convert external doses from rodents to administered doses in humans; however, residual uncertainty related to potential pharmacodynamic differences remains.
UF_H	10	A UF_H of 10 is applied for developmental immune and thyroid effects. This is to account for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFHxS exposure in humans. (See discussion below for additional details).
UF_S	1	A UF_S of 1 is applied to reduced antibody responses in children (Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012). The developmental period is recognized as a susceptible lifestage when exposure during a critical window of development is more relevant than lifetime exposure in adulthood (U.S. EPA, 1991). Additional considerations for the UF_s for immune effects are discussed below.
	1	A UF_S of 1 is applied to thyroid effects observed in the F1 animals from reproductive study (Ramhøj et al., 2018); the developmental period is a susceptible lifestage where exposure during certain time windows (e.g., pregnancy and gestation) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL as is the case for developmental immune endpoint or POD is a NOAEL as is the case for the thyroid endpoint.

	Value	Justification
UF_D	3	A UFD of 3 is applied to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of high/medium confidence short-term studies in rodents and a high confidence developmental study in mice. The database for PFHxS also includes several high/medium confidence epidemiological studies most informative for immune and developmental effects, which are sensitive effects of PFHxS exposure. However, uncertainties remain regarding the lack of studies examining effects with long-term exposure in adults—including in women of reproductive age (which may have increased susceptibility), studies of potential multigenerational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity during developmental lifestages. In all, the data are too sparse to conclude with certainty that the quantified developmental effects are likely to be the most sensitive; thus, a UFD of 1 was not selected. However, a UFD of 10 was also not selected given the availability of data from well-conducted studies on a range of health outcomes in multiple species, including sensitive evaluations of developmental and immune endpoints in humans. See discussion below for additional details.
UF_C	See Table 5-8	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans, and accounts for uncertainty regarding the pharmacokinetic and pharmacodynamic differences across species. As is usual in the application of this uncertainty factor, the pharmacokinetic uncertainty is mostly accounted for through the application of dosimetric approaches for estimation of HEDs. This leaves some residual uncertainty around the pharmacokinetics and the uncertainty surrounding pharmacodynamics. For developmental immune effects, a $UF_A = 1$ was applied to the POD as these responses were observed in epidemiological studies. For thyroid effects, a $UF_A = 3$ was applied to the POD derived from rodent studies to account for interspecies uncertainty. While uncertainty in the pharmacokinetic processes has largely been accounted for by using a DDEF to convert external rodent doses to human administered doses, a $UF_A = 3$ was applied to address the remaining pharmacokinetic uncertainty and to address the pharmacodynamic uncertainty in extrapolating those effects to humans (see Uncertainty in HED Calculations for more details.).

For developmental immune effects in children, a UF_H of either 3 or 10 was considered. Specifically, it can be argued that the PODs are derived from susceptible individuals because children's immune systems are not fully formed and are presumably more sensitive to these effects than most other populations, and thus, the UF_H should be reduced (although uncertainty regarding differences across individuals exposed during this sensitive lifestage would still remain). However, a counter argument is that currently there are no data to compare the responses in children with other populations or lifestages, so it is unclear whether these individuals are indeed particularly susceptible to these specific effects. As described in U.S. EPA (2020), other factors, in addition to lifestage, may increase susceptibility, including: demographics, genetic variability, health status, behavior or practices, and social determinants. Ultimately, since the current evidence is insufficient to address these uncertainties, a UF_H of 10 is applied for developmental immune effects. For thyroid effects, a UF_H of 10 is applied to address differences due to intraspecies variability, including potentially more sensitive or severe effects in susceptible populations or lifestages.

The duration extrapolation factor (UF_s) accounts for the uncertainty in extrapolating from less than chronic PFHxS exposure to lifetime exposure. A UF_s = 1 was applied to the PODs for thyroid effects as the selected POD was derived from a reproductive study with exposure encompassing the critical window of gestation (Ramhøj et al., 2018). This developmental window is recognized as a susceptible lifestage when exposure is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). The reduced antibody responses were measured in children 5–7 years of age, which also constitutes a sensitive lifestage. However, given the slow clearance rates for this chemical, particularly in humans (see Table 3-5), PFHxS is expected to accumulate in the body through adulthood. Therefore, it is plausible that longer exposure durations can result in effects at lower exposure levels. Although the MOA for PFHxS-induced immunosuppressive responses in humans is unknown, early-life exposures may alter the immune system and lead to unpredictable outcomes later in life or during other susceptible lifestages of reduced immunocompetence such as pregnancy, advanced lifestages, or immunocompromised states (IPCS, 2012) that show increased sensitivity with continuous, longer-term exposures. Still, given the expectation that the children and their mothers have been exposed to elevated levels of PFHxS for many years, the observed effects on immune response are considered the result of a cumulative, prolonged PFHxS exposure to the subjects from conception until the age when the response was evaluated. Further, the consequences of perturbed immune system function (in this case, suppressed antibody responses leading to potentially increased risk of disease) during development are expected to be generally more severe and longer lasting that those that manifest in healthy adults. Thus, a UF_s of 1 was considered appropriate.

The database uncertainty factor (UF_D) is applied to account for the potential of deriving an under-protective reference value as a result of incomplete characterization of a chemical's toxicity (U.S. EPA, 2002). For PFHxS, a UF_D of 3 was selected to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of *high/medium* confidence short-term studies in rodents and a *high* confidence developmental study in mice. The database for PFHxS also includes several *high/medium* confidence epidemiological studies most informative for immune and developmental effects, which are sensitive effects of PFHxS exposure. However, uncertainties remain regarding the lack of studies examining effects with long-term exposure in adults—including in women of reproductive age (which may have increased susceptibility), studies of potential multigenerational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity during developmental lifestages. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. While the PFHxS database does include *high* confidence reproductive/developmental toxicity studies in rats and mice, these only span one-generation. Therefore, despite their quality, these studies fail to cover potential transgenerational impacts of longer-term exposures evaluated in two-generation studies. The availability of a two-generation multigenerational reproductive study could result in reference values below those currently derived for PFHxS. However, the concern over a lack of two-generation study in the available literature is diminished when the PFHxS, PFDA, PFOA, and PFOS evidence bases are considered together. Although limited in their ability to assess reproductive health or function, measures of possible reproductive toxicity occurred at doses equal to or higher than those that resulted in effects in other organ systems (e.g., thyroid, liver) when measured after exposure to PFHxS in utero through PND 22 (Ramhøj et al., 2018). Similar results were observed for the animal databases for PFOA and PFOS indicating reproductive effects were not uniquely sensitive markers of toxicity for these long-chain PFAS (ATSDR, 2018b). Further, no notable male or female reproductive effects were observed in epidemiological or toxicological studies investigating exposure to PFHxS (MDH, 2019). Given these overall uncertainties with the database, a 3-fold UF was applied.

The uncertainty factors described in Table 5-6 and the text above were applied and the resulting candidate values are shown in Table 5-7. The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor:

Candidate values for PFHxS $= POD_{HED} \div UF_C$.

Table 5-7. Lifetime candidate values for PFHxS

Endpoint	Study/ confidence	Strain/ species/sex	Free Acid POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)
Thyroid										
Decreased Total T4	Ramhøj et al. (2018), *high* confidence Wistar rat, combined F_1	Wistar rat, Combined F_1 (PND 16/17)	2.49×10^{-5}	3	10	1	1	3	100	2×10^{-7}
Decreased T3	Multigenerational Study Ramhøj et al. (2020), *high* confidence	Wistar rat, Combined F_1 (PND 16/17)	2.68×10^{-3}	3	10	1	1	3	100	3×10^{-5}
Developmental Immune Effects										
Decreased serum anti-tetanus antibody concentration in children at age 7	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012); *medium* confidence	Human (children), male and female	1.16×10^{-8}	1	10	1	1	3	30	4×10^{-10}
Decreased serum anti-diphtheria antibody concentration in children at age 7	Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012); *medium* confidence	Human (children), male and female	1.23×10^{-8}	1	10	1	1	3	30	4×10^{-10}

Selection of Lifetime Toxicity Value(s)

Selection of organ-/system-specific oral reference doses (osRfDs)

Table 5-7 shows osRfDs selected for the individual organ systems identified in Section 3.2 (i.e., thyroid and developmental immune effects).

The value of 4×10^{-10} mg/kg-day (rounded from 3.9×10^{-10} and, separately, 4.1×10^{-10} mg/kg-day in Table 5-7 for decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children (male and female) at age 7 years and PFHxS measured at age 5 years from the Grandjean et al. (2012) and Budtz-Jørgensen and Grandjean (2018) was selected as the osRfD for developmental immune effects. The respective POD_{HED} values for these two endpoints (decreased anti-tetanus as well as decreased anti-diphtheria antibodies) were close in value (1.16×10^{-8} versus 1.23×10^{-8}, respectively) and the candidate values round to the same toxicity value.

For the thyroid effects, an osRfD of 2×10^{-7} mg/kg-day (rounded from 2.49×10^{-7} in Table 5-7) was selected based on decreased total T4 in F1 pups exposed to PFHxS in the Ramhøj et al. (2018). As there was no other reason to select one POD over the other (e.g., different levels of confidence in the POD calculations), the more sensitive POD for total T4 was selected over the POD for T3.

The confidence decisions about the study, evidence base, quantification of the POD, and overall RfD for these organ-/system-specific values are described in detail in Table 5-8, along with the rationales for selection of confidence levels. In deciding overall confidence, confidence in the evidence base is prioritized over the other confidence decisions. The overall confidence in the osRfDs for both immune and thyroid effects is judged as *medium*. Selection of the overall RfD is described in the following section.

Table 5-8. Confidence in the organ-/system-specific RfDs for PFHxS

Confidence categories	Designation	Discussion
Thyroid 2×10^{-7} RfD = mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in Ramhøj et al. (2018) was *high* and is based on a well-designed experimental design using established approaches, recommendations, and best practices (HAWC link).
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for thyroid effects is medium based on consistent findings in animals of decreases in T3 and T4 in adult and juvenile rats in the absence of effects on TSH (NTP, 2018a; Ramhøj et al., 2018), but with unexplained inconsistency in the available epidemiological studies and other uncertainties (see Table 3-6).
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD_{HED} and osRfD is *medium* given POD was based on a NOAEL (data did not fit BMD models) and because a DDEF was applied to estimate the POD_{HED}. The uncertainty associated with the use of a DDEF is less than the uncertainty introduced from the use of a NOAEL because the DDEF is based on PFHxS-specific pharmacokinetic data (see Uncertainty in HED Calculations). Considering these limitations, confidence in the POD was *medium*.
Overall confidence in osRfD	*Medium*	The overall confidence in the osRfD is *medium*. The *medium* confidence in the POD derivation is offset by the *high* confidence in the study and *medium* confidence in the evidence base for thyroid effects.
Developmental Immune RfD = 4×10^{-10}		
Confidence in study[a] used to derive osRfD	Medium	Confidence in Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018) was rated as *medium* based on some concerns for sensitivity from narrow exposure contrast, which decreases confidence in null associations only (HAWC link).
Confidence in evidence base supporting this hazard	Medium	Confidence in the evidence base for immune effects is *medium* based on consistent findings of reduced antibody responses from two *medium* confidence birth cohort studies (Grandjean et al., 2017b; Grandjean et al., 2017a; Grandjean et al., 2012) and a *low* confidence study in adults (Grandjean et al., 2017b). Limitations in this evidence base include the lack of epidemiological studies in adults or long-term/chronic studies in animals, and a general lack of studies examining effects on the immune system across different developmental immunotoxicity categories, including sensitization and allergic response and autoimmunity and autoimmune disease.

Confidence categories	Designation	Discussion
Confidence in quantification of the POD_{HED}	Medium	The POD is based on BMD modeling within the range of the observed data and a $BMDL_{½SD}$ estimate that is associated with little uncertainty due to potential confounding by PFOA or PFOS (see Appendix D, Section 1.1 for more details). The POD_{HED}s for decreased anti-tetanus and decreased anti-diphtheria antibodies were close in value (1.16×10^{-8} vs. 1.23×10^{-8}, respectively) which increases confidence in the quantification of the POD_{HED}. There is uncertainty as to the most sensitive window of vulnerability with respect to the exposure/outcome measurement timing (BMDs/BMDLs were estimated from PFHxS levels measured at age 5 or perinatally and anti-tetanus antibody concentrations measured at age 7 or 5) and the effect on antibodies at age 7 were more sensitive that those measured at age 5 (see Appendix D, Section 1.1 for more details); however, Grandjean et al. (2017b) reported that *estimated* PFOS and PFOA "concentrations at 3 m and 6 m showed the strongest inverse associations with antibody concentrations at age 5 yrs, particularly for tetanus." Thus, it is possible that adverse effects of PFHxS during infancy could be more sensitive than between ages 5 and 7 yrs.
Overall confidence in osRfD	Medium	The overall confidence in the osRfD is *medium* and is driven by *medium* confidence in the evidence base for immune effects, the quantification of the POD, and the study used for BMD modeling.

[a]All study evaluation details can be found on HAWC.

Selection of overall reference dose (RfD) and confidence statement

Table 5-9. RfD and organ-/system-specific RfDs for PFHxS

Reference Dose (RfD)					
Basis		**RfD (mg/kg-d)**		**Confidence**	
Immune (developmental) effects		**4×10^{-10}**		***Medium***	
Organ-/system-specific RfDs (osRfDs)					
Organ / System	**Outcomes and studies**	**POD_{HED} (mg/kg-d)**	**UFC**	**osRfD (mg/kg-d)[a]**	**Confidence**
Thyroid	Decreased serum Total T4 in F1 Wistar rats (Ramhøj et al., 2018)	2.49×10^{-5}	100	2×10^{-7}	*Medium*
Immune (developmental)	Decreased serum anti-tetanus and anti-diphtheria antibody concentrations measured in children at age 7 with PFHxS exposure measured at age 5 Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018); Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012)	1.16×10^{-9} and 1.23×10^{-9}	30	4×10^{-10}	*Medium*

[a]The RfD or osRfD values for different salts of PFHxS would be calculated by multiplying the RfD or osRfD values for the free acid of PFHxS (i.e.., the toxicity values in the table above) by the ratio of molecular weights. For example, for the potassium salt the ratio would be: $\frac{MW\ apotassium\ salt}{MW\ free\ acid} = \frac{438}{400} = 1.095$. This same method of conversion can be applied to other salts of PFHxS, such as the ammonium or sodium salts, using the corresponding molecular weights.

From the identified human health effects of PFHxS and derived osRfDs for thyroid and developmental immune effects (see Table 5-10), an RfD of 4 × 10^{-10} mg/kg-day was selected based on decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children. As described in Table 5-9, confidence in the RfD is *medium*, based on *medium* confidence in the developmental immune osRfD. This osRfD is based on the two lowest POD_{HED}s available on PFHxS immune effects (an evidence based interpreted with *medium* confidence) using a study considered *medium* confidence. The selected osRfD is based on effects in children and expected to be protective across all lifestages. The selection considered both available osRfDs as well as the overall confidence and composite uncertainty for those osRfDs. The thyroid osRfD was based on application of a composite uncertainty threefold greater than that applied in deriving the immune osRfD. Further, when comparing the sensitivity of thyroid and immune osRfDs, the thyroid value is over 3,000-fold higher. Had the osRfD for thyroid effects been chosen as the overall RfD, this would have raised concerns over the ability of the thyroid RfD to be protective against potential immune effects (and it may not be protective against other developmental effects, such as decreased birth weight (see Table 5-6) if those other effects could be reliably quantified). Selection of the RfD on the basis of developmental immune effects is presumed to be protective of possible thyroid and other potential adverse health effects (including potential effects on birth weight) in humans. Finally, since the developmental immune osRfD is based on effects observed in males and females, the overall RfD would be protective for both sexes.

5.2.2. Subchronic Toxicity Values for Oral Exposure (Subchronic Oral Reference Dose [RfD]) Derivation

In addition to providing an RfD for lifetime exposure in health systems, this document also provides an RfD for less-than-lifetime ("subchronic") exposures. These candidate subchronic toxicity values were based on the endpoints and PODs in Table 5-5 including the shorter duration studies that were not advanced for consideration in developing the lifetime RfD. Given that the immune and thyroid effects considered for the RfD were observed after exposure to PFHxS during susceptible lifestages, these endpoints were also considered for the derivation of candidate subchronic toxicity values, applying identical uncertainty factors to those used for the lifetime RfDs (see Table 5-6).

The datasets advanced for derivation of the subchronic toxicity values were selected on the basis of several considerations, including whether there is an endpoint with less uncertainty and/or greater sensitivity, and whether the endpoint is protective of both sexes and all lifestages. Ultimately, similar to the datasets advanced for the lifetime thyroid osRfD derivation, decreased total T4 and decreased T3 endpoints from the Ramhøj et al. (2018) study was advanced over identical endpoints from the *high* confidence NTP (2018a) study. This is because the Ramhøj et al. (2018) study included exposure to PFHxS during gestation, this exposure is interpreted as a critical sensitive window for effects on the developing thyroid system. Further, consistent with the decision when estimating the lifetime osRfD, the POD for total T4 was advanced over the POD for T3 from

Ramhøj et al. (2018) given the increased sensitivity of the POD. The $NOAEL_{HED}$ of 2.49×10^{-5} mg/kg-day for decreased total T4 in F1 generation rats in the Ramhøj et al. (2018) study was selected for the thyroid subchronic osRfD (see Table 5-5). The UFs applied to the derivation of a subchronic RfD thyroid POD in rat offspring are the same as those applied in the derivation of lifetime RfD values. See Table 5-6 for details.

Likewise, the same datasets on developmental immune effects were advanced for derivation of the subchronic osRfD, with the same inherent confidence and uncertainties.

Selection of Subchronic Toxicity Value(s)

As described above, subchronic osRfDs associated with each health effect are presented as they may be useful for certain decision purposes (i.e., site-specific risk assessments with less-than-lifetime exposures). The osRfD values selected were associated with decreased serum anti-tetanus antibody concentrations for immune effects and decreased total T4 levels for thyroid effects. Confidence in each osRfD is described in Table 5-8 and consider confidence in the study used to derive the quantitative estimate, the overall health effect, specific evidence base, and quantitative estimate for each osRfD.

Selection of Subchronic RfD and Confidence Statement

Organ-/system-specific subchronic RfD values for PFHxS selected in the previous section are summarized in Table 5-10.

Table 5-10. Subchronic RfD organ-/system-specific RfD values for PFHxS

Subchronic Reference Dose (RfD)		
Basis	**RfD (mg/kg-d)**	**Confidence**
Immune (developmental) effects	**4×10^{-10}**	***Medium***

Subchronic organ-/system-specific RfDs					
Organ / system	**Outcomes and studies**	**POD_{HED} (mg/kg-d)**	**UF_C**	**osRfD (mg/kg-d)**	**Confidence**
Thyroid	Decreased serum T4 (free) in F1 Wistar rats Ramhøj et al. (2018)	2.49×10^{-5} (NOAEL)	100	2×10^{-7}	*Medium*
Immune (developmental)	Decreased serum anti-tetanus and anti-diphtheria antibody concentrations measured in children at age 7 with PFHxS exposure measured at age 5 Grandjean et al.	1.16×10^{-8} and 1.23×10^{-8} ($BMDL_{½SD}$)	30	4×10^{-10}	*Medium*

Subchronic Reference Dose (RfD)		
Basis	**RfD (mg/kg-d)**	**Confidence**
Immune (developmental) effects	**4×10^{-10}**	***Medium***

Subchronic organ-/system-specific RfDs					
Organ / system	**Outcomes and studies**	**POD_{HED} (mg/kg-d)**	**UF_C**	**osRfD (mg/kg-d)**	**Confidence**
	(2012); Budtz-Jørgensen and Grandjean (2018); Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018) $1.16–1.23 \times 10^{-9}$				

From the identified targets of PFHxS toxicity and derived subchronic osRfDs (see Table 5-10), an RfD of 4×10^{-10} mg/kg-day based on decreased serum anti-tetanus and diphtheria antibody concentrations in children is selected for less-than-lifetime exposure. Confidence in the RfD is *medium*, based on *medium* confidence in the immune osRfD, as described in Table 5-8. The considerations for selecting the immune osRfD for the lifetime RfD are the same as those applied in selecting the subchronic RfD.

5.2.3. Inhalation Reference Concentration (RfC) Derivation

No studies examining inhalation effects of short-term, subchronic, chronic, or gestational exposure for PFHxS in humans or animals have been identified, precluding the derivation of an RfC.

5.3. CANCER TOXICITY VALUES

Considering the limitations in the PFHxS evidence base on cancer (see Section 3.3) and in accordance with the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), EPA concluded that based on the available evidence, a classification of "Inadequate Information to Assess Carcinogenic Potential" of PFHxS in humans. The lack of adequate carcinogenicity data for PFHxS precludes the derivation of quantitative estimates of cancer for either oral (e.g., an oral slope factor [OSF]) or inhalation (e.g., an inhalation unit risk [IUR]) PFHxS exposure.

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Attachment

B

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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

AFFIDAVIT OF CAROL HAWBAKER

I, Carol Hawbaker, certify under penalty of perjury pursuant to Section 1-109 of the Illinois Code of Civil Procedure, 735 ILCS 5/1-109, that the statements set forth in this affidavit are true and correct.

1. I am the Manager of the Office of Toxicity Assessment ("OTA") of the Illinois Environmental Protection Agency's ("Illinois EPA") Associate Director's Office ("ADO").
2. I have been employed at Illinois EPA since September of 2000, first as a Project Manager in Bureau of Land's Leaking Underground Storage Program; then, as a Lead Worker and Environmental Risk Assessor in OTA. Finally, I became Manager of OTA in November of 2023.
3. As the Lead Worker, and now Manager, for OTA, my primary responsibilities include the development and use of procedures for toxicity and environmental risk assessments, review of toxicity and risk data in support of Illinois EPA programs, review of human health and ecological risk assessment for projects enrolled in state and federal programs, and review of exposure, risk assessment and fate and transport models.
4. I am a member of U.S. Environmental Protection Agency's ("U.S. EPA") Environmental Council of States and Association of State and Territorial Health Officials PFAS Science Group, a participant in State Risk Assessors Teleconference

Group, and a member of the Interstate Technology and Regulatory Council ("ITRC") PFAS Team, currently working on updates to its PFAS webpage and Fact Sheets. I also participate in workgroups within the Illinois EPA focusing on updates to cleanup objectives and procedures utilized in developing cleanup objectives, including taking a lead role in drafting updates to 35 Ill. Adm. Code 742.

5. I received a Bachelor in Science in History from Illinois State University in 1995 and subsequently received 48 hours of educational credit toward a Master's in Science degree in Environmental Studies with a focus on environmental risk assessment and toxicology; after which I received certification in Environmental Risk Assessment.
6. I have provided the Agency's answers to the Board's questions 2, 3, 4, 5, 6, 7 and 10.

FURTHER AFFIANT SAYETH NOT

Carol Hawbaker

CAROL HAWBAKER

4/25/24

DATE

OFFICIAL SEAL
MOLLY S. GILLELAND
NOTARY PUBLIC, STATE OF ILLINOIS
MY COMMISSION EXPIRES 10-08-2025

State of Illinois
County of Sangamon

Subscribed and Sworn to before me this 25 day of April, 2024.

Molly Gilleland

Notary Public

Attachment

C

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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

AFFIDAVIT OF LYNN DUNAWAY

I, Lynn Dunaway, certify under penalty of perjury pursuant to Section 1-109 of the Illinois Code of Civil Procedure, 735 ILCS 5/1-109, that the statements set forth in this affidavit are true and correct.

1. I have a Bachelor of Science in Geology.
2. I have been a licensed geologist since 1997.
3. I began working for the Illinois EPA in the Groundwater Section in 1988 and retired from the Groundwater section in 2023.
4. I have been working on contract in the Groundwater Section since January 2024.
5. I have used and participated in Part 620 updates since adoption of the regulation in 1991.
6. I have provided the Agency's answers to the Board's questions 1, 8, and 10.

OFFICIAL SEAL
MOLLY S. GILLELAND
NOTARY PUBLIC, STATE OF ILLINOIS
MY COMMISSION EXPIRES 10-08-2025

FURTHER AFFIANT SAYETH NOT

Lynn Dunaway

LYNN DUNAWAY

4/24/24

DATE

State of Illinois
County of Sangamon

Subscribed and Sworn to before me this 24 day of April, 2024.

Molly Gilleland

Notary Public