EPA/635/R-23/148b
External Review Draft
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IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 355-46-4) and Related Salts Supplemental Information—Appendices A–G

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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ABBREVIATIONS

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)
CI	confidence interval
CL	confidence limit
CNS	central nervous system
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	food expiratory volume of one second
GDH	glutamate dehydrogenase
GGT	conflict of interest
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of one second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GLP	Good Laboratory Practice
GSH	glutathione
GST	glutathione-*S*-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HBCD	hexabromocyclododecane
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
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
NCEA	National Center for Environmental Assessment
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
PBPK	physiologically based pharmacokinetic
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
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum 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

APPENDIX A. SYSTEMATIC REVIEW PROTOCOL

A single systematic review protocol was used to guide the development of five, separate IRIS PFAS assessments (i.e., PFBA, PFHxA, PFHxS, PFNA, and PFDA). This "systematic review protocol for the PFAS IRIS assessments" was released for public comment and subsequently updated. The updated protocol and prior revisions can be found at the following location: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065.

APPENDIX B. LITERATURE SEARCH STRATEGY AND POPULATIONS, EXPOSURES, COMPARATORS, AND OUTCOMES (PECO) CRITERIA

B.1. LITERATURE SEARCH AND SCREENING STRATEGY

Table B-1. Summary of detailed search strategies for perfluorohexanesulfonic acid and related salts (PubMed, Web of Science, Toxline, TSCATS, Toxcenter)

Database	Terms	Hits
Initial strategy		
PubMed 7/24/17	108427-53-8[rn] OR 355-46-4[rn] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid"[tw] OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-"[tw] OR "1-Hexanesulfonic acid, tridecafluoro-"[tw] OR "1-Perfluorohexanesulfonic acid"[tw] OR "Perfluoro-1-hexanesulfonate"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "Perfluorohexanesulfonate"[tw] OR "Perfluorohexanesulfonic acid"[tw] OR "Perfluorohexylsulfonate"[tw] OR "Tridecafluorohexanesulfonic acid"[tw] OR "tridecafluoro-1-Hexanesulfonic acid"[tw] OR "PFHxS"[tw]	396

Database	Terms	Hits
PubMed 04/29/2020	(("108427-53-8"[rn] OR "355-46-4"[rn] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid"[tw] OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-"[tw] OR "1-Hexanesulfonic acid, tridecafluoro-"[tw] OR "1-Perfluorohexanesulfonic acid"[tw] OR "Perfluoro-1-hexanesulfonate"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "Perfluorohexanesulfonate"[tw] OR "Perfluorohexanesulfonic acid"[tw] OR "Perfluorohexylsulfonate"[tw] OR "Tridecafluorohexanesulfonic acid"[tw] OR "tridecafluoro-1-Hexanesulfonic acid"[tw] OR "PFHxS"[tw]) AND ("2019/05/03"[Date - Publication] : "3000"[Date - Publication]) ((((((((((((((((((((((((((("108427-53-8"[rn]) OR "423-50-7"[rn]) OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-, ion(1-)"[tw]) OR "PFHxS ion(1-)"[tw]) OR "PFHxS_ion"[tw]) OR "Perfluorohexanesulfonate"[tw]) OR "Tridecafluorohexane-1-sulfonate"[tw]) OR "perfluorohexyl sulfonate"[tw]) OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonyl fluoride"[tw]) OR "1-Hexanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-"[tw]) OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonic acid"[tw]) OR "EC 206-587-1"[tw]) OR "EINECS 206-587-1"[tw]) OR "PFHS"[tw]) OR "Perfluorhexan-1-sulfonsaure"[tw]) OR "Perfluorohexane sulfonic acid (PFHxS)"[tw]) OR "Perfluorohexane-1-sulphonic acid"[tw]) OR "acide perfluorohexane-1-sulfonique"[tw]) OR "acido perfluorohexano-1-sulfonico"[tw]) OR "perfluorohexane-1-sulphonic acid"[tw]) OR "perfluorohexanesulfonic acid"[tw]) OR "Ammonium Perfluorohexanesulfonate"[tw]) OR "Ammonium perfluorohexanesulfonate"[tw]) OR "PFHxS-H3N"[tw]) OR "PFHxS-K"[tw]) OR "Potassium Perfluorohexanesulfonate"[tw]) OR "Potassium perfluorohexanesulfonate"[tw]) OR "Lithium Perfluorohexanesulfonate"[tw]) OR "Lithium perfluorohexanesulfonate"[tw]) OR "PFHxS-Li"[tw]) AND ("2019/05/03"[Date - Publication] : "3000"[Date - Publication])	116

Database	Terms	Hits
PubMed 04/06/2021	(("108427-53-8"[rn] OR "355-46-4"[rn] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid"[tw] OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-"[tw] OR "1-Hexanesulfonic acid, tridecafluoro-"[tw] OR "1-Perfluorohexanesulfonic acid"[tw] OR "Perfluoro-1-hexanesulfonate"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "Perfluorohexanesulfonate"[tw] OR "Perfluorohexanesulfonic acid"[tw] OR "Perfluorohexylsulfonate"[tw] OR "Tridecafluorohexanesulfonic acid"[tw] OR "tridecafluoro-1-Hexanesulfonic acid"[tw] OR "PFHxS"[tw]) AND ("2020/04/29"[Date - Publication] : "3000"[Date - Publication]) ("108427-53-8"[rn] OR "423-50-7"[rn] OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-, ion(1-)"[tw] OR "PFHxS ion(1-)"[tw] OR "PFHxS_ion"[tw] OR "Perfluorohexanesulfonate"[tw] OR "Tridecafluorohexane-1-sulfonate"[tw] OR "perfluorohexyl sulfonate"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonyl fluoride"[tw] OR "1-Hexanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonic acid"[tw] OR "EC 206-587-1"[tw] OR "EINECS 206-587-1"[tw] OR "PFHS"[tw] OR "Perfluorhexan-1-sulfonsaure"[tw] OR "Perfluorohexane sulfonic acid (PFHxS)"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "acide perfluorohexane-1-sulfonique"[tw] OR "acido perfluorohexano-1-sulfonico"[tw] OR "perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Ammonium Perfluorohexanesulfonate"[tw] OR "Ammonium perfluorohexanesulfonate"[tw] OR "PFHxS-H3N"[tw] OR "PFHxS-K"[tw] OR "Potassium Perfluorohexanesulfonate"[tw] OR "Potassium perfluorohexanesulfonate"[tw] OR "Lithium Perfluorohexanesulfonate"[tw] OR "Lithium perfluorohexanesulfonate"[tw] OR "PFHxS-Li"[tw]) AND ("2020/04/29"[Date - Publication] : "3000"[Date - Publication])	28

Database	Terms	Hits
Web of Science 7/27/2017	TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid" OR TS="1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR TS="1-Hexanesulfonic acid, tridecafluoro-" OR TS="1-Perfluorohexanesulfonic acid" OR TS="Perfluoro-1-hexanesulfonate" OR TS="Perfluorohexane sulfonic acid" OR TS="Perfluorohexane-1-sulphonic acid" OR TS="Perfluorohexanesulfonate" OR TS="Perfluorohexanesulfonic acid" OR TS="Perfluorohexylsulfonate" OR TS="Tridecafluorohexanesulfonic acid" OR TS="tridecafluoro-1-Hexanesulfonic acid" OR TS="PFHxS"	394
Web of Science 04/29/2020	((TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid" OR TS="1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR TS="1-Hexanesulfonic acid, tridecafluoro-" OR TS="1-Perfluorohexanesulfonic acid" OR TS="Perfluoro-1-hexanesulfonate" OR TS="Perfluorohexane sulfonic acid" OR TS="Perfluorohexane-1-sulphonic acid" OR TS="Perfluorohexanesulfonate" OR TS="Perfluorohexanesulfonic acid" OR TS="Perfluorohexylsulfonate" OR TS="Tridecafluorohexanesulfonic acid" OR TS="tridecafluoro-1-Hexanesulfonic acid" OR TS="PFHxS") AND PY=2019-2020) ((TS="1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-, ion(1-)" OR TS="PFHxS ion(1-)" OR TS="PFHxS_ion" OR TS="Perfluorohexanesulfonate" OR TS="Tridecafluorohexane-1-sulfonate" OR TS="perfluorohexyl sulfonate" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonyl fluoride" OR TS="1-Hexanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonic acid" OR TS="EC 206-587-1" OR TS="EINECS 206-587-1" OR TS="PFHS" OR TS="Perfluorhexan-1-sulfonsaure" OR TS="Perfluorohexane sulfonic acid (PFHxS)" OR TS="Perfluorohexane-1-sulphonic acid" OR TS="acide perfluorohexane-1-sulfonique" OR TS="acido perfluorohexano-1-sulfonico" OR TS="perfluorohexane-1-sulphonic acid" OR TS="perfluorohexanesulfonic acid" OR TS="Ammonium Perfluorohexanesulfonate" OR TS="Ammonium perfluorohexanesulfonate" OR TS="PFHxS-H3N" OR TS="PFHxS-K" OR TS="Potassium Perfluorohexanesulfonate" OR TS="Potassium perfluorohexanesulfonate" OR TS="Lithium Perfluorohexanesulfonate" OR TS="Lithium perfluorohexanesulfonate" OR TS="PFHxS-Li") AND PY=2019-2020)	90

Database	Terms	Hits
Web of Science 04/06/2021	((TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid" OR TS="1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR TS="1-Hexanesulfonic acid, tridecafluoro-" OR TS="1-Perfluorohexanesulfonic acid" OR TS="Perfluoro-1-hexanesulfonate" OR TS="Perfluorohexane sulfonic acid" OR TS="Perfluorohexane-1-sulphonic acid" OR TS="Perfluorohexanesulfonate" OR TS="Perfluorohexanesulfonic acid" OR TS="Perfluorohexylsulfonate" OR TS="Tridecafluorohexanesulfonic acid" OR TS="tridecafluoro-1-Hexanesulfonic acid" OR TS="PFHxS") AND PY=2020-2021) ((TS="1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-, ion(1-)" OR TS="PFHxS ion(1-)" OR TS="PFHxS_ion" OR TS="Perfluorohexanesulfonate" OR TS="Tridecafluorohexane-1-sulfonate" OR TS="perfluorohexyl sulfonate" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonyl fluoride" OR TS="1-Hexanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonic acid" OR TS="EC 206-587-1" OR TS="EINECS 206-587-1" OR TS="PFHS" OR TS="Perfluorhexan-1-sulfonsaure" OR TS="Perfluorohexane sulfonic acid (PFHxS)" OR TS="Perfluorohexane-1-sulphonic acid" OR TS="acide perfluorohexane-1-sulfonique" OR TS="acido perfluorohexano-1-sulfonico" OR TS="perfluorohexane-1-sulphonic acid" OR TS="perfluorohexanesulfonic acid" OR TS="Ammonium Perfluorohexanesulfonate" OR TS="Ammonium perfluorohexanesulfonate" OR TS="PFHxS-H3N" OR TS="PFHxS-K" OR TS="Potassium Perfluorohexanesulfonate" OR TS="Potassium perfluorohexanesulfonate" OR TS="Lithium Perfluorohexanesulfonate" OR TS="Lithium perfluorohexanesulfonate" OR TS="PFHxS-Li") AND PY=2020-2021)	69
Toxline 7/21/2017	(108427-53-8[rn] OR 355-46-4[rn] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid" OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR "1-Hexanesulfonic acid, tridecafluoro-" OR "1-Perfluorohexanesulfonic acid" OR "Perfluoro-1-hexanesulfonate" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "Perfluorohexanesulfonate" OR "Perfluorohexanesulfonic acid" OR "Perfluorohexylsulfonate" OR "Tridecafluorohexanesulfonic acid" OR "tridecafluoro-1-Hexanesulfonic acid" OR "PFHxS") AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) [not] PubMed [org] [not] pubdart [org]	0

Database	Terms	Hits
SCOPUS (new search) 4/26/2021	("108427-53-8" OR "355-46-4" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-Hexanesulfonic acid" OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR "1-Hexanesulfonic acid, tridecafluoro-" OR "1-Perfluorohexanesulfonic acid" OR "Perfluoro-1-hexanesulfonate" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "Perfluorohexanesulfonate" OR "Perfluorohexanesulfonic acid" OR "Perfluorohexylsulfonate" OR "Tridecafluorohexanesulfonic acid" OR "tridecafluoro-1-Hexanesulfonic acid" OR "PFHxS") ("108427-53-8" OR "423-50-7" OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-, ion(1-)" OR "PFHxS ion(1-)" OR "PFHxS_ion" OR "Perfluorohexanesulfonate" OR "Tridecafluorohexane-1-sulfonate" OR "perfluorohexyl sulfonate" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonyl fluoride" OR "1-Hexanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexanesulfonic acid" OR "EC 206-587-1" OR "EINECS 206-587-1" OR "PFHS" OR "Perfluorhexan-1-sulfonsaure" OR "Perfluorohexane sulfonic acid (PFHxS)" OR "Perfluorohexane-1-sulphonic acid" OR "acide perfluorohexane-1-sulfonique" OR "acido perfluorohexano-1-sulfonico" OR "perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonic acid" OR "Ammonium Perfluorohexanesulfonate" OR "Ammonium perfluorohexanesulfonate" OR "PFHxS-H3N" OR "PFHxS-K" OR "Potassium Perfluorohexanesulfonate" OR "Potassium perfluorohexanesulfonate" OR "Lithium Perfluorohexanesulfonate" OR "Lithium perfluorohexanesulfonate" OR "PFHxS-Li")	1,208
TSCATS2, TSCA recent notices 7/21/2017	84-66-2	10
	84-66-2 (8E OR FYI) TSCA	0 recent notice

Table B-2. Processes used to augment the search of core databases for PFHxS (355-46-4)

System used	Selected key reference(s) or sources	References identified
TSCATS[a]	TSCATS2 (https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm)	2
	Chemical Data Access Tool (CDAT) (https://java.epa.gov/oppt_chemical_search/)	1
	ChemView (https://java.epa.gov/chemview)	1
Resources searched for physiochemical property information	Agency for Toxic Substances and Disease Registry (ATSDR) (https://www.atsdr.cdc.gov/) Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (https://www.nicnas.gov.au/chemical-information) CAMEO Chemicals (https://cameochemicals.noaa.gov/) Canada DSL List (http://webnet.oecd.org/CCRWEB/Search.aspx) ChemIDplus (https://chem.nlm.nih.gov/chemidplus/)	5

System used	Selected key reference(s) or sources	References identified
	ChemSpider (http://www.chemspider.com/) Chemical Risk Information Platform (CHRIP) (http://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop) CRC Handbook of Chemistry and Physics (http://hbcponline.com/faces/contents/ContentsSearch.xhtml;jsessionid=9408875156F724E0E945D3A6D0454891) ECHA Information on Chemicals (https://echa.europa.eu/) eChemPortal (https://www.echemportal.org/echemportal/index.action) SRC Fate Pointers (http://esc.syrres.com/fatepointer/search.asp) Hazardous Substances Data Bank (HSDB) https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) HSNO Chemical Classification and Information Database (CCID) (http://www.epa.govt.nz/search-databases/Pages/HSNO-CCID.aspx) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris) IARC Monographs (http://www.inchem.org/pages/iarc.html) J-Check (http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en) Kirk-Othmer Encyclopedia of Chemical Technology (http://onlinelibrary.wiley.com/mrw/advanced/search?doi=10.1002/0471238961) NIEHS (https://www.niehs.nih.gov/) OSHA Occupational Chemical Database (https://www.osha.gov/chemicaldata/) PubChem (https://pubchem.ncbi.nlm.nih.gov/search/index.html) Ullmann's Encyclopedia http://onlinelibrary.wiley.com/mrw/advanced/search?doi=10.1002/14356007) USEPA ACToR (https://actor.epa.gov/actor/home.xhtml) USEPA ChemView (https://java.epa.gov/chemview) USEPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) USEPA CDAT (https://java.epa.gov/oppt_chemical_search/) USEPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) Web based search for chemical manufacturer documents	
Resources searched for health effects, toxicokinetic, and mechanistic information	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA OEHHA (http://www.oehha.ca.gov/risk.html) OEHHA Toxicity Criteria Database (http://www.oehha.ca.gov/tcdb/index.asp) CPSC (http://www.cpsc.gov) ECHA (http://echa.europa.eu/information-on-chemicals) European Union Risk Assessment Reports (https://ec.europa.eu/jrc/en/publications-list) EFSA Europe (http://www.efsa.europa.eu/) eChemPortal[b](http://www.echemportal.org/echemportal/participant/page.action?pageID=9) eChemPortal[b](http://www.echemportal.org/echemportal/participant/page.action?pageID=9) Environment Canada (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36)	2

System used	Selected key reference(s) or sources	References identified
	Health Canada (https://www.canada.ca/en/health-canada.html) USEPA NSCEP (https://www.epa.gov/nscep) FDA (http://www.fda.gov/) Federal Docket (http://www.regulations.gov) IARC (http://monographs.iarc.fr/ENG/Classification/index.php) ITER (http://www.tera.org/iter/) Japan Existing Chemical Data Base (http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp) NIEHS (http://www.niehs.nih.gov/) NICNAS (http://www.nicnas.gov.au/chemical-information) NTP (http://ntpsearch.niehs.nih.gov/home) WHO (http://www.who.int/ipcs/assessment/en/)	

[a]Only relevant TSCATS studies from these interfaces were added to the HERO project page.
[b]eChemPortal includes the following databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, EPA-IRIS, and EPA-SRS.

B.2. TITLE AND ABSTRACT LEVEL SCREENING CRITERIA FOR THE INITIAL LITERATURE SEARCHES

Table B-3. Title and abstract level screening criteria for the initial literature searches

	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog • Alternative animal models in standard laboratory conditions (e.g., Xenopus, zebrafish, minipig) • Human or animal cells, tissues, or organs (not whole animals); bacteria, nonmammalian eukaryotes; other nonmammalian laboratory species	• Ecological species
Exposure	• Exposure is to PFHxS compound • Exposure via oral, inhalation, dermal, intraperitoneal, or intravenous injection routes • Exposure is measured in air, dust, drinking water, diet, gavage, or injection vehicle or	• Study population is not exposed to a PFHxS compound • Exposure is to a mixture only

	Inclusion criteria	Exclusion criteria
	via a biomarker of exposure (PFHxS levels in whole blood, serum, plasma, or breastmilk)	
Outcome	• Studies that include a measure of one or more health effect endpoints including, but not limited to, effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer • In vivo and/or in vitro studies related to toxicity mechanisms, physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action (MOAs) • Qualitative or quantitative description of absorption, distribution, metabolism, excretion, toxicokinetic and/or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD) • Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations	
Other	• Structure and physiochemical properties • Reviews and regulatory documents	• Not on topic, including: • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant • Bioremediation, biodegradation, or chemical or physical treatment of PFHxS compounds, including evaluation of wastewater treatment technologies and methods for remediation or contaminated water and soil • Ecosystem effects, studies in ecological species that are not relevant to health effects in humans • Studies of environmental fate and transport of PFHxS compounds in environmental media • Analytical methods for detecting/measuring PFHxS compounds in environmental media and use in sample preparations and assays.

	Inclusion criteria	Exclusion criteria
		• Studies describing the manufacture and use of PFHxS compounds • Not chemical-specific (studies that do not involve testing of PFHxS compounds) • Studies that describe measures of exposure to PFHxS compounds without data on associated health effects

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Table B-4. Example DistillerSR form questions to be used for title/abstract level and full text level screening for literature search updates from 2019

Used in title/abstract and full text screening						Used in full text screening only	
Question	**Source of study if not identified from database search?**	**Does the article meet PECO criteria?**	**If meets PECO, what type of evidence?**	**If supplemental, what type of information?**	**Which PFAS did the study report?**	**If meets PECO, which health outcome(s) apply?**	**If meets PECO and endocrine outcome, which endocrine tags apply?**
Answer options (can select multiple options)	• Source other than HERO database search	• Yes • No • Unclear • Tag as potentially relevant supplemental information	• Human • Animal (mammalian models) • In vitro or in silico genotoxicity • PBPK or PK model	• In vivo mechanistic or MOA studies, including nonPECO routes of exposure (e.g., injection) and populations (e.g., nonmammalian) • In vitro or in silico studies (nongenotoxicity) • ADME/toxicokinetic (excluding models) • Exposure assessment or characterization (no health outcome) • PFAS mixture study (no individual PFAS comparisons) • Human case reports or case series • Ecotoxicity studies • Environmental fate or occurrence (including food)	• PFBA • PFHxA • PFHxS • PFNA • PFDA	• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and development • Hematological, including nonimmune/hepati/renal clinical chemistry measures • Hepatic, including liver measures and	• Adrenal • Sex hormones (e.g., androgen; estrogen; progesterone) • Neuroendocrine • Pituitary • Steroidogenesis • Thyroid

Used in title/abstract and full text screening						Used in full text screening only	
				• Manufacture, engineering, use, treatment, remediation, or laboratory methods • Other assessments or records with no original data (e.g., reviews, editorials, commentaries)		serum markers (e.g., ALT; AST) • Immune/ inflammation • Musculoskeletal • Nervous system, including behavior and sensory function • Nutrition and metabolic • Ocular • PBPK or PK model • Renal, including urinary measures (e.g., protein) • Reproductive • Respiratory • Skin and connective tissue effects	

B.3. DOCUMENTATION OF LITERATURE SEARCH UPDATES AFTER APRIL 2022

Table B-5 documents the decisions regarding studies identified after April 2022, including a literature search update in April 2023 and studies identified in public comments received through the EPA docket on the draft IRIS PFDA assessment[1]. The table focuses primarily on the new studies that met the assessment PECO criteria. Specifically, epidemiology studies that meet the PECO criteria were identified; no experimental animal studies that meet the PECO criteria were identified. Table B-5 provides EPA's disposition on the decision to incorporate these studies into the assessment as defined in draft Peer Review Charge question 1 (i.e., only incorporating studies that may potentially change which hazards are identified, or notably affect the RfDs, or studies that directly inform the identified key science issues); the charge question asks the peer reviewers to weigh in on EPA's disposition. These same criteria were applied to certain categories of newly identified supplemental studies (i.e., ADME and mechanistic studies, including non-PECO exposure route studies). Thus, in addition to providing this characterization for all studies that meet PECO criteria, Table B-5 also includes studies from supplemental evidence categories that were determined to warrant inclusion in the assessment based on the criteria described above. The decision to exclude other recently identified studies that meet these specific supplemental evidence categories is documented in HAWC. Recently identified studies that meet supplemental evidence categories other than those above (e.g., exposure-only) were not evaluated in this way and are tagged in HERO and HAWC along with other screening decisions (e.g., excluded studies).

[1] A total of 186 studies were submitted by the State of New Jersey Department of Environmental Protection and the Natural Resources Defense Council (NRDC). There was a large amount of overlap between these studies and those already identified and screened for inclusion in the draft IRIS PFHxS assessment by EPA before April 2022. Those not already identified were screened using the PFHxS assessment PECO criteria. Table B-5 lists as "Commenter (on PFDA)" those studies meeting PECO criteria that were not identified before April 2022 and not identified through the routine EPA literature update in 2023.

Table B-5. Summary of decisions regarding studies identified after April 2022, including characterization of all studies meeting PECO criteria and all supplemental studies added to the assessment syntheses

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Immune Effects				
Kaur et al. (2023)	Lit update	Antibody levels to SARS-COV2 in adults	Inverse association (beta -0.68, 95% CI -1.18, -0.18)	No. Findings are consistent with existing evidence and have no impact on immunosuppression conclusions, particularly given that two of the new studies are in adults and the draft conclusions are primarily based on studies in children.
(Porter et al., 2022)	Lit update	Antibody levels to SARS-COV2 in adults	Inverse association with IgG and neutralizing antibodies in response to COVID vaccination (statistical significance varied based on model)	
(Zhang et al., 2023b)	Lit update	Vaccine response	Inverse association with rubella antibodies (-6.48% change, 95% CI -10.69, -2.07). Inverse but not statistically significant association with mumps antibodies in sub-population with lower folate.	
(Zhang et al., 2022)	Lit update	Infectious disease	Positive association with common cold at 3-11 years (OR 1.31, 95% CI 1.05, 1.63) but not 12-19 years	No. Existing evidence on infectious disease is inconsistent and new studies do not change current draft judgment.
(Pan et al., 2023)	Lit update	Asthma	No association with current asthma (OR 0.97, 95% CI 0.57, 1.65 in Q4 vs Q1) or wheezing. Inverse association with asthma attacks and emergency visits.	No. Existing evidence on asthma is inconsistent and new studies do not change current draft judgment.
Gaylord et al. (2019)	Commenter (on PFDA)	Asthma	No association with asthma diagnosis (OR 0.96, 95% CI 0.65, 1.44)	
Averina et al. (2019)	Commenter (on PFDA)	Asthma	Positive association with asthma (OR 2.18, 95% CI 1.08, 4.42 in Q4 vs Q1). No association with allergies or eczema.	
(Ammitzbøll et al., 2019)	Commenter (on PFDA)	Multiple sclerosis	No association with multiple sclerosis (2% change, 95% CI -9,15)	No.

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Gaylord et al. (2020)	Commenter (on PFDA)	Celiac disease	Positive association with celiac disease (OR 1.72, 95% CI 0.85, 3.49) with stronger effect in women (OR 3.24, 95% CI 1.04, 10.11)	Mixed results for autoimmune conditions in new studies would not influence draft conclusions on immune effects.
Developmental Effects				
Wang et al. (2022)	Lit update	Fetal growth restriction (Birth length (BL); head circumference (HC); birthweight (BWT))	No sex-specific associations were observed for birth length (BL), birth weight (BWT) and head circumference (HC) endpoints. BL Male β = −0.080; 95%CI: −0.062, 0.222; BL Female β = −0.004; 95%CI: −0.310, 0.303. HC Male β = 0.005; 95%CI: −0.180, 0.191; HC Female β = −0.110; 95%CI: −0.345, 0.125. BWT Male β = 0.024; 95%CI: −0.140, 0.188; BWT Female β = −0.062; 95%CI: −0.291, 0.166.	No. Null results observed for birth length, birth weight and head circumference endpoints in both female and male neonates would not change the current draft judgment for fetal growth restriction.
Peterson et al. (2022)	Lit update	Fetal growth restriction	No associations were evident across fetal measures in relation to PFHxS exposures.	No. Null results for fetal biometric endpoints would not change the current draft judgment for fetal growth restriction.
Padula et al. (2023)	Lit update	Fetal growth restriction, gestational duration	No associations were evident across fetal growth and gestational duration endpoints [gestational age β= 0.02; 95%CI: −0.19, 0.23; birth weight for gestational age β= −0.06; 95%CI: −0.18, 0.06; term low birth weight OR= 1.14; 95%CI: 0.46, 2.84; small for gestational age OR= 1.25; 95%CI: 0.84, 1.87; large for gestational age OR= 0.86; 95%CI: 0.59, 1.25; preterm birth OR= 0.97; 95%CI: 0.61, 1.55.	No. Null results for all fetal growth and gestational duration endpoints would not change the current draft judgment for either gestational duration or fetal growth restriction.
Ouidir et al. (2020)	Commenter (on PFDA)	Fetal growth restriction	Per each PFHxS IQR increase, a statistically significant longitudinal decrease in head circumference (β = −0.22 mm; p-value: <0.05) and increases in longitudinal biparietal diameter (β = 0.07 mm; p-value: <0.05), and femur length (β = 0.12 mm; p-value: <0.001)	No. Study population was previously reported in a publication already in the assessment Buck Louis et al. (2018). New results for longitudinal in utero measurements from

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
			were detected. Results were null for abdominal circumference (β = 0.11 mm), occipital-frontal diameter changes (β = −0.04 mm) and estimated fetal growth (β = 3.27 g) (p-value/CIs not provided).	ultrasonography would not change the current draft judgment.
Petroff et al. (2023)	Lit update	Gestational age	No association between PFHxS exposure and gestational age (ß = 0.04 ± 0.21; p=0.85).	No. Null results for gestational age would not change the current draft judgment for gestational duration.
Yu et al. (2022)	Lit update	Preterm birth	Results were mixed with a non-significant increase in risk seen for untransformed data (OR=1.76; 95%CI: 0.91, 3.40 per each ng/mL increase) only; transformed results were null (OR=0.93; 95%CI: 0.80, 1.08 per each ln-unit increase).	No. Small increased risks here along with the null results in Padula et al. (2023) and Liao et al. (2022b) would not change the current draft judgment for gestational duration.
Liao et al. (2022b)	Lit update	Preterm birth	Results were mixed with a statistically significant decrease in preterm birth per each log10 increase (OR=0.73; 95%CI: 0.39, 1.38) driven by tertile 3 (OR=0.60; 95%CI: 0.37, 0.98); results were null for tertile 2 (OR=0.97; 95%CI: 0.63, 1.50) relative to tertile 1.	No. Inconsistent new results in three new studies including decreased risk reported here combined with increased risk by Yu et al. (2022) and null results in Padula et al. (2023) above would not change the current draft judgment for gestational duration.
Wang et al. (2016)	Commenter (on PFDA)	Gestational duration	This exposure study showed a statistically significant increase between gestational age and concentrations of PFHxS in cord blood.	No. Although an association was reported in an exposure prediction model examining gestational age and PFHxS, the study design and analyses would likely preclude this from inclusion into the synthesis. These data would not contribute to the gestational duration judgments.
Hong et al. (2022)	Lit update	Spontaneous abortion	Inverse association (OR=0.05; 95% CI: 0.00, 7.36)	No. Updated analysis of study that is already included in the draft assessment.

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Li et al. (2022a)	Lit update	Anogenital distance	Positive association with two AGD measures ($p<0.05$)	No. New study adds to existing inconsistency in the AGD evidence and would not change the draft judgment.
Hepatic				
Borghese et al. (2022)	Lit update	Liver enzymes	Positive association with AST, GGT, and ALP, positive but not statistically significant association with ALT and bilirubin	No. New studies are consistent with the existing studies and would not change the draft judgment.
Liao et al. (2023)	Lit update	Liver enzymes	Positive association with bilirubin but not ALT, AST, or GGT	
Kim et al. (2023b)	Lit update	Liver enzymes	Positive but not statistically significant associations with ALT, AST, and GGT	
Yao et al. (2020)	Commenter (on PFDA)	Liver enzymes	Positive association with ALT, AST, GGT (statistically significant for GGT)	
Salihović et al., (2019, 6324314)	Commenter (on PFDA)	Bile acid levels (liver)	Inverse correlations with most bile acids (statistically significant for GDCA)	
Rantakokko et al. (2015)	Commenter (on PFDA)	Non-alcoholic fatty liver disease	Inverse association with lobular inflammation (OR 0.02, 95% CI <0.01, 0.53 for 2–4 foci per 200× field)	No. While there are no studies of clinical liver disease available for PFHxS in the current draft, the new studies are inconsistent and would not change the draft judgment of *slight* for hepatic effects.
E et al. (2023)	Lit update	Liver disease	No association with liver problems (OR 0.97, 95% CI 0.72, 1.30). Positive but not statistically significant association with ALT.	
Nilsson et al. (2022b)	Lit update	Liver problems	Positive association with non-alcoholic fatty liver disease in women but not men, with strongest association in postmenopausal women (OR 2.50, 95% CI 1.29, 4.85 in Q4 vs Q1)	
Cancer				
Feng et al. (2022a)	Lit update	Breast cancer	No association with breast cancer (OR = 0.93, 95% CI: 0.79, 1.09) per unit increase in ln-transformed plasma PFHxS levels.	No. Inconsistent results across the new studies showing increased risk, decreased risk and

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Li et al. (2022b)	Lit update	Breast cancer	Decreased risk for breast cancer (OR = 0.73, 95% CI: 0.63, 0.87) per SD increase in ln-transformed PFHxS from the adjusted model – without LASSO (see Table S3).	no association of PFHxS and breast cancer. In addition, one new breast cancer study reports on the same study population as a publication already in the assessment Wielsøe et al. (2017). One previous study reported significantly increased risk of breast cancer among women <= 50 years of age who were estrogen receptor positive; and non-significantly decreased risk of breast cancer among women who were estrogen receptor negative and > 50 years of age. Another study reported significantly decreased risk for some genotypes. The only study reporting on liver cancer did not find an association with PFHxS. The only study of renal cancer reported a significant association observed for renal cancer that dissipated when controlling for other PFAS. The available epidemiologic evidence on PFDA and the risk of cancer remains inadequate; the new studies are not impactful.
Wielsøe et al. (2018)	Commenter (on PFDA)	Breast cancer	Increased risk for breast cancer (OR 5.45, 95% CI 1.26, 23.8) in high vs. low PFHxS exposure for one genotype).	
Lee et al. (2020)	Commenter (on PFDA)	Breast cancer	No association of PFHxS with mammographic density, a strong predictor of breast cancer (beta -0.02, p-value 0.95).	
Goodrich et al. (2022)	Lit update	Liver cancer	No association of PFHxS with liver cancer (OR = 1.10, 95% CI: 0.56, 2.30) for PFHxS greater than the 90th% vs less than 90th%.	
Shearer et al. (2021)	Commenter (on PFDA)	Renal cancer	Increased risk of renal cell carcinoma with PFHxS per unit increase in log_2-transformed serum PFHxS (OR=1.27; 95% CI: 1.03, 1.56) that attenuated when controlling for other PFAS (OR=1.12; 95% CI: 0.88, 1.43).	
Neurodevelopment				
Luo et al. (2022a)	Lit update	Broad neurodevelopmental scale	Inverse but not statistically significant association with cognitive, language, motor, and social-emotional scores, but statistically significant positive association with adaptive behavior score	No. There is inconsistency for neurodevelopmental effects in the current draft, and the new studies would not

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Oh et al. (2022b)	Lit update	Autism, developmental delay	Positive but not statistically significant associations with autism spectrum disorder and developmental delay	influence the draft judgment of *slight* evidence.
Zhou et al. (2023)	Lit update	Broad neurodevelopmental scale	Inverse association with communication and motor at 6 mos but inconsistent findings for other measures (problem solving, personal-social) and other visits (2, 12, and 24 mos)	
Li et al. (2023c)	Lit update	Broad neurodevelopmental scale	Positive association with persistently low trajectory for communication (p<0.05), gross motor, problem solving ability (p<0.05), and personal-social skills, but not fine motor	
Oulhote et al. (2019)	Commenter (on PFDA)	Broad neurodevelopmental scale	Positive association with Boston Naming Test. No association with Strengths and Difficulties Questionnaire.	
(van Larebeke et al., 2022)	Lit update	Broad neurodevelopmental scale	Inverse (favorable) association with incorrect responses on the Continuous Performance Test but not other test results	
Kim et al. (2023a)	Lit update	ADHD scale	Positive though non-monotonic association with ADHD rating scale at 8 yrs, dependent on age at exposure measurement and sex	
Male Reproductive				
Luo et al. (2022b)	Lit update	Semen parameters	No association with sperm concentration of motility	No. Evidence is inconsistent and the new studies would not influence the draft conclusion.
Ma et al. (2021)	Commenter (on PFDA)	Semen parameters	No association sperm concentration, motility, or morphology	
Rivera-Núñez et al. (2023)	Lit update	Reproductive hormones	Positive association with T (p<0.05), no association with free T, E1, E2, E3	No. Evidence is inconsistent in existing studies and the new studies would not influence
Guo et al. (2023)	Lit update	Reproductive hormones	No association with testosterone or estradiol (included boys and girls)	

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Nian et al. (2020)	Commenter (on PFDA)	Reproductive hormones	No association with total testosterone (beta 0.079, 95% CI -0.009, 0.166 per ln-unit change), FSH, or LH	the draft synthesis conclusion of *indeterminate* evidence.
Female Reproductive				
Hong et al. (2022)	Lit update	In vitro fertilization outcomes	No association with oocyte maturation rate, fertilization rate, high quality embryo rate. Inverse but not statistically significant (OR=0.60, 95% CI 0.12, 2.96) for clinical pregnancy	No. Evidence of an association with fecundity and infertility is inconsistent across new studies and was similarly inconsistent across existing studies. The conclusion of *indeterminate* evidence would likely remain the same.
Cohen et al. (2023)	Lit update	Fecundity, pregnancy	No association with time to pregnancy or odds of clinical pregnancy	
Luo et al. (2022c)	Lit update	Fecundity, infertility	Lower odds of infertility (OR 0.61, 95% CI 0.45, 0.82) and higher fecundability	
Tan et al. (2022)	Lit update	Infertility	Lower odds of infertility (non-monotonic across quartiles and not statistically significant)	
(Whitworth et al., 2016)	Commenter (on PFDA)	Fecundity	No association (FR 0.97, 95% CI 0.90, 1.1)	
Ma et al. (2021)	Commenter (on PFDA)	In vitro fertilization outcomes, pregnancy	Fewer zygotes and good quality embryos with higher exposure. No association with clinical pregnancy.	
Wang et al. (2019)	Commenter (on PFDA)	Polycystic ovarian syndrome	Positive but not statistically significant association with PCOS-related infertility (OR 2.08, 95% CI 0.88, 4.93 in 3rd vs. 1st tertile)	No. Existing evidence on gynecological conditions is inconsistent and there is considerable uncertainty due to potential reverse causation. The new study does not inform this uncertainty.
Rivera-Núñez et al. (2023)	Lit update	Reproductive hormones	Positive association with E1, E2, E3 (p<0.05), no association with T, FT	No.

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Nian et al. (2020)	Commenter (on PFDA)	Reproductive hormones	No association with total testosterone (beta -0.029, 95% CI -0.090, 0.032 per ln-unit change), FSH, or LH	New studies would not change the current draft judgment.
Liu et al. (2020a)	Commenter (on PFDA)	Reproductive hormones	Positive association with estradiol (6.8% change, 95% CI 2.2, 11.6)	
Lin et al. (2022)	Lit update	Postpartum hemorrhage	Higher odds of postpartum hemorrhage (OR 3.42, 95% CI 1.45, 8.07)	No. Single study of the outcome and evidence is not strong enough to increase certainty in the evidence for female reproductive effects.
Urinary				
(Nilsson et al., 2022b)	Lit update	Kidney disease, urate	No association with kidney disease (OR 0.90, 95% CI 0.76, 1.08) or urate	No. Existing studies are inconsistent with considerable uncertainty due to potential reverse causation. The new studies do not inform this uncertainty.
Liang et al. (2023)	Lit update	Glomerular filtration rate	Higher GFR (not statistically significant)	
Sood et al. (2019)	Commenter (on PFDA)	Glomerular filtration rate	Inverse but not statistically significant association with eGFR (beta -10.3, 95% CI -23.6, 3.0)	
Feng et al. (2022b)	Lit update	Hyperuricemia	No association with hyperuricemia	
Arrebola et al. (2019)	Commenter (on PFDA)	Hyperuricemia	Positive but not statistically significant association with hyperuricemia (OR 1.33, 95% CI 0.70, 2.54)	
Yao et al. (2020)	Commenter (on PFDA)	Uric acid	Positive association with uric acid (beta 8.44, 95% CI 2.17, 15.09)	
Cardiometabolic				
Haug et al. (2023)	Lit update	Serum lipids	No association with HDL or LDL cholesterol	No. Mixed results from the new studies would not change the current draft judgment.
Donat-Vargas et al. (2019b)	Commenter (on PFDA)	Serum lipids, hypertension	No association with total cholesterol, triglycerides, or hypertension	

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
(Batzella et al., 2022)	Lit update	Serum lipids	Positive association with total cholesterol (beta 1.74, 95% CI 1.36, 2.13) and LDL-cholesterol	
(Morgan et al., 2023)	Lit update	Serum lipids	No association with total cholesterol or LDL-cholesterol (crude analysis only)	
(Nilsson et al., 2022b)	Lit update	Serum lipids, blood pressure, cardiovascular disease	Positive association with total cholesterol and LDL-cholesterol in cross-sectional but not prospective analysis. No association with high blood pressure (OR 0.92, 95% CI 0.83, 1.03) or cardiovascular disease (OR 0.96, 95% CI 0.81, 1.15)	
Yao et al. (2020)	Commenter (on PFDA)	Serum lipids, blood glucose	Positive association with total cholesterol (beta 6.98, 95% CI 3.06, 11.14), triglycerides, and blood glucose	
Mitro et al. (2020)	Lit update	Blood pressure	No association with blood pressure, BMI, waist circumference, mid-upper arm circumference, or skinfold thickness	
Sood et al. (2019)	Commenter (on PFDA)	Blood pressure	No association with blood pressure (beta 0.3, 95% CI -0.1, 0.7)	
Lind et al. (2018)	Commenter (on PFDA)	Carotid artery intima-media thickness	Positive association with IMT thickness (beta 0.015, 95% CI 0.005, 0.0025)	No. These results support coherence with serum lipids but would not change the current draft judgment.
Li et al. (2023b)	Lit update	Cardiovascular disease	No association with acute coronary syndrome	No. New study contributes to existing inconsistency and would not change the current draft judgment.
Yang et al. (2022)	Lit update	Gestational hypertension	Lower odds of gestational hypertension (OR 0.66, 95% CI 0.35, 1.24) and lower continuous blood pressure	No.

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Huo et al. (2020)	Lit update	Gestational hypertension	No association with gestational hypertension (OR 0.80, 95% CI 0.44, 1.47) or preeclampsia (OR 1.05, 95% CI 0.60, 1.83)	New studies contribute to existing inconsistency and would not change the current draft judgment.
Zhu and Bartell (2022)	Lit update	Gestational hypertension	Small positive association with hypertensive disorders in pregnancy (OR 1.03, 95% CI 1.02, 1.04)	
Xu et al. (2022)	Lit update	Gestational diabetes	Inverse association with gestational diabetes (OR 0.09, 95% CI 0.03, 0.22 in third tertile), inverse association with continuous glucose levels in oral glucose tolerance test	No. Existing studies are inconsistent and new studies would not change the current draft judgment.
Zhang et al. (2023a)	Lit update	Gestational diabetes	Positive association with gestational diabetes (OR 3.46, 95% CI 1.64, 6.30 in 3rd tertile)	
Xu et al. (2020)	Lit update	Gestational diabetes	No association with gestational diabetes (OR 0.79, 95% CI 0.46, 1.31 in Q4 vs Q1)	
Li et al. (2020)	Commenter (on PFDA)	Gestational blood glucose	Positive but not statistically significant association with blood glucose in oral glucose tolerance test (beta 0.07, 95% CI -0.06, 0.21)	
Dunder et al. (2023)	Lit update	Blood glucose	No association with blood glucose	No. Existing and new studies are primarily null, and the current draft judgment is unlikely to change.
Christensen et al. (2016)	Commenter (on PFDA)	Diabetes	No association with diabetes (OR 0.98, 95 % CI 0.69, 1.16) or pre-diabetes (OR 1.00, 95% CI 0.77, 1.16)	
(Park et al., 2022)	Lit update	Diabetes	Positive association with incident diabetes (OR 1.58, 95% CI 1.13, 2.21 in T3 vs T1) but not monotonic across tertiles	
Donat-Vargas et al. (2019a)	Commenter (on PFDA)	Diabetes risk, insulin resistance	No increase in diabetes risk or HOMA-IR	
Kim et al. (2015)	Commenter (on PFDA)	Insulin resistance	No association with HOMA (beta -0.08, 95% CI -0.68, 0.52)	

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Mehta et al. (2021)	Commenter (on PFDA)	Insulin resistance	No association with blood glucose or HOMA-IR	
Brosset and Ngueta (2022)	Lit update	Glycemic control	No association with poor glycemic control	
Ye et al. (2021)	Commenter (on PFDA)	Metabolic syndrome	No association with metabolic syndrome (OR 1.02, 95% CI 0.93, 1.13) or blood glucose, blood pressure, serum lipids, or waist circumference	No. Existing and new studies are primarily null, and the current draft judgment is unlikely to change.
Schillemans et al. (2022)	Lit update	Adiposity	No association with BMI z-score	No. Existing and new studies are primarily null, and the current draft judgment is unlikely to change.
Zeng et al. (2023)	Lit update	Adiposity	No association with BMI z-score trajectory	
(Harris et al., 2017)	Commenter (on PFDA)	Adiposity	Lower PFHxS levels in obese (-8.0% difference, 95% CI -26.6, 15.2 for obese vs normal)	
Ji et al. (2012)	Commenter (on PFDA)	Adiposity	Higher PFHxS concentrations in overweight participants, but no statistical analysis	
Pirard et al. (2020)	Commenter (on PFDA)	Adiposity	No association with BMI (quantitative results not presented)	
Liu et al. (2020b)	Commenter (on PFDA)	Adiposity	No association with BMI	
Endocrine				
Jensen et al. (2022)	Lit update	Thyroid hormones	No association with free T4, positive but non-monotonic and not statistically significant association with TSH (beta 4.05, 95% CI -1.58, 10.00)	No. Existing and new studies are primarily null, and the current draft judgment is unlikely to change.
Derakhshan et al. (2022)	Lit update	Thyroid hormones	Positive association with free T4 (beta 0.13, 95% CI -0.01, 0.28) but no association with TSH or free T3	
Li et al. (2023a)	Lit update	Thyroid hormones	No association with TSH or free T4	

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Tillaut et al. (2022)	Lit update	Thyroid hormones	No association with free T4, free T3, or TSH	
Jain and Ducatman (2019)	Commenter (on PFDA)	Thyroid hormones	Positive association with Total T3 in participants at higher glomerular filtration stages.	
Dufour et al. (2020)	Commenter (on PFDA)	Thyroid disease	Inverse association with hyperthyroidism (OR 0.14, 95% CI 0.03, 0.63)	
(Christensen et al., 2016)	Commenter (on PFDA)	Thyroid disease	Inverse association with thyroid disease (OR 0.59, 95% CI 0.20, 1.06)	
(Nilsson et al., 2022b)	Lit update	Thyroid problems, thyroid hormones	No association with thyroid problems (OR 0.94, 95% CI 0.73, 1.21). Inverse but not statistically significant association with T4 but not T3 or TSH.	
Other				
Højsager et al. (2022)	Lit update	Bone mineral density	Inverse association with bone mineral content and density (p>0.05), stronger in boys	No. Available studies are inconsistent, and evidence would likely be *indeterminate*.
Zhao et al. (2022)	Lit update	Bone mineral density	Inverse association (p>0.05) with femur bone mineral density in women without menopause/hysterectomy	
Colicino et al. (2020)	Lit update	Bone mineral density	No association with lumbar spine or femur density	
Xiong et al. (2022)	Lit update	Bone mineral density	Positive association with femur density and inverse association with lumbar spine density in girls only	
Fan et al. (2023)	Lit update	Bone mineral density, osteoporosis	Positive but not statistically significant association with osteoporosis (OR 1.23, 95% CI 0.95, 1.60), inverse association with bone mineral density (beta -0.23, 95% CI -0.33, -0.12)	

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
Shiue (2015d)	Commenter (on PFDA)	Oral health	No association with teeth health, ache, tooth loss	
Liao et al. (2022a)	Lit update	Hematology	Positive but not statistically significant association with gestational anemia in the 1st and 3rd but not 2nd trimesters. No association with hemoglobin concentration during pregnancy	No. Inconsistent results in new studies. Evidence would likely be *indeterminate* overall.
Cui et al. (2022)	Lit update	Hematology	Positive association with hematocrit (3.51% change, 95% CI 1.82, 5.24) and hemoglobin (3.14% change, 95% CI 1.33, 4.99) during pregnancy	
Liu et al. (2022)	Lit update	Hematology	No association with white blood cells and lymphocytes	
Shiue (2015a)	Commenter (on PFDA)	Neurologic; Remembering condition	No association with difficulty remembering (RR 0.45, 95% CI 0.25–0.81 for >3 times per week)	No. Lack of association in available studies and would likely be *indeterminate* overall.
Shiue (2015b)	Commenter (on PFDA)	Neurologic; Depression	No association with adult depression	
Shiue (2015c)	Commenter (on PFDA)	Neurologic; Hearing disturbance	No association with trouble hearing	
(Gaylord et al., 2019)	Commenter (on PFDA)	Pulmonary function	No association with FEV or FVC (FEV1 beta -0.01, 95% CI -0.10, 0.08, FVC beta 0.03, 95% CI -0.08, 0.13)	No. Lack of association in available studies and would likely be *indeterminate* overall.
Shi et al. (2023)	Lit update	Pulmonary function	No association with forced expiratory volume or forced volume capacity	
ADME/PBPK Studies				
Chiu et al. (2022)	Lit update	One-compartment PK model fit to data from highly	GM (95% CI) for $t_{1/2}$, Vd and CL are 8.30 (5.38–13.5) yr, 0.29 (0.17–0.45) L/kg and 0.068 (0.033–0.107) mL/kg-d. The CL is higher than	Yes.

Reference	Source[1]	Health outcome	Results summary	EPA disposition on incorporation and characterization of impact[2]
		exposed communities (after intervention)	our previous GM and health-protective lower bound, but in the range of other studies.	Incorporated clearance value into calculation of overall average clearance. See Section 3.1 in main document
Jain and Ducatman (2022)	Lit update	PFHxS serum levels in US females vs. males as a function of age (NHANES).	In males a slow, steady increase from age 12 to ≥ 75, but in females the levels decline from age 12 to 30, reaching ~ ½ the levels in males, then begin to increase around age 45.	Yes. Semi-quantitative support for impact of menstrual fluid loss: data are consistent with estimated clearance values. Including *mean* non-menstrual clearance. See Section 3.1 in main document
Oh et al. (2022a)	Lit update	Change in maternal PFHxS levels from conception to 2 yrs post-partum	Mean PFHxS serum levels decline slightly (0.6%) during pregnancy, decline 5.6% (statistically significant) from 0–6 mos post-partum, then increase 0.5% from 6–24 mos post-partum.	Yes. Maternal concentrations at or below concentration at conception: should be predicted with menstrual clearance included. See Section 3.1 in main document
Li et al. (2022c)	Lit update	PFHxS half-life in Swedish population after end of high drinking water exposure.	Mean (95% CI) $t_{1/2}$ = 4.52 (4.14, 4.99) y. Median (5^{th}, 95^{th} %tile) = 5.4 (2.34, 9.29) y. This is a bit shorter than some other studies but overlap to a fair extent. Results may be less impacted by ongoing background exposure than other data.	Incorporated clearance (from reported half-life) into calculation of overall average clearance. See Section 3.1 in main document
Nilsson et al. (2022a)	Lit update	PFHxS half-lives in Australian fire-fighters after change in foam formulation.	Mean (95% CI) $t_{1/2}$ estimated to be 7.8 (7.3, 8.3) yrs. Rate of decline vs. initial serum level also evaluated and appeared to be independent. Mean $t_{1/2}$ in high exposure group was 7.7 yrs vs. 8.2 yrs in low exposure group.	Yes. Incorporated clearance (from reported half-life) into calculation of overall average clearance. See Section 3.1 in main document

[a]No animal studies were identified in the April 2023 literature search.

APPENDIX C. SUPPLEMENTAL APPROACHES AND DATA ANALYSES

C.1. PFAS CO-EXPOSURE CONSIDERATIONS AND META-ANALYSIS OF PFHXS EFFECTS ON BIRTH WEIGHT

As noted in the polyfluoroalkyl substances (PFAS) protocol, the potential for confounding by co-occurring PFAS to bias effect estimates is a concern in epidemiological studies despite a lack of scientific consensus on how best to address PFAS co-exposures (and other co-occurring contaminants) especially those exposures that may derive from different sources. The potential for confounding across PFAS is incorporated in individual study evaluations and assessed across studies in evidence syntheses and in the characterizations of the strength of evidence. For other covariates like glomerular filtration rate, in general, more confidence was placed in studies that adjusted for pregnancy hemodynamics, or if they considered this potential source of bias by sampling PFAS levels earlier in pregnancy. More details on the considerations of the potential effect of PFAS co-exposures and pregnancy hemodynamics follow.

C.1.1. Confounding Directionality and PFAS Co-exposure Statistical Approaches

A source of uncertainty in the epidemiological database was the potential for confounding by other PFAS (and other co-occurring contaminants). Co-occurring PFAS that are actual confounders (i.e., associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway between the two) would be considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the main primary PFAS of interest. In this example, such PFAS are considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of interest. If positive confounders are not accounted for in the epidemiological study design or analysis phase, the anticipation is that any resultant bias would be away from the null.

Statistical approaches can help address the challenges of evaluating the effects of numerous PFAS that may be present in the environment and estimated via different biomarkers and other exposure measures. For example, multi-pollutant models (i.e., those that adjust for at least one co-exposure) can theoretically provide an estimate of the independent association for specific pollutants with the endpoint of interest by controlling for the independent effect of one or more co-exposures, thereby removing the potential confounding bias (assuming proper functional forms of and limited measurement error related to the confounding variables). However, under certain circumstances, when highly correlated co-exposures are included in the same model, controlling for one co-exposure can potentially amplify the potential confounding bias of another confounder

rather than removing it (Weisskopf et al., 2018). This may be especially problematic when confounding is present and simultaneously adjusted PFAS exposures come from different exposure sources. Dimension-reducing statistical approaches (e.g., principal component analysis and penalized modeling based on elastic net regression) are increasingly being used for screening large groups of chemical classes and help to prioritize specific mixtures. However, as noted by Meng et al. (2018), these approaches might be better suited as "prediction models to screen for a wide range of chemicals from different sources, and the interpretation of results might become less straightforward due to the necessary standardization of exposure values." These regression model outputs also do not provide confidence intervals (CIs), which precludes evaluations of precision. Given these interpretation difficulties and potential for co-exposure amplification bias, it remains unclear whether certain mutually adjusted models give a more accurate representation of the independent effect of specific pollutants for complex PFAS mixture scenarios.

In the main developmental epidemiological syntheses, the evaluation of between-study heterogeneity is based on single pollutant models to increase comparability of available data. An evaluation of single-pollutant (i.e., perfluorohexanesulfonic acid [PFHxS] alone) models and other approaches are detailed below. The objective herein is to assess whether there is any direct evidence for confounding in the studies comparing multi-pollutant (mutually adjusted for other PFAS) and single-pollutant (i.e., PFHxS alone with other confounders adjusted for) model results under the assumption that multi-pollutant models may provide a better reflection of the underlying risk in the absence of any co-amplification bias. Additional objectives of this Appendix were to consider the potential for confounding by examining the strength of associations between co-occurring PFAS as well as those between each PFAS and the primary endpoint of interest (e.g., birth weight-related measures).

C.1.2. PFAS Co-exposure Correlations with PFHxS

In general, the stronger the correlation or association observed between co-exposures, and the larger the associations between the co-exposure and endpoints such as fetal growth restriction, the more concern there would be for potential confounding. A preliminary analysis of 22 studies in the inventory informs Table C-1, which illustrates the direction and magnitude of the correlations between PFAS co-exposures in the PFHxS studies that examined these measures. While it shows that some PFAS co-occur with PFHxS (as expected given some similar anticipated sources), it also illustrates that the magnitude of these relationships can vary across studies. For example, with the exception of two studies for which correlations ranged from 0.30 to 0.34, most studies showed moderate or high correlations of PFHxS with PFOA and PFOS (range: 0.47–0.75). PFHxS was consistently weakly or moderately correlated with both PFDA and PFNA in all of these studies (range: 0.22–0.51). These data suggest that among the other PFAS that have been evaluated, PFOS, and to a lesser extent, PFOA, might be the co-exposures of most concern in many study settings. The stronger correlations between PFHxS and PFOS is unsurprising because, of these chemicals, PFOS shares the greatest structural similarity with PFHxS. In addition to the impact of the structural

similarity on the physico-chemical properties that determine disposition of the chemicals in the environment, these chemicals may also occur together due to their co-production in the manufacturing process (Boucher et al., 2019; 3M, 1999). Similarly, although co-occurrence data may be unavailable, other sulfonated PFAS that are unmeasured or less well characterized in biomarkers may likewise represent potential co-exposures of concern.

Table C-1. PFAS correlation coefficients in nine mutually adjusted PFAS studies

Reference	Study confidence	Correlations with PFHxS			
		PFOS	PFOA	PFNA	PFDA
Ashley-Martin et al. (2017)	*High*	0.55	0.47		
Luo et al. (2021)	*High*	0.01	0.02	-0.04	-0.03
Manzano-Salgado et al. (2017)	*High*	0.56	0.40	0.36	
Shoaff et al. (2018)[a]	*High*	~0.6	~0.4	~0.3	~0.2
Starling et al. (2017)	*High*	0.65	0.61	0.45	0.27
Hamm et al. (2010)	*Medium*	0.54	0.55		
Lenters et al. (2016)	*Medium*	0.34	0.34	0.22	0.36
Meng et al. (2018)	*Medium*	0.30	0.33	0.28	
Callan et al. (2016)	*Low*	0.75	0.71	0.51	0.44

[a]Shoaff et al. (2018) Pearson correlation coefficients ranged from 0.32 (PFNA and PFHxS) to 0.60 (PFOA and PFOS). The estimated correlation coefficients above are based on their related publication (Woods et al., 2017); thus, this may slightly over-estimate the PFDA and PFNA correlation given the initial range provided by Shoaff.

C.1.3. PFHxS and PFAS Co-exposure Study Results

Nine of the PFHxS studies that were evaluated examined PFAS co-exposures including one *low* confidence (Callan et al., 2016), three *medium* confidence (Meng et al., 2018; Lenters et al., 2016; Hamm et al., 2010), and five *high* confidence studies (Luo et al., 2021; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017; Starling et al., 2017). The studies by Hamm et al. (2010) and Luo et al. (2021) did not provide both single-pollutant and multi-pollutant model results for the continuous exposures of interest; this lack of a direct comparison precluded further evaluations of the potential confounding by co-occurring PFAS in that study. The results for the seven other studies based on continuous PFHxS unit changes are compared and summarized below to assess whether any patterns of evidence for larger associations with other PFAS occurred and/or any direct evidence for confounding in the mutually adjusted PFAS studies examining mean birth weight given the primary focus on this endpoint (Table C-2). Two of these studies (Starling et

al., 2017; Lenters et al., 2016) examined PFAS co-exposures through elastic net regression, while the remaining studies performed multi-pollutant modeling using ordinary least squares regression.

As shown in Table C-2, Callan et al. (2016) found large mean birth weight deficits (β = −72 g; 95%CI: −194, 50) in their single-pollutant model of PFHxS, which became *stronger* (39% increase) following adjustment only for perfluoroundecanoic acid (β = −100 g; 95%CI: −221, −22). Meng et al. (2018) reported nonsignificant deficits (β = −12.4 g; 95%CI: −46.2, 21.4) in a single-pollutant PFHxS model, which became *stronger* (30% increase) upon adjustment of PFOS, PFOA, and PFNA in their multi-pollutant model (β = −16.4 g; 95%CI: −61.0, 28.1). Starling et al. (2017) reported largely null findings for PFHxS based on either single-pollutant or multi-pollutant models and this PFAS was not selected based on their elastic net regression. Lenters et al. (2016) reported null results for PFHxS in both their single-pollutant model and their elastic net regression of mutually adjusted PFAS with only PFOA retained in the latter model. Shoaff et al. (2018) reported that their marginally significant single-pollutant PFHxS birth weight z-score was *attenuated* and became more imprecise upon multi-pollutant adjustment. Meng et al. (2018) reported largely null results for PFHxS in single-pollutant models, whereas a nonsignificant increase in mean birth weight was seen upon multi-pollutant adjustment for PFOS, PFOA, PFNA, perfluoroheptane sulfonic acid (PFHpS), and PFDA.

Among these limited studies, there were no consistent patterns detected in the birth weight data for PFHxS following mutual adjustment for other correlated PFAS. For example, two of the five studies that reported birth weight deficits in single-pollutant models were strengthened in similar fashion (30-39%) following statistical adjustment for other PFAS. In contrast, associations in two other studies were attenuated and another study went from an overall mean decreased birthweight to increased birthweight following adjustment. The other two studies were null in both single and multi-pollutant models. There were also no clear patterns of larger associations for other PFAS examined in these studies (data not shown). Thus, strong, and consistent evidence of confounding by other PFAS is not demonstrated in these studies, which is also supported by the lack of differences in the PFHxS-stratified pooled estimates evaluated in the meta-analysis; if confounding were present, there would likely be more variability detected across studies and strata given the variable correlations noted above. While there is still uncertainty due to reported correlations between PFHxS and some PFAS (e.g., PFOS), based on the available evidence, it seems unlikely that the consistency of birth weight deficits demonstrated from (categorical and continuous results) in the full set of 27 PFHxS studies examined here can be fully attributed to confounding by PFAS co-exposures.

Table C-2. Impact of co-exposure exposure adjustment on birth weight results (change in mean birth weight or birth weight Z-scores) per unit change (ng/mL) in PFHxS levels

Reference	Study confidence	Birth weight measure	Single-PFAS model results with 95%CIs	Multi-PFAS results with 95%CIs	Elastic net regression results	Effect of adjustment on PFHxS birth weight results	PFAS adjustments
Ashley-Martin et al. (2017)	*High*	Birth Weight z-scores	0.08 (−0.12, 0.20)[a]	0.04 (-0.13, 0.21)[a]	N/A	Slightly Attenuated	PFOS, PFOA
Shoaff et al. (2018)	*High*	Birth Weight z-scores[b]	-0.08 (-0.18, 0.01)[a]	-0.05 (-0.17, 0.06)[a]	N/A	Slightly Attenuated	PFOS, PFOA, PFNA
Manzano-Salgado et al. (2017)	*High*	Mean Birth Weight	-12.4 (-46.2, 21.4)	-16.4 (-61.0, 28.1)	N/A	Slightly Strengthened	PFOS, PFOA, PFNA
Starling et al. (2017)	*High*	Mean Birth Weight	-13.5 (-50.7, 23.7)	11.5 (-38.9, 61.9)	N/S	Changed direction from Negative to Positive	PFOS, PFOA, PFNA, PFDeA
Lenters et al. (2016)	*Medium*	Mean Birth Weight	-19.1 (-40.7, 2.3)	N/A	N/S	Attenuated	PFOS, PFOA, PFNA, PFUnDA, PFDoDA, PFDA
Meng et al. (2018)	*Medium*	Mean Birth Weight	1.7 (-40.8, 44.3)	25.0 (-10.1, 60.1)	N/A	Changed from Null to Positive	PFOS, PFOA, PFNA, PFDA, PFHpS
Callan et al. (2016)	*Low*	Mean Birth Weight	-72 (-194, 50)	-100 (-221, 22)	N/A	Strengthened	PFUnDA

Abbreviations: N/A: not available; N/S: PFAS not selected in final elastic net regression model. PFUnDA: perfluoroundecanoic acid; PFDeA: perfluorodecanoic acid; PFDoDA: perfluorododecanoic acid; PFHpS: perfluoroheptanesulfonic acid.

[a]The Ashley-Martin et al. (2017) study BWT z-score results are per log-10 unit increases and the Shoaff et al. (2018) study BWT z-score results are per a log-2 increase (i.e., a doubling of PFHxS exposure); all other studies presented here were for each ln-unit increase based on original results from publication or EPA re-expressions.

[b]The mean birth weight result for the single-pollutant model in Shoaff et al. (2018) was -13.4 grams (95%CI: -35.9, 9.1) per each 1 ng/mL increase (JR Shoaff Personal Communication, 10-19-18 (Shoaff, 2018)).

C.1.4. Pregnancy Hemodynamics Background

Pregnancy-related hemodynamic changes that occur during pregnancy (e.g., increased blood plasma volume due to decreased mean arterial pressure, increased cardiac output, and systemic vasodilation (Sagiv et al., 2018; Sanghavi and Rutherford, 2014; Chapman et al., 1998)) are complex and can lead to challenges in data interpretability when timing of PFAS sampling differs within and across studies. These changes could lead to lower PFAS levels in plasma, due to dilution and increased renal filtration. A decrease in PFAS levels has been noted in serial measurements for most of some PFAS during pregnancy, namely PFOA, PFOS, and PFNA (Chen et al., 2021; Glynn et al., 2012). These hemodynamic changes have been proposed as a potential source of bias for associations between different PFAS and neonatal and early childhood growth measures, which is suggested by the association between glomerular filtration rate (GFR), a marker of renal function and, indirectly, of plasma volume expansion, and fetal growth independent of gestational age and other maternal covariates (Morken et al., 2014; Gibson, 1973). Because PFNA concentration in serum is expected to decrease during pregnancy due to plasma volume expansion, increased renal excretion, and transplacental transfer, time windows earlier in pregnancy prior to this decrease may reflect the largest insult to a developing fetus. Potential confounding is one possible explanation for the effects of pregnancy hemodynamics, but Steenland et al. (2018) also proposed that GFR may lead to reverse causality if increased fetal growth leads to increased maternal blood expansion and glomerular filtration rate. These potential sources of bias are anticipated to be of greater concern when maternal serum PFAS samples are collected later in pregnancy. Therefore, as part of the study quality evaluations, more confidence was placed in studies that adjusted for pregnancy hemodynamics or if they considered this potential source of bias by sampling PFAS levels earlier in pregnancy.

Only three of the 21 PFHxS studies examined in the Developmental Effects section collected were able to analyze maternal hemodynamic data such as GFR and albumin (i.e., a marker of plasma volume expansion). None of these studies showed evidence of substantial confounding of the associations between PFAS and fetal growth following statistical adjustment for GFR (Manzano-Salgado et al., 2019; Gyllenhammar et al., 2018b), or for GFR and albumin (Sagiv et al., 2018). Although early pregnancy measures may limit this potential source of bias, the first trimester sampling of plasma albumin and GFR in two of these studies (Manzano-Salgado et al., 2019; Sagiv et al., 2018) may not be best-suited to examine potential confounding if the sample timing did not fully reflect pregnancy-related hemodynamic changes. However, the study by (Gyllenhammar et al., 2018b) with postpartum samples along with another measurement of PFOA and PFOS based on mid-pregnancy samples (Whitworth et al., 2012) have also shown no evidence of confounding by albumin or GFR. These data run counter to meta-analyses for both PFOA (Steenland et al., 2018) and PFOS (Dzierlenga et al., 2020), which have detected larger birth weight deficits for later trimester sampling (e.g., beyond trimester one) compared with early periods. To examine whether the overall pooled estimates for mean birth weight reported in the synthesis varied across different

sampling periods and overall study confidence levels, EPA examined stratum-specific pooled estimates for PFHxS.

C.1.5. Meta-Analysis Methods

Study Inclusion

Thirty-eight developmental epidemiological publications of PFHxS that were identified met our aforementioned inclusion criteria. Some birth cohorts reported BWT analyses for different subsets of their populations in different publications. To avoid double-counting of overlapping study populations, we restricted the meta-analysis to the largest study population sample where multiple publications existed. Specifically, the Woods et al. (2017) study overlapped with the Shoaff et al. (2018) study from the Health Outcomes and Measures of the Environment cohort, and the Bjerregaard-Olesen et al. (2019) study overlapped with (Bach et al., 2016a) from the Aarhus Birth Cohort. Following exclusion of (Bjerregaard-Olesen et al., 2019) and (Woods et al., 2017), there were 36 non-overlapping studies with developmental endpoints of mean BWT changes in relation to PFHxS exposure which advanced to study evaluation. As shown in Figure 1, five studies ((Maekawa et al., 2017; Alkhalawi et al., 2016; Lee and Viberg, 2013; Monroy et al., 2008)) were classified as uninformative largely due to critical study deficiencies in at least one risk of bias domain (e.g., confounding and participant selection) or multiple domain deficiencies. Among the remaining 31 studies, study confidence ratings included 7 low confidence studies (Marks et al., 2019b; Workman et al., 2019; Xu et al., 2019; Cao et al., 2018; Gao et al., 2018; Shi et al., 2017; Callan et al., 2016) , 11 medium confidence studies (Chang et al., 2022; Chen et al., 2020; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018a; Meng et al., 2018; Li et al., 2017a; Kwon et al., 2016; Lenters et al., 2016; Maisonet et al., 2012; Hamm et al., 2010), and 13 high confidence studies (Yao et al., 2021; Eick et al., 2020; Wikström et al., 2020; Buck et al., 2018; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017; Manzano-Salgado et al., 2017; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016b).

Of the 31 informative epidemiological studies with mean BWT data, three studies (Eick et al., 2020; Gao et al., 2019; Cao et al., 2018) reported categorical results only. Our primary analysis was restricted to BWT studies that were most comparable, i.e., those based on continuous PFHxS exposures. Among the 28 studies with results based on continuous data, 24 provided effect estimates in the overall population (i.e., male and female combined). Data were pooled for the four studies that only reported sex-specific findings (Marks et al., 2019b; Ashley-Martin et al., 2017; Lind et al., 2017; Maisonet et al., 2012) within each study using inverse-variance weighting to provide an effect estimate in each study's overall population. This included male and female-specific results in studies by (Lind et al., 2017) and (Ashley-Martin et al., 2017). The study by Maisonet et al. (2012) and Marks et al. (2019b) reported sex-specific estimates for girls and boys in different publications from the same Avon Longitudinal Study of Parents and Children (ALSPAC) study population. These two studies were also pooled to obtain an effect estimate in the overall population and included in

the meta-analysis labeled henceforth, herein, as (Maisonet et al., 2012). Including these mean birthweight estimates pooled across sexes from these three birth cohorts (Ashley-Martin et al., 2017; Lind et al., 2017; Maisonet et al., 2012) resulted in the inclusion of 27 nonoverlapping informative PFHxS studies (from 28 publications) with continuous exposure expressions for the meta-analysis.

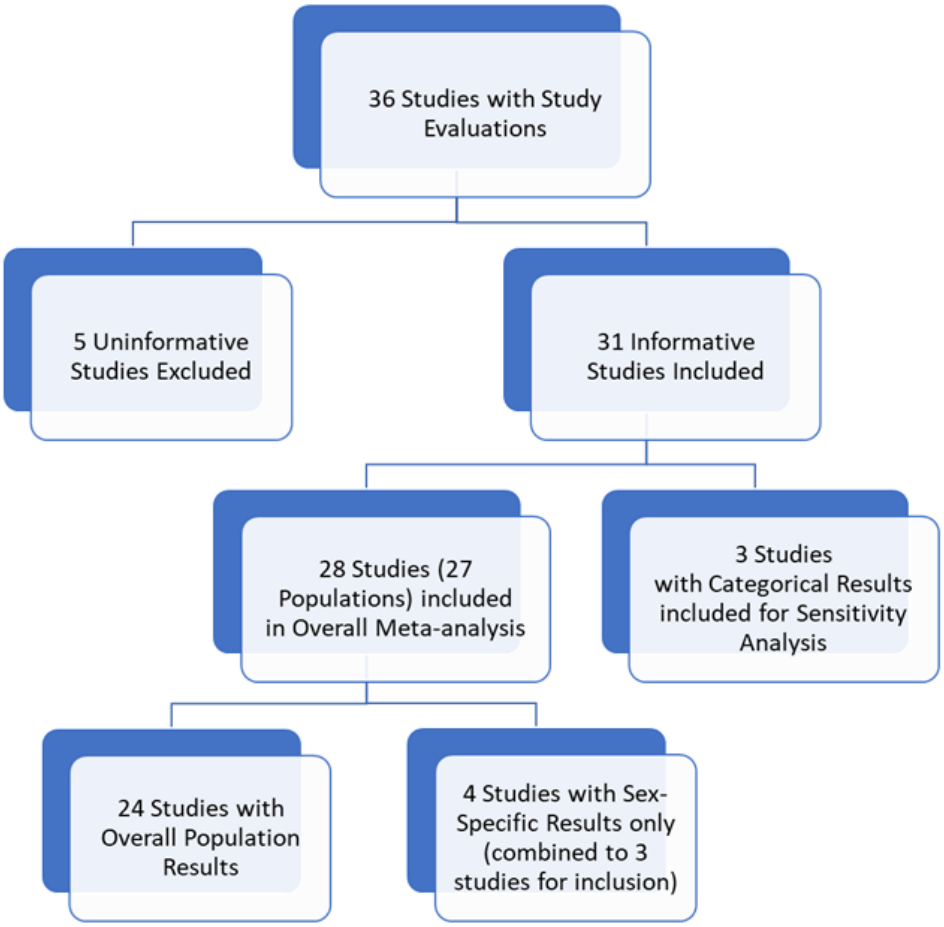

Figure C-1. Twenty-seven informative nonoverlapping perinatal studies of birth weight measures and continuous PFHxS exposure results included in meta-analysis.

Data Preprocessing

EPA converted the exposure-response functions quantifying the results for the 27 available studies (based on data from 28 publications) using different units into two common exposure metrics of natural (i.e., per ng/mL) or natural log units (i.e., per ln(ng/mL)). For example, to standardize the units and reduce between-study heterogeneity due to the choice of unit, different units such as $\log_2$, $\log_{10}$, and per SD- or IQR-unit changes were converted into a common logarithmic function (natural log) as described below. Four of the 27 included studies were based on natural scale PFHxS data (Sagiv et al., 2018; Shoaff, 2018; Bach et al., 2016a; Maisonet et al.,

2012), and EPA used those results to estimate what the results would have been had they been based on a one natural log (ln) unit transformation. This approach was developed by Dzierlenga et al. (2020) and involved plotting the reported linear function for the main effect using 25th–75th percentiles at 10 percentile intervals of the exposure distribution in each study and then fitting a natural logarithmic function to those points. This process was repeated using the reported upper and lower CIs to estimate the bounds of the natural log function and thus the estimated standard error of the natural log function (i.e., standard error = (upper confidence limit – lower confidence limit) / 3.92 (Higgins et al., 2022)).

The meta-analysis was carried out on the natural log scale because the majority (23 out of 27) of the studies reported results on the log scale. Transformations to the log-normal scale are typically employed in epidemiological studies to satisfy regression assumptions. However, the re-scaling methods used by Dzierlenga et al. (2020) and Steenland et al. (2018) can also be used to express ln-transformed data on the natural scale, which may have useful implications for dose response. A sensitivity analysis was conducted to test the robustness of our analysis to selection of natural or natural log scale (see Sensitivity Analysis section below).

Statistical Analysis

The meta-analysis of the 27 developmental PFHxS studies reporting mean birth weight differences was carried out using a random effects model, which follows the assumption that each study produced an estimate of a study-specific true effect that varies across studies (Borenstein et al., 2009). Inverse-variance weighting was employed to minimize the influence of both sampling variance and between-study variance on the pooled effect estimate. The amount of variation due to study heterogeneity was captured by two metrics: the I^2 statistic and Cochran's Q test. The I^2 statistic represents the percent of variation in the pooled estimate due to between-study heterogeneity. The range of values shown in the Cochran's I^2 guidelines (Higgins et al., 2022) informed EPA's consideration of I^2 statistics <40% to represent "low" potential heterogeneity, with values from 40–69% being "moderate," and values ≥70% to represent "high" heterogeneity. Cochran's Q test evaluates whether the dispersion of study-specific estimates about the pooled effect estimate is statistically significant via a p-value (p_Q), based on significance level (α) of 0.05. Both metrics may suffer from low statistical power when few studies are available, potentially complicating interpretation of the examinations of heterogeneity. Thus, consideration of both measures in conjunction is recommended to identify situations for which heterogeneity may be present (Huedo-Medina et al., 2006). While the number of studies for the overall analysis may be large enough (n = 27) to not be subject to these concerns, some uncertainty exists for the stratified analyses with considerably fewer studies per strata.

EPA conducted stratified analyses to evaluate whether the summary effect estimate varied by the study confidence rating or by the timing of maternal serum sampling. As detailed in Section 3.2.3, study confidence designations included four *low* confidence studies (Workman et al., 2019; Xu et al., 2019; Shi et al., 2017; Callan et al., 2016), eleven *medium* confidence studies (Chang et al.,

2022; Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018b; Meng et al., 2018; Li et al., 2017c; Kwon et al., 2016; Lenters et al., 2016; Maisonet et al., 2012; Hamm et al., 2010), and twelve *high* confidence studies (Luo et al., 2021; Yao et al., 2021; 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., 2017; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016a).

Sample timing strata were defined according to two strategies based on reported gestational age (weeks) at time of biomarker collection. Strategy 1 was a three-strata approach with subgroups, *early* (n = 12), *late* (n = 10) and *post* (n = 5) pregnancy. Strategy 2 was a two-strata approach, using the same definition of *early* pregnancy as in Strategy 1, but combining late- and post-pregnancy into a single stratum, *late + post* (n = 15). Early pregnancy included studies reporting samples from preconception (0 days), the first trimester (0 days to 13 weeks and 6 days) or a mixture of the first and second trimesters (0 days to 27 weeks and 6 days); late pregnancy studies sampled in the second trimester (14 weeks and 0 days to 27 weeks and 6 days), a mixture of the second and third trimester (14 weeks and 0 days to birth), or the third trimester only (28 weeks and 0 days to birth); post-pregnancy studies sampled at or after birth (ACOG, 2020). Studies were assigned to sample timing strata based on reported trimesters of sampling as well as sampling ranges and interquartile ranges or measures of centrality when measures of spread were unavailable (see Table C-3 below for details on sample timing distributions and strata assignments).

The two-strata sample timing approach was also used by previous PFAS meta-analyses (Dzierlenga et al., 2020; Steenland et al., 2018). However, as noted in Wright et al. (2023), there may be value in differentiating late maternal samples from post-partum measures and further refining what constitutes early sampling when a sufficient number of studies allow other alternatives. Thus, EPA apportioned studies with late pregnancy samples from those with post-pregnancy samples to better understand differences in sampling matrices, i.e., maternal serum sampled during pregnancy versus umbilical cord samples or postpartum maternal serum samples (i.e., termed post-pregnancy here). Furthermore, the use of more subgroups provides more detail on the gradient of changes that sample timing may be associated with. A sensitivity analysis was employed to assess the robustness of the meta-analysis results to using three strata instead of two.

Strata-specific estimates that allowed for heterogeneity were calculated using a random effects model and a subsequent fixed effects model was used to test for statistically significant differences across the subgroups (Borenstein et al., 2009). A *p*-value less than 0.05 from this hypothesis test was indicative of no statistically significant differences between any of the strata. Strata-specific statistical tests conducted on subgroups with lower sample sizes are subject to lower power, susceptible to higher uncertainty and therefore should be interpreted with caution. For full details on the computations involved in both the stratified and overall meta-analyses, please refer to the R code developed by EPA (Larsen, 2022).

All statistical analyses are carried out using the open-source platform, R (Version 4.0.3), and all meta-analytic techniques are carried out using the meta-analysis R package, metafor (Viechtbauer, 2010).

Table C-3 Details on study sample timings and strata assignments

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
Ashley-Martin et al. (2017)	N/R (N/R)	Trimester 1	Early	Sampling time of 9.9 wks was estimated from the trimester 1 midpoint with a range of 6 wks to 13 wks and 6 d.
Bach et al. (2016a)	12 (9, 20) *Mode (Min, Max)*	Trimesters 1 and 2	Early	
Buck Louis et al. (2018)	10, 13.9 *(Min, Max)*	Trimester 1	Early	Value of 11.9 wks was estimated as midpoint of the range (10 to 13.9 wks).
Chang et al. (2022)	11.4 (9.6, 12.6) *Median (*25%, 75%*)*	Trimesters 1, 2	Early	
Chen et al. (2021)	16.3 (13.85, 20.43) *Median (Min, Max)*	Trimesters 1 and 2	Early	The authors provided additional data, which showed their serial measures included overlapping trimesters, e.g., their trimester 1 results encompassed trimesters 1 and 2 samples (see Zhang (2022)).

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
Hjermitslev et al. (2020)	15.39 (7, 40) *(Min, Max)*	Trimesters 1, 2, and 3	Early	This study was assigned to the *early* strata because sampling predominantly occurred earlier in pregnancy: study authors report that the mean gestational week of sampling in 2010–2011 was week 26.2, and in 2013–2015 all samples were collected before the end of week 13. 38% of samples were taken in 2010–2011; 62% was collected in 2013–2015 (Bonefeld-Jørgensen, 2022).
Lind et al. (2017)	10 (5, 12) *Median (Min, Max)*	Trimester 1	Early	
Maisonet et al. (2012); Marks et al. (2019a)[a]	10, 228 (25%, 75%); 12, 33 (25%, 75%)	Trimester 1, 2, and 3	Early	
Manzano-Salgado et al. (2017)	12.3 (5.6) *Mean (SD)*	Trimester 1, 2, and 3	Early	Sampling is reported to be all in the first trimester (Manzano-Salgado et al., 2017) supporting information clarifies

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
				that some sampling outside of the 1st trimester also occurred (Wright and Larsen, 2022). However, first trimester sampling was predominant, so this study is designated as conducting "early" sample timing.
Meng et al. (2018)	8 (4, 14) *Mean*	Trimesters 1 and 2	Early	The mean is reported in related publication by Liew et al. (2020).
Sagiv et al. (2018)	9 (5, 19) *Median (Min, Max)*	Trimesters 1 and 2	Early	
Wikström et al. (2020)	10 *Median*	Trimesters 1 and 2	Early	
Callan et al. (2016)	37.7 (33, 40) *(Min, Max)*	Trimester 3	Late	Samples were taken 2 wks before due date, which ranged from 35 to 42 wks; estimate measure of centrality used here of 37.7 wks.
Hamm et al. (2010)	15.5 (15, 16) *(Min, Max)*	Trimester 2	Late	Measure of centrality of 15.5 wks estimated from

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
				the midpoint of the reported range.
Kashino et al. (2020)	29 *Median*	Trimester 3	Late	
Lenters et al. (2016)	25.2 *Weighted mean of medians*	Trimesters 2 and 3	Late	Study authors reported country-specific medians: 33 wks (Poland, 18%), 25 weeks (Greenland, 32%), 23 wks (Ukraine, 49%).
Luo et al. (2021)	39.3 *Mean*	Trimester 3	Late	
Shoaff et al. (2018)	18.1 *Weighted average*	Trimesters 2 and 3, and at delivery	Late	Study was assigned to the *late* strata instead of *post* because only 5% of samples taken at delivery, and sensitivity analysis conducted by study authors found results robust to Trimester 2 only. The weighted average was derived the following data provided in the manuscript: "16-week serum samples

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
				if available (86%), the 26-wk sample if the 16-wk sample was not available (9%), and if neither of those was available, then the samples from delivery were used (5%)."
Starling et al. (2017)	27 (20, 34) *Median (Min, Max)*	Trimesters 2 and 3	Late	
Valvi et al. (2017)	34 *Exact*	Trimester 3	Late	
Workman et al. (2019)	28.6 (14.3, 39.6) *Median (Min, Max)*	Trimesters 2, 3	Late	Data provided by authors: Mean = 27.7 wks. Median = 28.6 wks; Range = 14.3–39.6 wks.
Yao et al. (2021)	39.4 *Mean*	Trimester 3	Late	
Gyllenhammar et al. (2018b)	43 (37.9, 46.1) *Mean (Range)*	Post-Birth	Post	Samples were taken 3 wks after delivery; mean (range) delivery date = 40 wks (34.9, 43.1).
Kwon et al. (2016)	40 *Exact*	At Delivery	Post	
Li et al. (2017c)	39	At Delivery	Post	

Study	Sampling distribution - estimates and measures of centrality (and spread) in gestational weeks	Time period	Sample timing start[a]	Notes
	Mean			
Shi et al. (2017)	39.8 (4.2) *Mean (SD)*	At Delivery	Post	
Xu et al. (2019)	39.4 (1.4) *Mean (SD)*	At delivery	Post	

Abbreviations: N/R: not reported; 25th, 75th%; 25th percentile, 75th percentile of exposure distribution; SD: standard deviation

[a]Maisonet et al. (2012) was rated as *Medium* confidence, while Marks et al. (2019b) was *Low*. This combined population was evaluated as *Medium* for stratified analyses.

C.1.6. Meta-Analysis Results

As shown in Figure C-2, the overall pooled birth weight effect estimate for the 27 studies based on the random effects model was −7.7 g (95%CI: -14.8, -0.5) per ln ng/mL increase in PFHxS exposure. The tests for heterogeneity showed that between-study variability was negligible (I^2 = 0%, p_Q = 0.84). The meta-analysis results stratified by study confidence are displayed in Table C-4. The 12 *high* confidence studies yielded a smaller pooled effect estimate of decreased birthweight (β = −6.8 g; 95%CI: -16.3, 2.8) than the 11 *medium* (β = −9.6 g; 95%CI: -20.8, 1.6) confidence studies; however, the differences between strata were not statistically significant (p = 0.85). There was negligible between-study heterogeneity for the *high* (I^2 = 0%, p_Q = 0.94) and *medium* confidence studies, while the *low* confidence subgroup exhibited "low" heterogeneity (I^2 = 20.1%, p_Q = 0.30). Given the small sample size of the strata (n = 4) and larger and more divergent results, the *low* confidence combined estimate and heterogeneity statistics are subject to relatively more uncertainty and should be interpreted with caution.

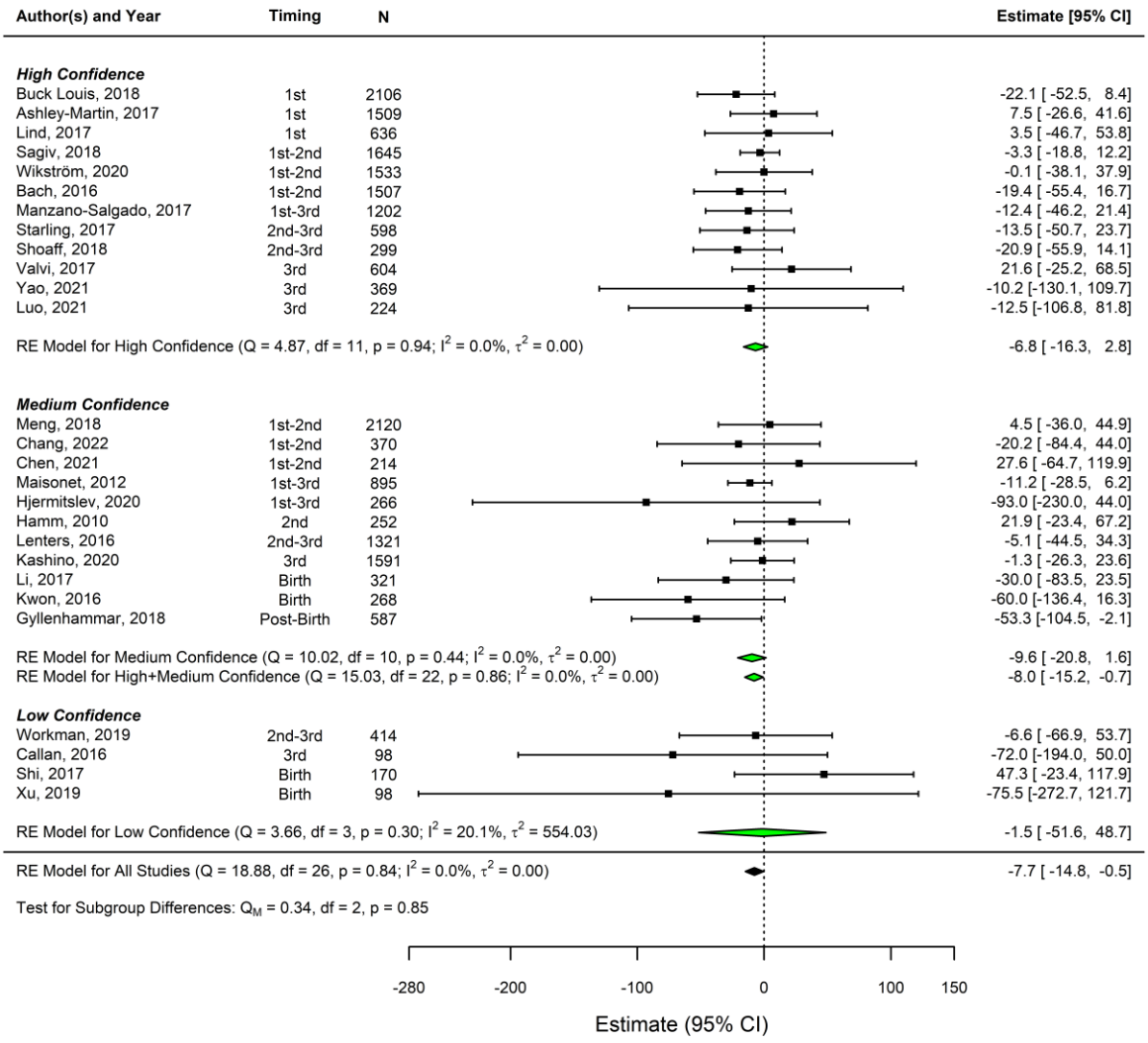

Figure C-2. Forest plot of 27 studies included in the meta-analysis on PFHxS exposures and changes in birth weight.

Table C-4. Meta-analysis of PFHxS on birth weight changes (in g per ln(ng/mL)) stratified by study confidence

Set of studies	*n*	β	95% confidence interval	I^2 (%)	p_Q
All Studies	27	-7.7	-14.8, -0.5	0.00	0.84
High Confidence	12	-6.8	-16.3, 2.8	0.00	0.94
Medium Confidence	11	-9.6	-20.8, 1.6	0.00	0.44
Low Confidence	4	-1.5	-51.6, 48.7	20.1	0.30
High + ***Medium*** Confidence	23	-8.0	-15.2, -0.7	0.00	0.86

Symbols and abbreviations: n = sample size; β = combined estimate of change in birth weight (g) per ln (ng/mL) PFHxS exposure; I^2 = % variation in the pooled effect due to study heterogeneity; p_Q = *p*-value for Cochran's Q test for heterogeneity.

The meta-analysis results stratified by sample timing are displayed in Table C-5. While there are no statistically significant differences between timing subgroups in either the two-strata or the three-strata approach (p = 0.88 and p = 0.54, respectively), there is some evidence that estimated birth weight deficits are greater in later sampling. For example, a fourfold difference in the three-strata approach was seen between the 12 *early* (β = −7.3 g; 95%CI: -16.0, 1.4) studies and 5 studies based on *post*-pregnancy samples (β= −28.3 g; 95%CI: -69.3, 12.7). Differences between early and later sampling are slightly more pronounced in the three-strata approach than in the two-strata approach for which the pooled estimate for the *late* and *post*-pregnancy samples combined was -8.5 g (95%CI: -21.0, 4.1). The estimate for the *late* pregnancy strata based on 10 studies was null (β= −3.9 g; 95%CI: -17.7, 9.9) but was more comparable in magnitude to the early sampled strata data (β= −7.3 g per each PFHxS ln-unit increase) versus the post-partum studies.

Except for the *post*-pregnancy strata, Heterogeneity observed in each subgroup was negligible for all strata expect the *post*-pregnancy stratum which had an estimated "low" percent of variation due to heterogeneity, and non-significant Cochran's Q test (I^2 = 20.1%, p_Q = 0.30). However, the *post*-pregnancy strata have a sample size of less than ten studies, so results from these heterogeneity tests are expected to be more uncertain.

Table C-5. Meta-analysis of PFHxS on birth weight (in g per ln(ng/mL)) stratified by sample timing

Set of studies	n	β	95% Confidence interval	I^2 (%)	p_Q
All Studies	27	-7.7	-14.8, -0.5	0.00	0.84

Set of studies	n	β	95% Confidence interval	I^2 (%)	p_Q
Early Pregnancy	12	-7.3	-16.0, 1.4	0.00	0.91
Late Pregnancy	10	-3.9	-17.7, 9.9	0.00	0.85
Post-Pregnancy	5	-28.3	-69.3, 12.7	39.8	0.19
Late + Post Pregnancy	15	-8.5	-21.0, 4.1	0.00	0.49

Symbols and abbreviations: n = sample size; β = combined estimate of change in birth weight in g per ln(ng/mL) PFHxS exposure; I^2 = % variation in the pooled effect due to study heterogeneity; p_Q = *p*-value for the Cochran's Q test for heterogeneity.

C.1.7. Sensitivity Analysis Results

The sensitivity of the meta-analysis results to re-expression was tested by comparing results based on effect estimates re-expressed to the natural log scale to those converted to the natural scale. Table C-6 illustrates that the pooled effect estimates of mean birth weight deficits in our primary analysis are smaller and closer to the null when based on the natural scale but that the patterns in magnitude seen across study confidence and sample timing strata are relatively comparable.

Table C-6. Sensitivity of natural log scale or natural scale re-expression for the overall and stratified meta-analyses of birth weight (in g per ln(ng/mL)

Set of studies	*n*	β (95%CI)	β (95%CI)
All Studies	27	-7.7 (-14.8, -0.5)	-2.9 (-6.4, 0.7)
Study Confidence Strata			
High	12	-6.8 (-16.3, 2.8)	-1.0 (-5.3, 3.2)
Medium	11	-9.6 (-20.8, 1.6)	-6.8 (-13.2, -0.3)
Low	4	-1.5 (-51.6, 48.7)	-15.9 (-55.2, 23.4)
High + Medium	23	-8.0 (-15.2, -0.7)	-2.8 (-6.4, 0.8)
Sample Timing Strata			
Early	12	-7.3 (-16.0, 1.4)	-3.1 (-8.0, 1.8)
Late	10	-3.9 (-17.7, 9.9)	0.1 (-5.7, 5.9)
Post	5	-28.3 (-69.3, 12.7)	-12.8 (-23.9, -1.6)
Late + Post	15	-8.5 (-21.0, 4.1)	-3.7 (-10.0, 2.6)

Symbols and abbreviations: n = sample size; β = pooled estimate of change in birth weight (g) per ln (ng/mL) or ng/mL PFHxS exposure; CI = confidence interval.

C.1.8. Summary of Meta-Analysis of PFHxS Effects on Birth Weight

Similar to the hazard synthesis of all the categorical and continuous birth weight results detailed in Section 3.2.2, the meta-analysis pooled estimate of 27 studies showed a statistically significant decrease in mean birth weight of 7.7 g (95%CI: −14.8, −0.5) per natural log-unit increase

in maternal serum PFHxS. This overall result was similar when studies were restricted to just the *medium* confidence studies (β = −9.6 g; 95%CI: -20.8, 1.6), *high* confidence studies (β = −6.8 g; 95%CI: −16.3, 2.8) or the *medium* + *high* confidence studies (β = −8.0 g; 95%CI: −15.2, -0.7). The smallest differences were null for the *low* confidence studies, with *high* confidence, *medium* confidence, and a combined estimate of those two being similar to the overall pooled estimate. These higher quality studies are anticipated to be the least susceptible to potential biases.

Similarly, although a consistent gradation in birth weight deficits was not seen across sample timing, the *early* sampled stratum has a pooled estimate similar in magnitude (β = −7.3 g) to the overall and the higher confidence studies. Interestingly, this value was fairly comparable to that seen in the later pregnancy sampled studies (β = −3.9 g) in contrast to large differences seen in the postpartum studies which were predominantly based on umbilical cord samples. A four-fold difference was seen when comparing pooled estimates from the twelve *early* sample studies (β = −7.3 g; 95%CI: −16.0, 1.4) and the five studies with *post*-pregnancy samples (β = −28.3 g; 95%CI: −69.3, 12.7); however, the CI for the *post*-pregnancy samples is wide and completely encompasses the CI for the *early* samples.

Overall, these data are suggestive of a pattern of later sampling times for PFHxS showing larger deficits in birth weight, a pattern that may suggest greater bias in later samples. And, although the postpartum sampled studies have considerably larger results, the small decrease of -7.3 grams (per each ln-unit PFHxS increase) from early pregnancy sample studies was not too dissimilar to what was seen amongst studies with maternal biomarkers sampled late in pregnancy. In comparison to meta-analyses of PFOA (Steenland et al., 2018) and PFOS (Dzierlenga et al., 2020), a strength of our meta-analysis was the ability to separate results across three different strata. Further investigation should be undertaken to identify if the large differences between late pregnancy and postpartum samples is unique to PFHxS or is common among PFAS. One interesting finding related to this topic is that a recent study reported no decrease in PFHxS serum concentration in serial sampling during pregnancy (Chen et al., 2021). This finding is in contrast to the other long-chain PFAS which uniformly decreased during pregnancy in the same study, which suggests that maternal physiological changes during pregnancy affect PFHxS differently than other long-chain PFAS.

There was a suggestion of an effect in the overall estimate of all 27 studies as well as the *medium* and *high* confidence study and the early sample subsets. The latter strata may still be impacted by potential bias from pregnancy hemodynamics since the categorization approach was based on samples that may have included a minority of late trimester participants. While the source of any differences between late pregnancy and postpartum remain unclear, the data do suggest that potential bias from pregnancy hemodynamics should continue to be examined as a source of uncertainty in epidemiological studies. Additional research on the slowly cleared PFAS such as PFHxS is needed to further delineate any differences and better delineate the potential impact of pregnancy hemodynamics across the class. The meta-analytical findings along with this research in

indicative of complex patterns of influence due to pregnancy hemodynamic differences that are not completely understood.

C.2. AOP-BASED APPROACH FOR EVALUATING POTENTIAL PFHxS-INDUCED MECHANISMS OF HEPATOTOXICITY MODE OF ACTION

C.2.1. Objective and Methodology

The goal of the qualitative analysis described here is to evaluate the available mechanistic evidence for PFHxS-induced liver effects to assess the biological plausibility of effects observed in animal models and identify mechanistic pathways that are conserved across species and strains of animals and liver cell culture models and are therefore more relevant to human health. The available mechanistic and toxicological evidence was organized and evaluated in concordance with the frameworks used for mode of action (MOA) analysis for noncancer effects and development of Adverse Outcome Pathways (AOP)[2] (Edwards et al., 2016; Boobis et al., 2008; IPCS, 2007). PFHxS-induced hepatic effects reported in in vivo and in cell culture studies was organized according to the following levels of biological organization: molecular interactions, cellular effects, organ effects, and organism effects[3]. As recommended in U.S. EPA (2005), the analysis described here was focused on the concordance of key events and adverse responses across species to obtain clarification on the relevance of animal studies to human health (U.S. EPA, 2005).

In addition to analyzing the available evidence published in the peer-reviewed literature EPA also considered mechanistic evidence from in vitro high-throughput screening (HTS) assays on PFHxS available from EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) (U.S. EPA, 2019). Bioactivity data from the ToxCast and Tox21 collaborative projects were also considered at the same levels of biological organization described below. A more detailed description of the HTS analysis and results is provided in Appendix C3.

C.2.2. Proposed AOP Approach for Evaluation of PFAS-Induced Liver Toxicity

The proposed MOA displayed in Figure C-3 is based on molecular initiating events, key events, and adverse outcomes identified in previous mechanistic evaluations and reviews on PFOS and PFOA (ATSDR, 2018; Li et al., 2017b; U.S. EPA, 2016a, b), which are structurally related to PFHxS and among the most well-studied PFAS. Additional reviews on biological pathways

[2]Although the World Health Organization (WHO)-International Programme on Chemical Safety (IPCS)-MOA and the Organization for Economic Co-operation and Development (OECD)-AOP frameworks are similar in the identification and analysis of key events following modified Bradford-Hill criteria (Meek et al., 2014), AOPs are chemical agnostic whereas MOA analyses are intended to inform health assessments of individual (or groups of) chemical(s) (Edwards et al., 2016).

[3]Mechanistic evidence from individual chemicals was summarized in a supplementary (MS Excel) database to facilitate the qualitative analysis of the outcomes reported in the available studies.

associated with chemical-induced cancer and noncancer liver effects were also consulted (see citations below). A summary of the MOA is presented below.

At the molecular level, experimental studies using in vivo and cell culture models have shown that perfluorinated compounds such as PFOS and PFOA can activate several nuclear receptor pathways including the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), the farnesoid X receptor (FXR), the peroxisome proliferator activated receptor alpha (PPARα) and gamma (PPARγ), estrogen receptor alpha (ERα) and other receptor-independent cell signaling pathways (e.g., phosphatidylinositol 3-kinase-serine/threonine protein kinase (PI3KAkt) signal transduction pathway, and the nuclear factor kappa B pathway [NFκB]) (ATSDR, 2018; Gleason, 2017; Li et al., 2017b; NJDWQI, 2017; U.S. EPA, 2016a, b). PFOS- and PFOA induced activation of PPARα is associated with hepatocellular hypertrophy caused by peroxisome proliferation, and increased peroxisomal fatty acid β oxidation and cytochrome P450 4A (CYP4A) expression and activity (ATSDR, 2018; U.S. EPA, 2016a, b), and altered cholesterol metabolism (Li et al., 2017b). Increased PPARα activity can lead to oxidative stress via induction of acyl CoA oxidase expression and activity, and H2O2 production in peroxisomes (Hall et al., 2012). Several studies have used genetically modified animal and cell culture models and immortalized human cell lines to evaluate potential PFOS or PFOA activation of the human PPARα. COS-1 cells transfected with the murine or human PPARα were responsive to PFAS exposure (U.S. EPA, 2016a, b), and F1 generation PPARαhumanized mice were responsive to PFOA-induced expression responsive genes on GD18, but unlike wild type animals this response was not apparent on PND 20 (U.S. EPA, 2016b; Takacs and Abbott, 2007). Studies using human liver cell lines or humanized animal models suggest that humans are less sensitive to PPARα activation by the perfluorinated compounds PFOS and PFOA (reviewed in Li et al. (2017b); U.S. EPA (2016a)). PPARα has also been shown to be activated by exposure to several PFAS, including PFOS, PFOA, PFNA, and PFHxS (ATSDR, 2018; Li et al., 2017b). Although PPARα is not expressed in high levels in the liver, its activation by pharmaceuticals and xenobiotic compounds has been proposed to be associated with hepatic steatosis caused by lipid accumulation (Angrish et al., 2016; Mellor et al., 2016).

As described above exposure to perfluorinated compounds such as PFOS and PFOA has also been shown to activate other nuclear receptor and cell signaling pathways, including the CAR, PXR, FXR, ERα, NFκB, and oxidative stress responsive nuclear factor erythroid 2 related factor 2 (Nrf2) (ATSDR, 2018; Li et al., 2017b; U.S. EPA, 2016a). Furthermore, experiments using null animal models exposed to several PFAS suggest that activation of CAR/PXR occurs independently of PPARα (ATSDR, 2018; Li et al., 2017b). Previous analyses of chemical-induced hepatotoxicity suggest that activation of these cell signaling pathways in experimental models is associated with increased expression and activity of xenobiotic metabolizing enzymes (XMEs) (Joshi-Barve et al., 2015; Hall et al., 2012), formation of reactive metabolites, alterations in cellular lipid metabolism (Angrish et al., 2016), and endoplasmic reticulum damage (Joshi-Barve et al., 2015).

At the cellular level, exposure to PFAS such as PFOS and PFOA has been shown to increase reactive oxygen species production and oxidative damage to cellular macromolecules (ATSDR, 2018; Li et al., 2017b; U.S. EPA, 2016b); promote mitochondrial damage, inhibit mitochondrial function, activate mitochondrial-mediated cell death (Li et al., 2017b; U.S. EPA, 2016a); increase endoplasmic reticulum stress (U.S. EPA, 2016b); induce DNA damage (ATSDR, 2018; U.S. EPA, 2016b); disrupt intercellular gap junction communication (ATSDR, 2018); elevate production/levels of pro-inflammatory cytokines (U.S. EPA, 2016a); alter lipid and glucose metabolism and bile acid biosynthesis (U.S. EPA, 2016a, b); and increase hepatocellular death (Li et al., 2017b; U.S. EPA, 2016b). These pathways/mechanisms are associated with toxicant-induced liver disease and can promote steatohepatitis and fibrosis (Bessone et al., 2019; Angrish et al., 2016; Cao et al., 2016; Joshi-Barve et al., 2015; Wahlang et al., 2013).

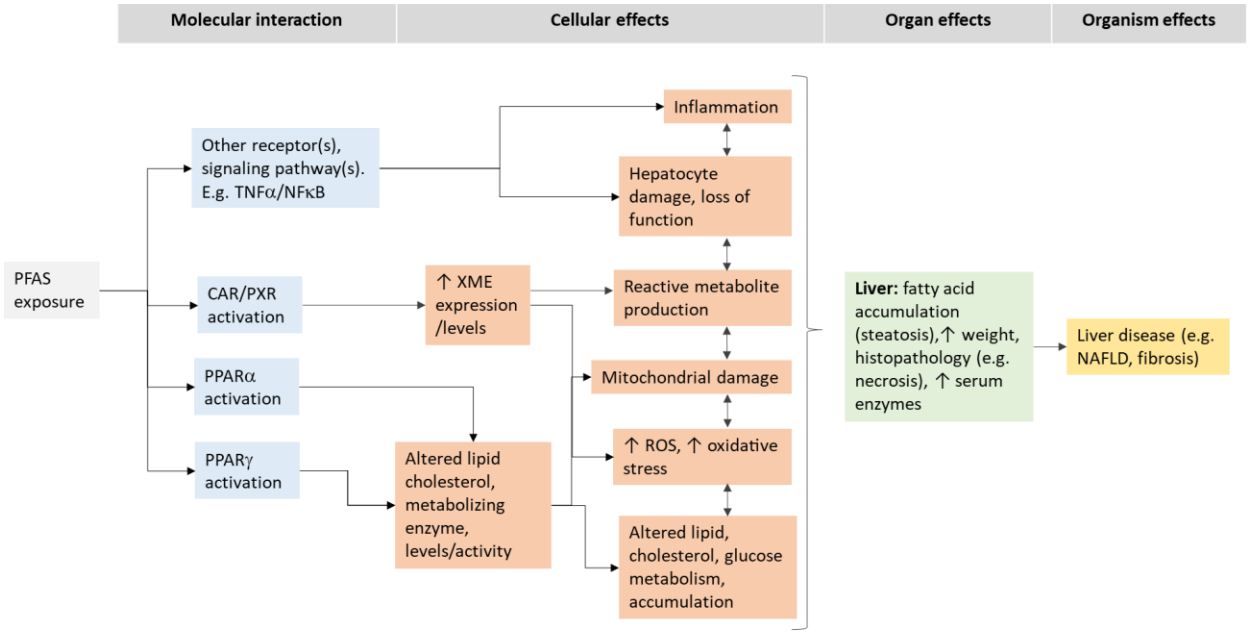

Figure C-3. The proposed MOA in the figure above is based on previous analyses on PFAS-induced (e.g., PFOA/PFOS) liver toxicity and the role of nuclear receptor pathways in hepatotoxicity.

C.3. SUMMARY OF RELEVANT HIGH-THROUGHPUT SCREENING ASSAYS FROM EPA'S COMPTOX CHEMICALS DASHBOARD

C.3.1. In vitro Bioreactivity Data Relevant to the Mechanisms of PFHxS-Induced Liver Effects

In vitro high-throughput screening (HTS) assays for PFHxS were downloaded from EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) (U.S. EPA, 2019), which provides bioactivity data from the ToxCast and Tox21 collaborative projects. Available information most pertinent to the analysis of the potential mechanisms of PFHxS-induced liver effects was extracted to supplement and augment mechanistic findings from studies in the peer-reviewed

literature previously described. Results (active/inactive, AC50 values, and scaled activity) from in vitro assays were obtained, filtering out background control assays and nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ('_dn')[4]. Bioactivity data were analyzed based on the type of biological response or biological target using the annotation structure within the ToxCast assay summary information (U.S. EPA, 2019).

PFHxS was active in 34 of 743 assays, of which 10 were performed in human liver tissues. PFHxS was active in 0 of 54 unique assay endpoints in human hepatoma HepG2 cells. The majority of the active liver relevant endpoint assays were nuclear receptor assays (see Figure C-4, Table C-9). PFHxS was able to induce reporter assays for multiple nuclear receptors including PPARα, PPARγ, RXR and LXR as well as transcriptional factors, FOS, and NRF2 (see Figure C-5, Table C-9). Nuclear receptor activities were further investigated to provide additional information to known interactions of PFHxS with these receptor-mediated signaling pathways in ToxCast/Tox21 assays profiling multiple endpoints (e.g., receptor binding, co-regulator recruitment and gene transactivation) and cell types (see Figure C-5, Table C-9). As previously mentioned, PFHxS induced activity of specific steroid/xenobiotic sensing receptors, most notably PXR, RXR and PPAR (see Figure C-5). PFHxS interacted with the retinoic acid (RAR, 1 out 19 assays) and the human RXR (1 out of 10) in receptor binding assays. PFHxS was active in 4 out of 27 PPAR-related assays, showing transcriptional activation, including induction of a PPAR-response element driven reporter gene assay, PPARα and α-dependent reporter gene expression and binding to the human PPARα.

[4]Inducible gene reporter assays were not optimized to detect loss of signal (U.S. EPA, 2019); therefore, responses from assays analyzed in the negative fitting direction relative to the control ('_dn') are considered non-specific and are not presented herein.

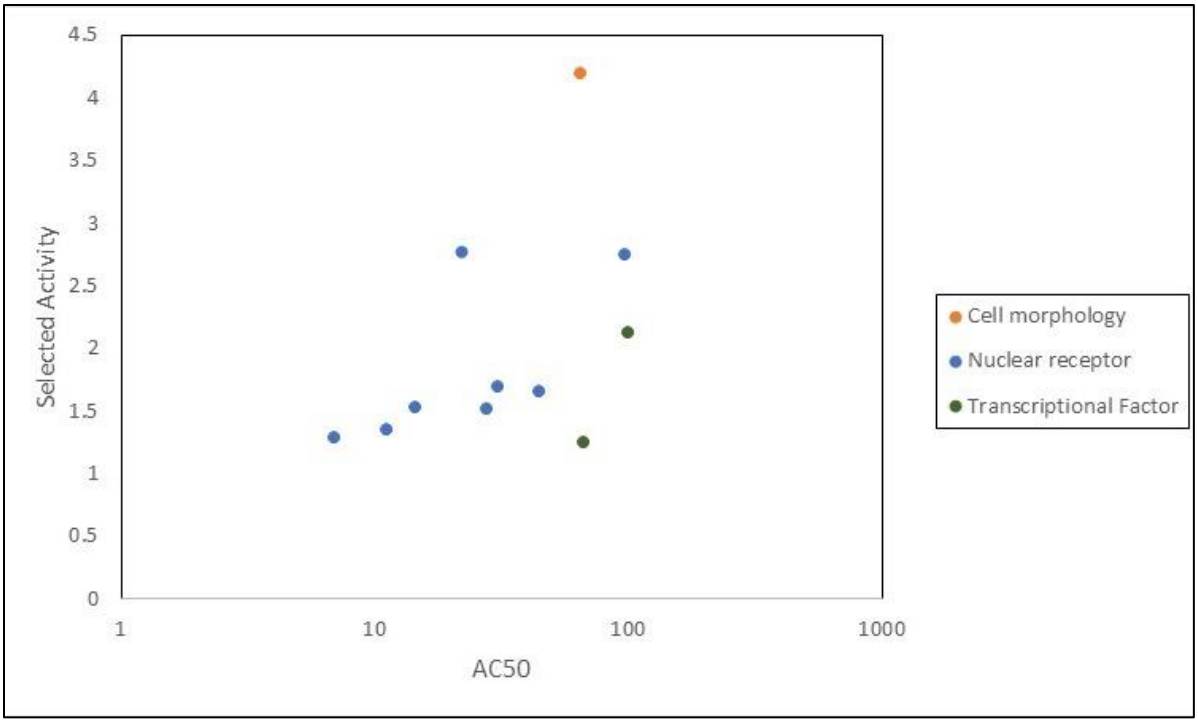

Figure C-4. Bioactivity data for PFHxS from in vitro HTS ToxCast/Tox21 assays in human liver tissues. Scatterplots show AC50 and scaled activity values from assays visualized according to the type of biological response or biological target. AC50 values refer to the concentration that elicits half maximal response and the scaled activity refers to the response value divided by the activity cutoff. Assays for which chemicals were inactive are not displayed. Additional information on these assays can be found in Table C-8.

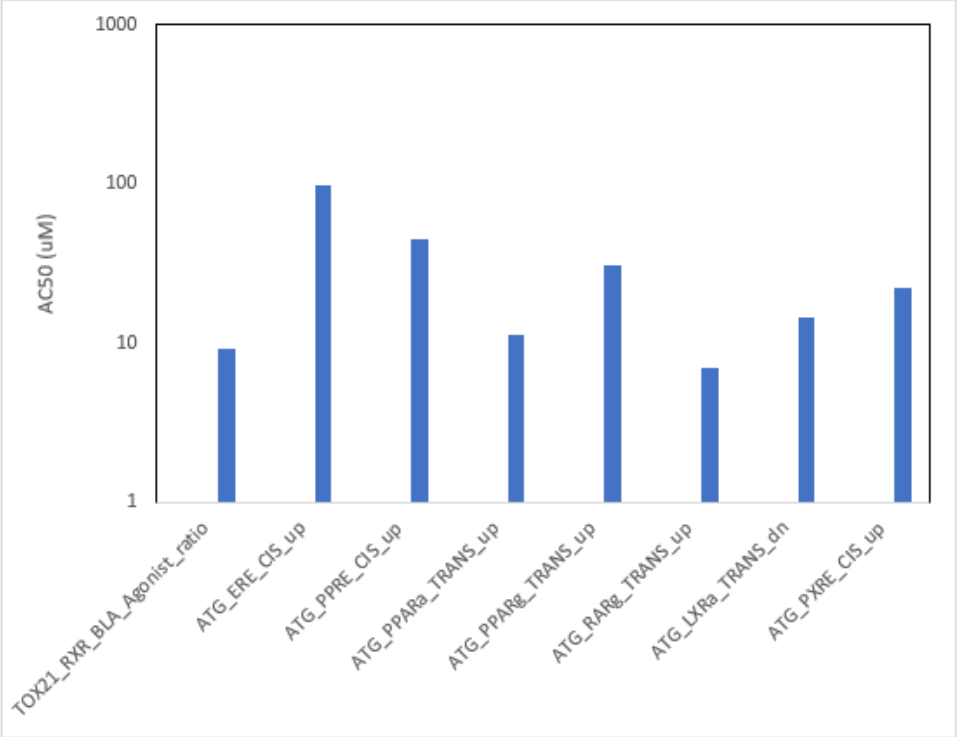

Figure C-5. Summary of positive nuclear receptor assays in human liver tissue. Additional information on these nuclear receptor assays may be found in Table C-9.

Table C-7. Bioactivity summary for PFHxS from in vitro HTS assays from ToxCast/Tox21 conducted in human liver tissue and grouped by biological response/target.

Assay name[a,b]	Activity call	Scaled activity	AC50 (µM)	Assay design
Cell morphology				
TOX21_MMP_ratio_up	ACTIVE	4.19	65.1	Membrane potential reporter
Nuclear receptor				
TOX21_RXR_BLA_Agonist_ratio	ACTIVE	1.31	9.28	Inducible reporter
ATG_ERE_CIS_up	ACTIVE	2.74	96.9	Inducible reporter
ATG_PPRE_CIS_up	ACTIVE	1.65	44.6	Inducible reporter
ATG_PPARa_TRANS_up	ACTIVE	1.35	11.2	Inducible reporter
ATG_PPARg_TRANS_up	ACTIVE	1.69	30.5	Inducible reporter
NVS_NR_hPPARg	ACTIVE	1.51	27.5	Binding reporter
ATG_RARg_TRANS_up	ACTIVE	1.28	6.91	Inducible reporter
ATG_LXRa_TRANS_dn	ACTIVE	1.53	14.4	Inducible reporter

Assay name[a,b]	Activity call	Scaled activity	AC50 (µM)	Assay design
ATG_PXRE_CIS_up	ACTIVE	2.77	22.0	Inducible reporter
Transcriptional factor				
ATG_AP_1_CIS_up	ACTIVE	1.25	66.5	Inducible reporter
ATG_NRF2_ARE_CIS_up	ACTIVE	2.12	99.5	Inducible reporter

[a]Data were sourced from EPA's CompTox Chemicals Dashboard (U.S. EPA, 2019).
[b]Background control assays, inactive and nonspecific responses from inducible reporter gene assays analyzed in the negative fitting direction relative to the control ('_dn') are not presented herein.
NA = not applicable.

C.3.2. In vitro Bioactivity Data Relevant to the Mechanisms of PFHxS-Induced Thyroid Effects

Table C-8. Endocrine disruptor screening program 21 assay summary results[a]

Assay name	Description	Cell model	Active/ inactive
ERE assays			
ACEA_ER_80hr	Growth reporter (proliferation) assay	T47D, human breast cell line	Inactive
ATG_ERE_CIS_up	Reporter Gene Assay	HepG2 human hepatoma cell line	**Active**
ATG_Era_TRANS_up	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
NVS_NR_hER	Binding reporter Assay	MCF7, human breast cell line	Inactive
OT_ER_ERaERa_0480	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_ER_ERaERa_1440	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_ER_ERbERb_0480	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_ER_ERbERb_1440	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_Era_EREGFP_0120	Reporter Gene Assay	HeLa, Human Cervix cell line	Inactive
OT_Era_EREGFP_0480	Reporter Gene Assay	HeLa, Human Cervix cell line	Inactive
TOX21_ERa_BLA_Agonist	Reporter Gene Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_ERa_BLA_Antagonist	Reporter Gene Assay	HEK293T, Human Kidney cell line	Inactive
Tox_ERa_LUC_VM7_Agonist	Reporter Gene Assay	VM7, Human Ovary cell line	Inactive
Tox_ERa_LUC_VM7_Antagonist	Reporter Gene Assay	VM7, Human Ovary cell line	Inactive
Tox_ERa_LUC_VM7_Antagonist	Reporter Gene Assay	VM7, Human Ovary cell line	Inactive
Tox_ERb_BLA_Agonist	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
Tox_ERb_BLA_Antagonist	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_ER_ERaERb_0480	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
OT_ER_ERaERb_1440	Binding Reporter Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_ERa_BLA_Antagonist_viability	Viability Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_ERa_LUC_VM7_Antagonist_0.5 nM_E2_viability	Viability Assay	VM7, Human Ovary cell line	Inactive
ATG_ERa_TRANS_dn	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
ATG_ERA_CIS_dn	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
ACEA_ER_AUC_viability	Viability Assay	T47D, Human Breast cell line	Inactive
TOX21_ERa_LUC_VM7_Antagonist_0.1 nM_E2_viability	Viability Assay	VM7, Human Ovary cell line	Inactive
TOX21_ERb_BLA_Agonist_viability	Viability Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_ERb_BLA_Antagonist_viability	Viability Assay	HEK293T, Human Kidney cell line	Inactive

Assay name	Description	Cell model	Active/ inactive
AR assays			
ATG_AR_TRANS_up	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
OT_AR_ARELUC_AG_1440	Reporter Gene Assay	CHO-K1, Chinese Hamster Ovary	Inactive
OT_AR_ARSRC1_0480	Binding Assay	HEK293T, Human Kidney cell line	Inactive
OT_AR_ARSRC1_0960	Binding Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_AR_BLA_Agonist_	Reporter Gene Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_AR_LUC_Antagonist_	Reporter Gene Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_AR_LUC_MDAKB2_Agonist	Reporter Gene Assay	MDA-kb2, Human Breast cell line	Inactive
TOX21_AR_LUC_MDAKB2_Antagonist	Reporter Gene Assay	MDA-kb2, Human Breast cell line	Inactive
TOX21_AR_LUC_MDAKB2_Antagonist	Reporter Gene Assay	MDA-kb2, Human Breast cell line	Inactive
TOX21_AR_LUC_MDAKB2_Agonist	Reporter Gene Assay	MDA-kb2, Human Breast cell line	Inactive
ACEA_AR_agonist_80hr	Signaling Assay	22Rv1, Human Prostate cell line	Inactive
TOX21_AR_BLA_Agonist	Viability Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_AR_LUC_MDAKB2	Viability Assay	MDA-kb2, Human Breast cell line	Inactive
ATG_AR_TRANS_up	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
TOX21_AR_LUC_MDAKB2	Viability Assay	MDA-kb2, Human Breast cell line	Inactive
TOX21_AR_LUC_MDAKB2	Viability Assay	MDA-kb2, Human Breast cell line	Inactive
Thyroid Assays			
ATG_THRa1_TRANS_up	Reporter Gene Assay		Inactive
NVS_NR_hTRa_Antagonist	Binding Assay	HepG2 human hepatoma cell line	Inactive
TOX21_TSHR_Agonist_ratio	Signaling Assay	Human cell line (no other information available)	Inactive
TOX21_TSHR_Antagonist_ratio	Signaling Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_TR_LUC_GH3_Agonist	Reporter Gene Assay	HEK293T, Human Kidney cell line	Inactive
TOX21_TR_LUC_GH3_Antagonist	Reporter Gene Assay	GH3, Rat pituitary cell line	Inactive
TOX21_TR_LUC_GH3_Antagonist_ viability	Viability Assay	GH3, Rat pituitary cell line	Inactive
ATG_THRa1_TRANS_dn	Reporter Gene Assay	HepG2 human hepatoma cell line	Inactive
TOX21_TSHR_wt_ratio	Background Control Assay	HEK293T, Human Kidney cell line	Inactive
NIS_RAIU_inhibition	Binding Assay	HEK293T, Human Kidney cell line	**Active**
NIS_HEK293T_CTG_Cytotoxicity	Viability Assay	HEK293T, Human Kidney cell line	Inactive
Steroid Assays			
NVS_ADME_hCYP19A1	Enzymatic Activity	Human, Cell Free	Inactive
TOX21_Aromatase_Inhibition	Reporter Gene Assay	MCF-7, Human Breast cell line	Inactive
TOX21_Aromatase_Inhibition_viability	Viability Assay	MCF-7, Human Breast cell line	Inactive

[a]CompTox Chemistry Dashboard accessed 3/5/2020 https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3037709#invitrodb-bioassays-toxcast-data.

APPENDIX D. BENCHMARK DOSE MODELING RESULTS

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints are modeled using EPA's Benchmark Dose Software (BMDS, Version 3.2). Sections E.1 (noncancer) and Section E.2 (cancer) describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance* document (U.S. EPA, 2012).

D.1. BENCHMARK DOSE MODELING SUMMARY FOR NONCANCER ENDPOINTS

D.1.1. Benchmark Dose Modeling Approaches

The endpoints selected for benchmark dose (BMD) modeling include decreased serum antibody concentrations for tetanus and diphtheria (Budtz-Jørgensen and Grandjean, 2018a; Grandjean et al., 2012) and decreased birth weight (Manzano-Salgado et al., 2019; Buck Louis et al., 2018; Shoaff et al., 2018; Starling et al., 2017; Bach et al., 2016a). The internal doses reported in the human studies were used in the BMD modeling and then converted to human equivalent doses (HEDs) using the pharmacokinetic (PK) model described in Section 3.1 of the main document; the modeling results are presented in this appendix.

Modeling Results for Decreased Tetanus Antibody Concentrations at 7 Years of Age and PFHxS Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of perfluorohexanesulfonic acid (PFHxS) measured at age 5 years, against $\log_2$-transformed anti-tetanus antibody concentrations measured at the 7-year-old examination controlling for sex, exact age at the 7-year-old examination, and booster type at age 5 years. Models were evaluated with additional control for PFOS (as $\log_2$[PFOS]) and PFOA (as $\log_2$[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median PFHxS concentration, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). There was evidence that the piecewise-linear model fit better than the linear model for both the PFHxS exposure model without adjustment for PFOS and PFOA (p = 0.002; see Budtz-Jørgensen and Grandjean (2018a) Table 3) and for the model that did adjust for PFOS and PFOA (p = 0.05).

Table D-1 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for PFHxS at age 5 years and tetanus antibodies at age 7 years. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a benchmark response (BMR) of 5% decrease in the $\log_2$-transformed antibody concentration in Table 1 of Budtz-Jørgensen and Grandjean (2018a).[5] As Budtz-Jørgensen and Grandjean (2018a) $\log_2$-transformed the outcome variable, the BMR was measured in unit of $\log_2$[tetanus antibody concentration] as $\log_2(1\text{-}0.05) = 0.074\ \log_2$(IU/mL).

Table D-1. Results specific to the low-dose slope from the piecewise- linear analyses of PFHxS measured at age 5 years and log2(tetanus antibody concentrations) measured at age 7 from Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a single-PFAS model and in a multi-PFAS model

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) fit	Lower bound slope (βLB)ng/mL
PFHxS at Age 5	Piecewise	No	-2.47	0.75	p = 0.001	-3.70
PFHxS at Age 5	Piecewise	Yes	-1.85	1.12	p = 0.100	-3.70

Interpretation of results in Table D-1:

- PFHxS is a significant predictor in the single-PFAS model (β = -2.47; p = 0.001).
- Effects of PFHxS in the single-PFAS model are attenuated when $\log_2$[PFOS] and $\log_2$[PFOA] are included in the model (β = -1.85; p = 0.100).
- The point estimate results for PFHxS (β) in the single-PFAS model are *potentially* confounded by PFOS and/or PFOA since there was a 25% reduction in the effect size for PFHxS from -2.47 to -1.85 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA was a confounder of the PFHxS effect and controlling for those co-exposures removed confounding.
    - Another possibility is that controlling for co-exposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
    - The reasons for the change in main effect size for PFHxS are not known. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFHxS.

[5]Budtz-Jørgensen and Grandjean (2018a) computed BMDs and BMDLs using a BMR of 5% decrease in the $\log_2$(antibody concentrations). Their formula, BMD = $\log_2(1\text{-BMR})/\beta$, can simply be reversed to solve for $\beta = \log_2(1\text{-BMR})/BMD$. For negative dose-response where more exposure results in lower antibody concentration, the BMDL is based on the lower bound of β, (β_{LB}). Thus, the $\beta_{LB} = \log_2(1\text{-BMR})/BMDL$. The $SE(\beta) = (\beta - \beta_{LB})/1.645$. The p-value is the two-sided probability that $Z <= SE(\beta)/\beta$.

- However, the lower bounds on the point estimates (β_{LB}) are the same for the single-PFAS and multi-PFAS models, minimizing the effect of the potential confounding given the lower bound is ultimately used for point-of-departure derivation.

    - The definition of the RfD, which is based upon the β_{LB}, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for potential confounding in the BMD from including, or excluding, PFOS and PFOA here is about 25%, while there is no uncertainty for potential confounding in the BMDL as those values are the same.

Selection of the Benchmark Response

The benchmark dose (BMD) approach involves dose-response modeling to obtain BMDs, i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data and the lower limit of the BMD (BMDLs) to serve as potential PODs for deriving quantitative estimates below the range of observation (U.S. EPA, 2012). Selecting a BMR to estimate the BMDs and BMDLs involves making judgments about the statistical and biological characteristics of the data set and about the applications for which the resulting BMDs and BMDLs will be used. An extra risk of 10% is recommended as a standard reporting level for quantal data for toxicological data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects as the basis of the POD for a reference value. However, a BMR of 1% has typically been used for quantal human data from epidemiology studies (U.S. EPA, 2012), although this is more typically used for epidemiologic studies of cancer mortality within large cohorts of workers which can support the statistical estimation of small BMRs.

A blood concentration for tetanus antibodies of 0.1 IU/mL is sometimes cited in the tetanus literature as a "protective level" and Grandjean et al. (2017) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL "cut-off" level "to determine whether antibody concentrations could be considered protective"; and Galazka and Kardymowicz (1989) mentions the same concentration), but Galazka et al. (1993) argues:

> "The amount of circulating antitoxin needed to ensure complete immunity against tetanus is not known for certain. Establishment of a fixed level of tetanus antitoxin does not take into consideration variable conditions of production and adsorption of tetanus toxin in the anaerobic area of a wound or a necrotic umbilical stump. A given serum level could be overwhelmed by a sufficiently large dose of toxin. Therefore, there is no absolute protective level of antitoxin and protection results when there is sufficient toxin-neutralizing antibody in relation to the toxin load (Passen and Andersen, 1986)."

In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 SD change from the control mean may be selected, as suggested in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012). As noted above, a lower BMR can also be used if it can be justified on a biological and/or statistical basis. Figure D-1 replicates a figure in the technical guidance (page 23; (U.S. EPA, 2012)) to show that in a

control population in which 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of one SD results in a ~10% extra risk of being at risk of having an adverse effect.

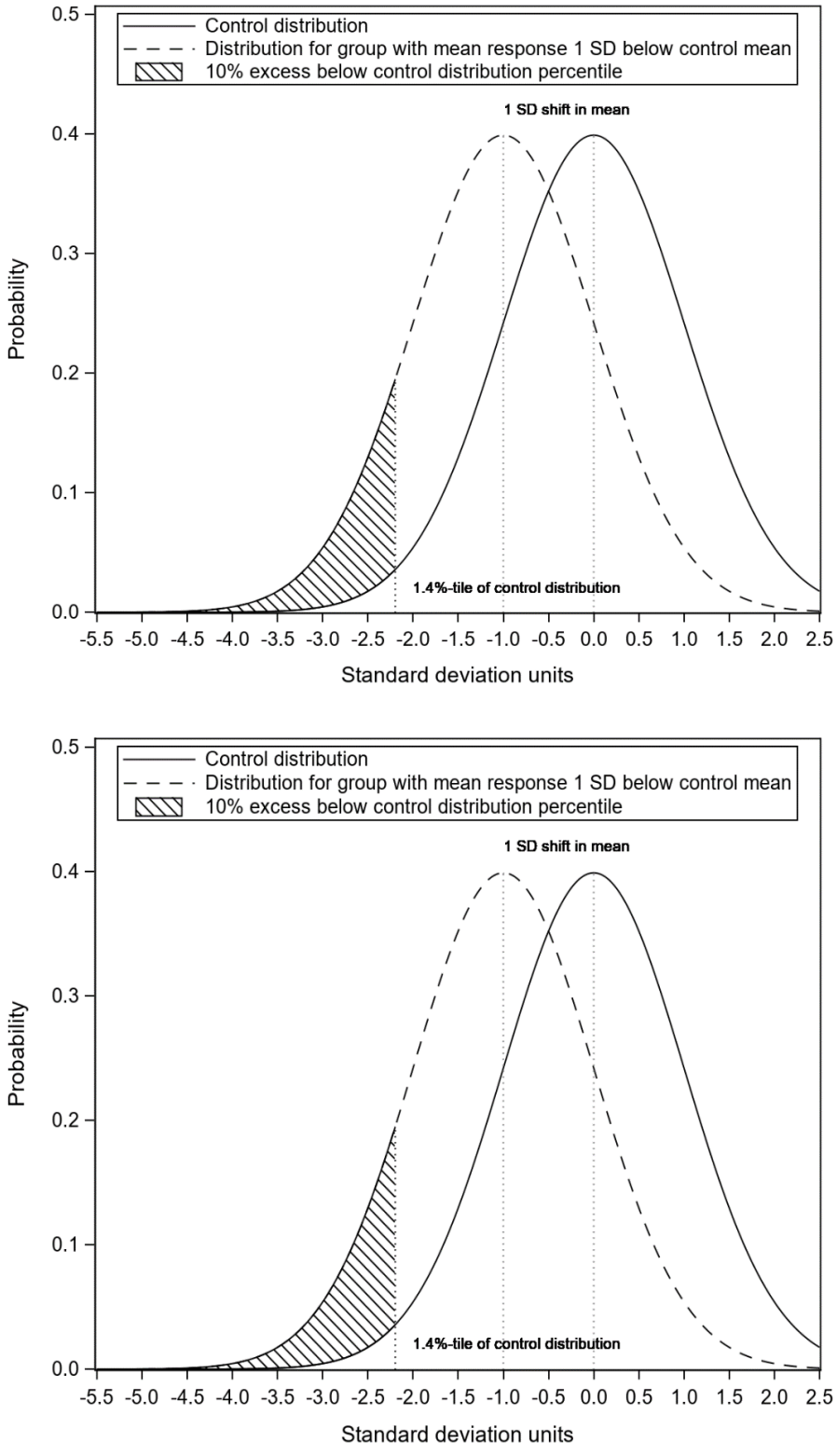

Figure D-1. Difference in population tail probabilities resulting from a 1 standard deviation shift in the mean from a standard normal distribution, illustrating the theoretical basis for a baseline BMR of 1 SD.

Statistically, the technical guidance additionally suggests that studies of developmental effects can support lower BMRs. Biologically, a BMR of ½ SD is a reasonable choice as anti-tetanus antibody concentrations prevent against tetanus, which is a rare, but severe and sometimes fatal infection, with a case-fatality rate in the United States of 13% during 2001–2008 (Liang et al., 2018). The case-fatality rate can be more than 80% for early lifestages cases (Patel and Mehta, 1999). Selgrade (2007) suggests that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards, which has the potential to be a more adverse effect that just a single immuno-toxic effect. Thus, decrements in the ability to maintain effective levels of tetanus antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFHxS. By contrast, a BMR of 1 SD may be more appropriate for an effect that would be considered "minimally adverse." A BMR smaller than ½ SD is generally selected for severe effects (e.g., 1% extra risk of cancer mortality); decreased antibody concentrations offer diminished protection from severe effects but are not themselves severe effects.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 SD change in the distribution of log2(tetanus antibody concentrations), and ½ SD change in the distribution of log2(tetanus antibody concentrations). The SD of the log2(tetanus antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the interquartile range (IQR) of the tetanus antibody concentrations at age 7 years in IU/mL was (0.65, 4.6). Log2-tranforming these values provides the IQR in log2(IU/mL) as (-0.62, 2.20). Assuming that these log2-transformed values are reasonably represented by a normal distribution, the width of the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of tetanus antibodies in log2(IU/mL) is (2.20 – (-0.62))/1.35 = 2.09 log2(IU/mL). To show the impact of the BMR on these results, Table D-2 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

While there was not a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, the value of 0.1 IU/mL is sometimes cited. As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cut-off value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies at age 7 years in log2(IU/mL), EPA calculated that 2.8% of those values would be below the cut-off value of 0.1 IU/mL which is -3.32 log2(IU/mL). A BMR of ½ SD resulted in 7.9% of the values being below that cutoff, which is 5.1% extra risk and shows that the generic guidance that a BMR of ½ SD can provide a reasonably good estimate of 5% extra risk. Figure D-2 shows an example.

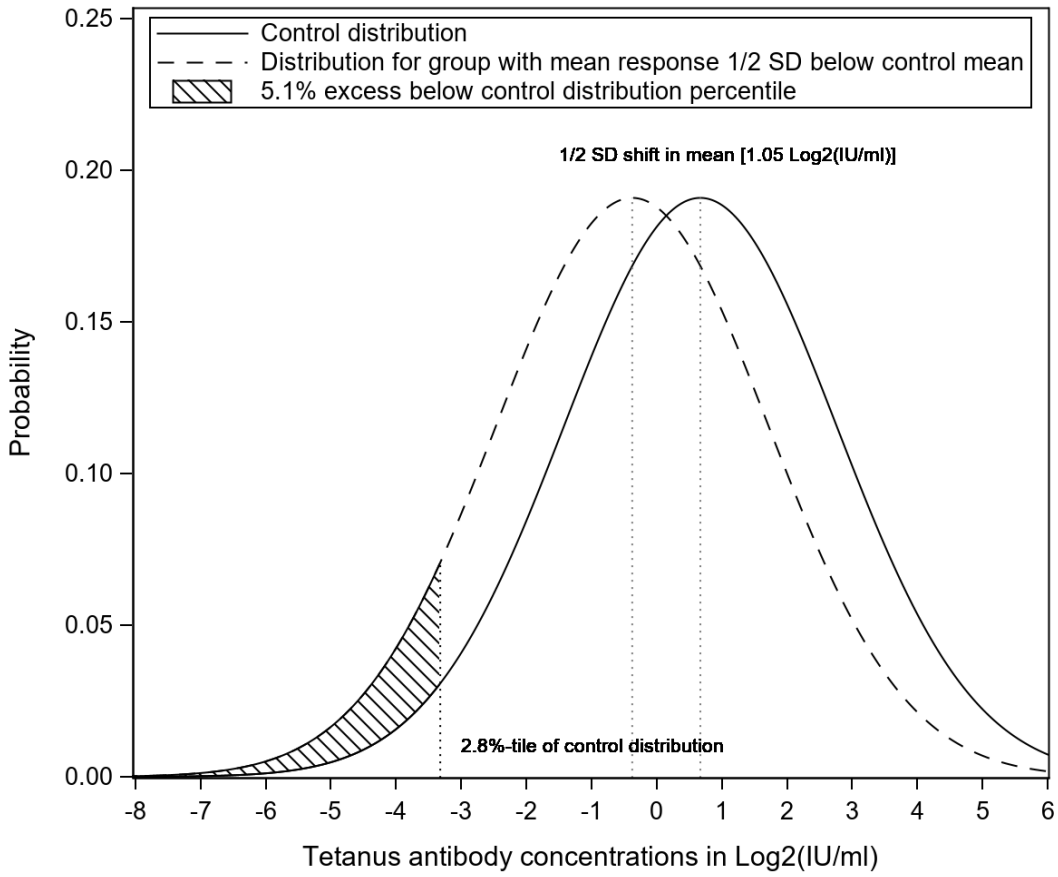

Figure D-2. Difference in population tail probabilities resulting from a ½ standard deviation shift in the mean from an estimation of the distribution of $\log_2$(tetanus antibody concentrations at age 7 years).

Table D-2. BMDs and BMDLs for effect of PFHxS at age 5 years on anti-tetanus antibody concentrations at age 7 years using a BMR of ½ SD change in $\log_2$(tetanus antibodies concentration) and a BMR of 1 SD $\log_2$(tetanus antibodies concentration)

	Estimated without control of PFOS and PFOA		**Estimated with control of PFOS and PFOA**	
BMR	**BMD (ng/mL)** β = -2.47 per ng/mL	**BMDL (ng/mL)** β_{LB} = -3.70 per ng/mL	**BMD (ng/mL)** β = -1.85 per ng/mL	**BMDL (ng/mL)** β_{LB} = -3.70 per ng/mL
½ SD	0.424	**0.282**[a]	0.565	0.282
1 SD	0.847	0.565	1.130	0.565

[a]Denotes the selected POD.

The lowest serum PFHxS concentration measured at age 5 years was 0.02 ng/mL, the 5th percentile was 0.2 ng/mL, and the 10th percentile was 0.3 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$) 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.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 33% higher than the $BMD_{½\ SD}$ estimate from the models with just PFHxS, but the $BMDL_{½\ SD}$ estimates are equal given the lower bounds for both models were equal. The change in BMD estimates may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which PFAS model provided "better" estimate of the point estimate of the effect of PFHxS, the two $BMDL_{½\ SD}$ estimates that serve as the PODs are equal and EPA advanced the derivation based on common $BMDL_{½\ SD}$ estimates of 0.282 ng/mL from both the single-PFAS and the multi-PFAS models. However, confidence was somewhat diminished by the potential confounding in the main effect—even though there was no observed confounding of the BMDL. *Medium* confidence in the BMDLs for PFHxS.

For immunotoxicity related to tetanus associated with PFHxS exposure measured at age 5 years, the POD is based on a BMR of ½ SD and a BMDL½ SD of 0.282 ng/mL.

Modeling Results for Decreased Diphtheria Antibody Concentrations at 7 Years of Age and PFHxS Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFHxS measured at age 5 years, against log_2-transformed anti-diphtheria antibody concentrations measured at the 7 year-old examination controlling for sex, exact age at the 7 year-old examination, and booster type at age 5 years. Models were evaluated with additional control for PFOS (as log_2[PFOS]) and PFOA (as log_2[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFHxS, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). There was evidence that the piecewise-linear model fit better than the linear model for the PFHxS exposure without adjustment for PFOS and PFOA (p = 0.05; see in Budtz-Jørgensen and Grandjean (2018a), Table 3), but not for the model that adjusted for PFOS and PFOA (p = 0.44). Table D-3 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a BMR of ½ SD in log_2(diphtheria antibody concentrations) in Table 1 of Budtz-Jørgensen and Grandjean (2018a).

Table D-3. Results specific to the low-dose slope from the piecewise- linear analyses of PFHxS measured at age 5 years and log2(diphtheria antibodies) measured at age 7 years from Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a single-PFAS model and in a multi-PFAS model

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) fit	Lower bound slope (β_{LB}) ng/mL
PFHxS at Age 5	Piecewise	No	-1.48	0.60	p = 0.0136	-2.47
PFHxS at Age 5	Piecewise	Yes	-0.67	1.09	p = 0.537	-2.47

Interpretation of results in Table D-3:

- PFHxS is a significant predictor in the single-PFAS model (β = -1.48; p = 0.01).
- Effects are attenuated when $\log_2$[PFOS] and $\log_2$[PFOA] are included in the model (β = -0.67; p = 0.54).
- The point estimate results for PFHxS are *potentially* confounded by PFOS and/or PFOA since there was a 55% reduction in the effect size for PFHxS from -1.48 to -0.67 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA was a confounder of the PFHxS effect and controlling for those co-exposures removed confounding.
    - Another possibility is that controlling for co-exposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
    - The reasons for the change in main effect size for PFHxS are not known. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFHxS.
- However, the lower bounds on the point estimates (β_{LB}) are the same for the single-PFAS and multi-PFAS models, minimizing the effect of the potential confounding given the lower bound is ultimately used for point-of-departure derivation.
    - The definition of the RfD, which is based upon the βLB, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for potential confounding in the BMD from including, or excluding, PFOS and PFOA here is about 55%, while there is no uncertainty for potential confounding in the BMDL as those values are the same.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(diphtheria antibody concentrations), and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). A blood concentration for diphtheria antibodies of 0.1 IU/ml is sometimes cited in the diphtheria literature as a "protective level" (Grandjean et al., 2017) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/ml "cut-off" level; and Galazka et al. (1993) mentions the same concentration), but Galazka et al. (1993) argues:

> "However, it has also been shown that there is no sharply defined level of antitoxin that gives complete protection from diphtheria (Ipsen, 1946). A certain range of variation must be accepted; the same degree of antitoxin may give an unequal degree of protection in different persons. Other factors may influence the vulnerability to diphtheria including the dose and virulence of the diphtheria bacilli and the general immune status of the person infected (Christenson and Böttiger, 1986). Thus, an antibody concentration between 0.01 and 0.09 IU/ml may be regarded as giving basic immunity, whereas a higher titer may be needed for full protection. In some studies that used in vitro techniques, a level of 0.1 IU/ml was considered protective (Cellesi et al., 1989; Galazka and Kardymowicz, 1989)."

Statistically, the technical guidance suggests that studies of developmental effects can support lower BMRs. Biologically, a BMR of ½ SD is a reasonable choice as anti-diphtheria antibody concentrations prevent against diphtheria, which is very rare in the United States, but can cause life-threatening airway obstruction, or cardiac failure (Collier, 1975). Among 13 cases reported in the United States during 1996–2016, no deaths were mentioned (Liang et al., 2018). However, diphtheria remains a potentially fatal disease in other parts of the world Galazka et al. (1993) mentions a case fatality rate of 5–10%) and PFHxS-related changes in anti-diphtheria antibody concentrations cannot be considered to be 'minimally adverse' given the historic lethality of diphtheria in the absence of vaccination. Selgrade (2007) suggests that specific immuno-toxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards—which has the potential to be a more adverse effect that just a single immuno-toxic effect.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(diphtheria antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the interquartile range (IQR) of the diphtheria antibody concentrations at age 7 years in IU/mL was (0.4, 1.6). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (-1.32, 0.68). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is

approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (0.68 – (-1.32))/1.35 = 1.48 $\log_2$(IU/mL). To show the impact of the BMR on these results, Table D-4 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table D-4. BMDs and BMDLs for effect of PFHxS at age 5 years on anti-diphtheria antibody concentrations at age 7 years using a BMR of ½ SD change in log2(diphtheria antibodies concentration) and a BMR of 1 SD log2(diphtheria antibodies concentration)

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL)** β = -1.48 per ng/mL	**BMDL (ng/mL)** β_{LB} = -2.47 per ng/mL	**BMD (ng/mL)** β = -0.67 per ng/mL	**BMDL (ng/mL)** β_{LB} = -2.47 per ng/mL
½ SD	0.500	**0.300**[a]	1.100	0.300
1 SD	1.000	0.600	2.200	0.600

[a]Denotes the selected POD.

The lowest serum PFHxS concentration measured at age 5 years was 0.02 ng/mL, the 5th percentile was 0.2 ng/mL, and the 10th percentile was 0.3 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$) in the single-PFAS model is at 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.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 2.2-fold higher than the $BMD_{½\ SD}$ estimate from the model with just PFHxS, but the $BMDL_{½\ SD}$ is the same, which may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which PFAS model provided the "better" estimate of the point estimate of the effect of PFHxS, the two $BMDL_{½\ SD}$ estimates which serve as the PODs are equal and EPA advanced POD based on common BMDL estimates of 0.300 ng/mL from both the single-PFAS and the multi-PFAS models because the uncertainty regarding potential confounding of the BMDL was low. However, confidence was diminished by the stronger potential confounding in the main effect—even though the was no observed confounding of the BMDL, and overall confidence in the BMDLs for diphtheria was judged to be *medium*/*low* confidence.

For immunotoxicity related to diphtheria, associated with PFHxS measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.300 ng/mL.

Modeling Results for Decreased Tetanus Antibody Concentrations at 5 Years of Age and Perinatal PFHxS

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFHxS measured perinatally in maternal serum, against $\log_2$-transformed anti-tetanus antibody concentrations

measured at the 5-year-old examination controlling for sex, and exact age at the 5-year-old examination, cohort, and interaction terms between cohort and sex, and between cohort and age. Models were evaluated with additional control for PFOS (as $\log_2$[PFOS]) and PFOA (as $\log_2$[PFOA]), and without PFOS and PFOA. Three model shapes of PFHxS were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). Compared to the linear model, the piecewise-linear model did not fit better than the linear model for either the PFHxS exposure without adjustment for PFOS and PFOA using a likelihood ratio test (p = 0.45; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS and PFOA ($\log_2$[PFOS] and $\log_2$[PFOA]) (p = 0.90).

Table D-5 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for tetanus in this exposure window. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a BMR of ½ SD change in $\log_2$(tetanus antibody concentrations) in Table 2 of Budtz-Jørgensen and Grandjean (2018a).

Table D-5. Results of the linear analyses of PFHxS measured perinatally and tetanus antibodies measured at age 5 years from Budtz-Jørgensen and Grandjean (2018a) in a single-PFAS model and in a multi-PFAS model.

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) fit	Lower bound slope (β_{LB}) ng/mL
Perinatal PFHxS	Linear	No	-0.0238	0.0183	p = 0.19	-0.0540
Perinatal PFHxS	Linear	Yes	-0.0190	0.0184	p = 0.30	-0.0492

Interpretation of results in Table D-5:

- PFHxS is a nonsignificant predictor in the single-PFAS model (β = -0.0238; p = 0.190).
- Effects are attenuated when log2[PFOS] and log2[PFOA] are included in the model (β = -0.019; p = 0.30).
- The point estimate results for PFHxS are *potentially* confounded by PFOS and/or PFOA since there was a 20% reduction in the effect size for PFHxS from -0.0238 to -0.0190 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA was a confounder of the PFHxS effect and controlling for those co-exposures removed confounding.
    - Another possibility is that controlling for co-exposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).

- The reasons for the change in main effect size for PFHxS are not known. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFHxS.

- However, the lower bound on the point estimates (β_{LB}) for the single-PFAS model is 35% lower than the multi-PFAS model estimate for PFHxS.

The definition of the RfD, which is based upon the βLB, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for potential confounding in the BMD from including, or excluding, PFOS and PFOA here is about 20%, while the uncertainty for potential confounding in the BMDL is about 9%.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(tetanus antibody concentrations), and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(tetanus antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018a). Grandjean et al. (2012) reported on 587 5-year-olds from the cohort of children born during 1997-2000 and in Grandjean et al. (2017) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately and then pooled to describe the common SD. The IQR of the tetanus antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.1, 0.51). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (-3.32, -0.97). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (-0.97 – (-3.32))/1.35 = 1.74 $\log_2$(IU/mL). The IQR of the tetanus antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (-3.32, -1.74), and the SD of tetanus antibodies in $\log_2$(IU/mL) is (-1.74 – (-3.32))/1.35 = 1.17 $\log_2$(IU/mL). The pooled variance is a weighted sum of the independent SDs and the pooled SD was estimated as 1.55 $\log_2$(IU/mL).[6] To show the impact of the BMR on these results, Table D-6 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table D-6. BMDs and BMDLs for effect of PFHxS measured perinatally and anti-tetanus antibody concentrations at age 5 years.

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL)** β = -0.0238 per ng/mL	**BMDL (ng/mL)** β_{LB} = -0.0540 per ng/mL	**BMD (ng/mL)** β = -0.0190 per ng/mL	**BMDL (ng/mL)** β_{LB} = -0.049 per ng/mL

[6]Pooled variance for tetanus in 5-year-olds = [(502-1)(1.74)^2+ (298-1)(1.17)^2]/[502+298-2] = 2.41. The pooled SD is the square root of 2.41 which is 1.55 $\log_2$(IU/mL).

½ SD	32.5	**14.4**[a]	40.8	15.7
1 SD	65.1	28.7	81.6	31.5

[a]Denotes the POD that corresponds to the analyses of PFHxS concentrations perinatally and tetanus antibodies at age 5 years.

The lowest perinatal maternal serum PFHxS concentration measured was 0.6 ng/mL, the 5th percentile was 1.2 ng/mL, and the 10th% was 1.5 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDLs for a BMR of ½ SD ($BMDL_{½\ SD}$ =14.4 ng/mL) in the single-PFAS model is well above the 10th% 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. The $BMDL_{½\ SD}$ estimate from the single-PFAS models was 14.4 ng/mL. The BMDL estimates from the multi-PFAS models were about 9% higher than for the single-PFAS model.

Low confidence in the BMDLs from the PFHxS-only model (14.4 ng/mL) and in the multi-PFAS model (15.7 ng/mL). Confidence is diminished by the low quality of the model fit for PFHxS in either model compared with the PFHxS results from tetanus in the 5-year to 7-year exposure-outcome window of time and there is some uncertainty regarding potential confounding.

For immunotoxicity related to tetanus, associated with PFHxS measured perinatally, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 14.4 ng/mL. Note that this result is based on a poorly fit PFHxS regression parameter (β) estimated as −0.024 per ng/mL (90%CI: −0.054, 0.0.0064; p = 0.19) (Budtz-Jørgensen and Grandjean, 2018b), and thus this POD is identified with low confidence.

For immunotoxicity related to tetanus associated with PFHxS exposure measured at age 5 years, the POD estimated for comparison purposes were based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 14.4 ng/mL.

Modeling Results for Decreased Diphtheria Antibody Concentrations at 5 Years of Age and Perinatal PFHxS

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFHxS measured perinatally, against log_2-transformed anti-diphtheria antibody concentrations measured at the 5 year-old examination controlling for sex and age. Models were evaluated with additional control for PFOS (as log_2[PFOS]) and PFOA (as log_2[PFOA]), and without PFOS and PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFHxS, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). The piecewise-linear model did not fit better than the linear model for the PFHxS exposure without adjustment for PFOS and PFOA using a likelihood ratio test (p = 0.70; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS and PFOA (log_2[PFOS] and log_2[PFOA]) (p = 0.11). Table D-7 summarizes the results from Budtz-

Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a BMR of ½ SD change in $\log_2$(diphtheria antibody concentrations) in Table 2 of (Budtz-Jørgensen and Grandjean, 2018a).

Table D-7. Results of the analyses of PFHxS measured perinatally and diphtheria antibodies measured at age 5 years from Budtz-Jørgensen Budtz-Jørgensen and Grandjean (2018b) in a single-PFAS model and in a multi-PFAS model.

Exposure	Model shape	PFOS and PFOA adjusted	Slope (β) per ng/mL	SE(β)	Slope (β) fit	Lower bound slope (β_{LB})
Perinatal PFHxS	Linear	No	-0.0378	0.0193	p = 0.05	-0.0696
Perinatal PFHxS	Linear	Yes	-0.0328	0.0193	p = 0.089	-0.0645

Interpretation of results in Table D-7:

- PFHxS is a significant predictor in the single-PFAS model (β = -0.0378; p = 0.05.)
- Effects of PFHxS are attenuated when PFOA and PFOA are in the model (β = -0.0328; p = 0.09).
- Results for PFHxS are *potentially* confounded by PFOS and/or PFOA since there was a 13% change in the effect size for PFHxS from -0.038 to -0.033 when controlling for PFOS and PFOA.
    - One explanation is that PFOS and/or PFOA was a confounder of the PFDA effect and controlling for those co-exposures removed confounding.
    - Another possibility is that controlling for co-exposures like PFOS and PFOA actually induced confounding (Weisskopf et al., 2018; Weisskopf and Webster, 2017).
- The reasons for the change in main effect size for PFDA are not known. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFDA. However, the lower bounds on the point estimates (β_{LB}) are similar with the lower bound on the multi-PFAS model effect estimate for PFHxS only 9% lower than the single-PFAS model effect estimate for PFHxS. This small difference suggests very little uncertainty attributable to potential confounding of the lower bound effect estimates.
    - The definition of the RfD, which is based upon the βLB, includes allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate and the uncertainty for potential confounding in the BMD from including, or excluding, PFOS and PFOA here is about 13%, while the uncertainty for potential confounding in the BMDL is about 9%.

Selection of the Benchmark Response

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(tetanus antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different birth cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018a). Grandjean et al. (2012) reported on 587 5-year-olds from the cohort of children born during 1997-2000 and Grandjean et al. (2017) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately and then pooled to describe the common SD. The IQR of the diphtheria antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.05, 0.4). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (-4.32, -1.32). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of diphtheria antibodies in $\log_2$(IU/mL) is (-1.32 – (-4.32))/1.35 = 2.22 $\log_2$(IU/mL). The IQR of the diphtheria antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (-3.32, -1.74), and the SD of diphtheria antibodies in $\log_2$(IU/mL) is (-1.74 – (-3.32))/1.35 = 1.17 $\log_2$(IU/mL). The pooled variance is a weighted sum of the independent SDs and the pooled SD was estimated as 1.90 $\log_2$(IU/mL).[7] To show the impact of the BMR on these results, Table D-8 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table D-8. BMDs and BMDLs for effect of PFHxS measured perinatally and anti-diphtheria antibody concentrations at age 5 years

	Estimated without control of PFOS and PFOA		Estimated with control of PFOS and PFOA	
BMR	**BMD (ng/mL)** β = -0.0378 per ng/mL	**BMDL (ng/mL)** β_{LB} = -0.0696 per ng/mL	**BMD (ng/mL)** β = -0.0328 per ng/mL	**BMDL (ng/mL)** β_{LB} = -0.0645 per ng/mL
½ SD	25.1	**13.7**[a]	29.0	14.7
1 SD	50.2	27.3	57.9	29.4

[a]Denotes the POD that corresponds to the analyses of PFHxS concentrations perinatally and diphtheria antibodies at age 5 years.

The lowest serum PFHxS concentration measured perinatally was 0.6 ng/mL, the 5th percentile was 1.2 ng/mL, and the 10th% was 1.5 ng/mL (Grandjean and Bateson, 2021) so the estimated BMD for a BMR of ½ SD ($\text{BMDL}_{½\text{ SD}}$) in the single-PFAS model is well within the observed range. 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.

[7]Pooled variance for tetanus in 5-year-olds = [(502-1)(1.74)^2+ (298-1)(1.17)^2]/[502+298-2] = 2.41. The pooled SD is the square root of 2.41 which is 1.55 $\log_2$(IU/mL).

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 15% higher than the $BMD_{½\ SD}$ estimated from the model with just PFHxS, and the $BMDL_{½\ SD}$ is 8% higher, which may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which estimate is "better," the BMDLs which serve as the PODs are similar (13.7 ng/mL versus 14.7 ng/mL) and EPA advanced the derivation based on results that did not control for PFOS and PFOA because this model appeared to fit PFHxS well (p = 0.05) and there was low uncertainty due to potential confounding in either the BMD or the BMDL. *Medium* confidence in the BMDLs from PFHxS linear model (13.7 ng/mL) since the model fit reasonably well and these BMDLs do not show meaningful uncertainty about confounding.

For immunotoxicity related to diphtheria, associated with PFHxS measured at age 5 years, the POD is based on a BMR of ½ SD and a BMDL½ SD of 13.7 ng/mL.

Modeling Results for Decreased Birth Weight Using Individual Studies

As noted in Section 5.2.1 five *high* confidence studies (Manzano-Salgado et al., 2019; Buck Louis et al., 2018; Shoaff et al., 2018; Starling et al., 2017; Bach et al., 2016a) reported decreased birth weight in infants whose mothers were exposed to PFHxS. All five studies reported their exposure metric in units of ng/mL. Three studies reported the β coefficients per ln(ng/mL) or per $\log_2$(ng/mL), one study reported a β coefficients per ln(1+ng/mL) and one study reported a β coefficients per ng/mL, along with 95% confidence intervals (CIs), estimated from linear regression models. The logarithmic transformation of exposure yields a negative value for low numbers, which can result in implausible results from dose-response modeling (i.e., estimated risks are negative and unable to determine the responses at zero exposure). EPA first re-expressed the reported β coefficients in terms of per ng/mL, if necessary, according to Dzierlenga et al. (2020). Then EPA used the re-expressed β and lower limit on the CI to estimate BMD and BMDL values using the general equation $y = mx + b$, where y is birth weight and x is exposure, substituting the re-expressed β values from these studies for m. The intercept b represents the baseline value of birth weight in an unexposed population and it can be estimated through $\overline{y} = m\overline{x} + b$ using an average birth weight from an external population as $\overline{y}$, an average exposure as $\overline{x}$ and re-expressed β from the studies as m.

The CDC Wonder site (https://wonder.cdc.gov/natality.html) provides vital statistics for babies born in the United States. There were 3,791,712 all live births in the United States in 2018 according to final natality data. The mean and standard deviation of birth weight were 3261.6 ± 590.7 g (7.19 ± 1.30 lb), with 8.27% of live births falling below the public health definition of low birth weight (i.e., <2,500 g, or 5.5 lb). The full natality data for the U.S. data on birth weight was used as it is more relevant for deriving toxicity values for the U.S. general population than the study-specific birthweight data. Also, the CDC Wonder database may be queried to find the exact percentage of the population falling below the cut-off value for clinical adversity. America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals report

(https://www.epa.gov/americaschildrenenvironment/ace-biomonitoring-perfluorochemicals-pfcs#B6) provides the median blood serum levels of PFHxS of 0.6 ng/mL in 2015–2016 in women aged 16 to 49, using National Health and Nutrition Examination Survey (NHANES) as data source. These values are assumed to be representative of women of reproductive age and are subsequently used in the estimation of BMD and BMDL values from the available five epidemiological studies.

Buck Louis et al. (2018) reported a β coefficient of -17.1 g (95%CI: -40.7, 6.5) per 1 SD increase of ln(1+ng/mL), corresponding to a rescaled β coefficient of -53.1 g (95%CI: -126.4, 20.2) per ln(1+ng/mL) increase, for the association between birth weight and maternal PFHxS serum concentrations (collected during 10 weeks to 13 weeks and 6 days of pregnancy with a median of 12 weeks) in a United States cohort, based on their multiple linear regression ($y = m * \ln(1 + x) + b$) analysis. The reported β coefficient can be re-expressed in terms of per ng/mL according to Dzierlenga et al. (2020). Given the median (0.71 ng/mL) and IQR (0.44-1.23 ng/mL) of the exposure from Buck Louis et al. (2018), EPA estimated the distribution of exposure by assuming the exposure follows a log-normal distribution and the natural logarithm of exposure is normally distributed with mean and standard deviation as:

$$\mu = ln(q_{50}) = ln(0.71) = -0.35 \quad (1)$$

$$\sigma = ln(q_{75}/q_{25})/1.349 = ln(1.23/0.44)/1.349 = 0.75 \quad (2)$$

Then, EPA estimated the 25th – 75th percentiles at 10 percentile intervals of the exposure distribution and corresponding responses of reported β coefficient. The re-expressed β coefficient is determined by minimizing the sum of squared differences between the curves generated by the re-expressed β and the reported β. This resulted in a re-expressed β coefficient of -29.9 g (95%CI: -71.1, 11.4) per ng/mL.

Typically, for continuous data, the preferred definition of the benchmark response (BMR) is to have a basis for what constitutes a minimal level of change in the endpoint that is biologically significant. For birth weight, there is no accepted percent change that is considered adverse. However, there is a clinical measure for what constitutes an adverse response: babies born weighing less than 2,500 g are considered to have low birth weight, and further, low birth weight is associated with a wide range of health conditions throughout life (Tian et al., 2019; Reyes and Mañalich, 2005; Hack et al., 1995). Given this clinical cut-off for adversity and that 8.27% of all live births in the United States in 2018 fell below this cut-off, the hybrid approach can be used to define the BMR. The hybrid approach is advantageous in that it harmonizes the definition of the BMR for

continuous data with that for dichotomous data[8]. Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cut-off for adversity in the tail of the response distribution. Application of the hybrid approach requires the selection of an extra risk value for BMD estimation. In the case of birth weight, an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from toxicology studies, and that low birthweight confers increased risk for adverse health effects throughout life, thus supporting a BMR lower than the standard BMR of 10% extra risk.

Therefore, given a background response and a BMR = 5% extra risk, the BMD would be the dose that results in 12.86% of the responses falling below the 2500 g cut-off value:

$$Extra\ Risk(ER) = (P(d) - P(0)) \,/\, (1 - P(0))$$

$$P(d) = ER(1 - P(0)) + P(0) = 0.05(1 - 0.0827) + 0.0827 = 0.1286$$

Using the mean birth weight for all births in the United States of 3,261.6 g with a standard deviation of 590.7 g, EPA calculated the mean response that would be associated with the 12.86th percentile of the distribution falling below 2,500 g. In this case, the mean birth weight would be 3169.2g. Given the median exposure of 0.60 ng/mL from ACE Biomonitoring on Perfluorochemicals as $\overline{x}$, the mean birth weight in the United States as $\overline{y}$ and the re-expressed β as m term, the intercept b can be estimated as:

$$b = \overline{y} - m\overline{x} = 3261.6\ g - \left(-29.9\ g(\tfrac{ng}{mL})^{-1}\right) 0.60\ \tfrac{ng}{mL} = 3279.6\ g \qquad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x, using 3279.6 g for the b term and -29.9 for the m term. This resulted in a value of 3.70 ng/mL:

$$x = (y - b)/m = (3169.2\ g - 3279.6\ g)/(-29.9\ g(\frac{ng}{mL})^{-1}) = 3.70\ ng/mL$$

To calculate the BMDL, the method is essentially the same except that the lower limit (LL) on the β coefficient (-71.1) is used for the m term. However, (Buck Louis et al., 2018) reports a two-sided 95%CI for the β coefficient, meaning that the lower limit of that CI corresponds to a 97.5% one-sided lower limit. The BMDL is defined as the 95% lower limit of the BMD (i.e., corresponds to a two-sided 90%CI), so the corresponding lower limit on the β coefficient needs to be calculated before calculating the BMDL. First, the standard error of the β coefficient can be calculated as:

[8]While the explicit application of the hybrid approach is not commonly used in IRIS dose/concentration/exposure-response analyses, the more commonly used SD-definition of the BMR for continuous data is simply one specific application of the hybrid approach. The SD-definition of the BMR assumes that the cut-off for adversity is the 1.4th percentile of a normally distributed response and that shifting the mean of that distribution by one standard deviation approximates an extra risk of 10%.

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{11.4\ g(\frac{ng}{mL})^{-1} - \left(-71.1\ g(\frac{ng}{mL})^{-1}\right)}{3.92} = 21.0\ g(\frac{ng}{mL})^{-1}$$

Then the corresponding 95% one-sided lower bound on the β coefficient can be calculated as:

$$95\%\ one - sided\ LL = \beta - 1.645\big(SE(\beta)\big) = -29.9\ g(\frac{ng}{mL})^{-1} - 1.645\left(21.0\ g(\frac{ng}{mL})^{-1}\right)$$
$$= -64.4 g(\frac{ng}{mL})^{-1}$$

Using this value for the m term results in a BMDL value of 1.71 ng/mL maternal serum concentration.

Shoaff et al. (2018) reported a β coefficient of -13.4 g (95%CI: -35.9, 9.1) per ng/mL increase for the association between birth weight and maternal PFHxS serum concentrations (collected during 16 weeks of pregnancy to delivery with a median of 16 weeks) in a United States cohort. A BMD of 7.50 ng/mL was calculated from Shoaff et al. (2018) using the same approach as above with the same values for the mean birth weight in the United States and the reported β coefficient directly without re-expression.

To calculate the BMDL, the same procedure as above is used to calculate the 95% one-sided lower limit for the reported β coefficient from the reported lower limit on the 95% two-sided CI of -35.9 g per ng/mL. Using the corresponding lower limit (-32.3 g per ng/mL), a BMDL of 3.12 ng/mL is calculated.

Starling et al. (2017) reported a β coefficient of -13.5 g (95%CI: -50.7, 23.7) per ln(ng/mL) for the association between birth weight and maternal PFHxS serum concentrations (collected during 20 to 34 weeks of pregnancy with a median of 27 weeks) in a United States cohort. Given median (0.8 ng/mL) and IQR (0.5–1.2 ng/mL) of the exposure, EPA estimated the mean (-0.22) and standard deviation (0.65) of the natural logarithm of exposure. The re-expressed β coefficient is -16.2 g (95%CI: -60.7, 28.4) per ng/mL and the intercept *b* is 3271.3 g. The 95% one-sided lower limits for the re-expressed β coefficient is -53.6 g per ng/mL. The values of the BMD and BMDL are 6.32 ng/mL and 1.91 ng/mL, respectively.

Manzano-Salgado (2019) reported a β coefficient of –8.6 g (95%CI: -32.0, 14.8) per $\log_2$ (ng/mL) for the association between birth weight and maternal PFHxS serum concentrations (collected during the first trimester of pregnancy with a mean of 12.3 weeks) in a Spanish cohort. Given the median (0.58 ng/mL) and SD (0.37 ng/mL) of the exposure, EPA estimated the mean (-0.72) and standard deviation (0.58) of the natural logarithm of exposure. The re-expressed β coefficient is –24.5 g (95%CI: -91.2, 42.2) per ng/mL and the intercept is 3276.3 g. The 95% one-sided lower limits for the re-expressed β coefficient is –80.5 g per ng/mL. The values of the BMD and BMDL are 4.37 ng/mL and 1.33 ng/mL, respectively.

Bach (2016b) reported a β coefficient of –11.0 g (95%CI: -32.0, 9.0) per IQR increase in PFHxS (ng/mL), corresponding to a rescaled β coefficient of -40.7 g (95%CI: -118.5, 33.3) per ng/mL increase, for the association between birth weight and maternal PFHxS serum concentrations (collected 9–20 weeks of pregnancy, 96% within 13 weeks) in a Danish cohort. The BMD of 2.87 ng/mL and BMDL 1.12 ng/mL was calculated using the rescaled β coefficient directly without re-expression.

For all of the above calculations, EPA used the exact percentage (8.27%) of live births in the United States in 2018 that fell below the cut-off of 2,500 g as the tail probability to represent the probability of extreme ("adverse") response at zero dose ($P(0)$). However, this exact percentage of 8.27% was calculated without accounting for the existence of background PFHxS exposure in the United States population (i.e., 8.27% is not the tail probability of extreme response at zero dose). Thus, EPA considers an alternative control-group response distribution ($N(\mu_c, \sigma_c)$), using the study-specific intercept b obtained through equation (3) (representing the baseline value of birth weight in an unexposed population) as μ_c and the standard deviation of U.S. population as σ_c , to estimate the tail probability that fell below the cut-off of 2500 g. EPA estimated the study-specific tail probability of live births falling below the public health definition of low birth weight (2,500 g) as:

$$P(0) = \frac{1}{\sigma_c\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2\sigma_c^2}\right)}\,dx = \frac{1}{590.7\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2*590.7^2}\right)}\,dx$$

$$b = \overline{y} - m\overline{x} = 3261.6 - (\beta_{re-expressed} * 0.60\frac{ng}{mL})$$

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\overline{x} = 0$). By using the median of serum PFHxS concentrations (0.60 ng/mL) from ACE Biomonitoring on Perfluorochemicals as background exposure ($\overline{x}$), the tail probabilities using this alternative approach were study-specific and ranged from 9.35% - 9.65%. As such, the results from this alternative approach, presented under the column of "Alternative Tail Probability" in Table D-9, are very similar to the main results, presented under the column of "Exact Percentage" in Table D-9, when background exposure was not accounted for while estimating the tail probability.

Table D-9 presents the BMDs and BMDLs for all individual studies considered for POD derivation, with and without accounting for background exposure while estimating the percentage of the population falling below the cut-off value. The BMDLs across the studies and approaches ranged from 1.12 ng/mL to 4.20 ng/mL.

Table D-9. BMDs and BMDLs for effect of PFHxS on decreased birth weight, by using percentage (8.27%) of live births falling below the public health definition of low birth weight, or alternative study-specific tail probability

Study	Exposure median (IQR or 33-67QR or SD)	Exposure distribution (μ, σ)	Reported β (95%CI)	Re-expressed β (95%CI) g/ng/mL	Intercept b	SE of β	95% one-sided LL of β	Exact percentage (P(0)=8.27%)		Alternative tail probability[a]		
								BMD (ng/mL)	BMDL (ng/mL)	$P(0)$	BMD (ng/mL)	BMDL (ng/mL)
(Bach et al., 2016b)	0.47 (0.36-0.63)	(-0.76, 0.41)	-11.0 (-32.0, 9.0) g/IQR(ng/mL)	-40.7 (-118.5, 33.3)	3,286.1	38.74	-104.5	2.87	**1.12**[b]	9.16 %	3.44	1.34
(Buck Louis et al., 2018)	0.71 (0.44-1.23)	(-0.35, 0.75)	-17.1 (-40.7, 6.5) g/SD (ln(1+ng/ml))	-29.9 (-71.1, 11.4)	3,279.6	21.0	-64.4	3.70	1.71	9.35 %	4.63	2.14
(Manzano-Salgado et al., 2019)	0.58 (0.37)	(-0.72, 0.58)	-8.6 (-32.0, 14.8) g/log2(ng/ml)	-24.5 (-91.2, 42.2)	3,276.3	34.0	-80.5	4.37	1.33	9.44 %	5.60	1.71
(Shoaff et al., 2018)	1.5 (1.0-2.0)	(0.41, 0.79)	-13.4 (-35.9, 9.1) g/ng/ml	-13.4 (-35.9, 9.1)	3,269.7	11.5	-32.3	7.50	3.12	9.63 %	10.11	4.20
(Starling et al., 2017)	0.8 (0.5-1.2)	(-0.22, 0.65)	-13.5 (-50.7, 23.7) g/ln(ng/ml)	-16.2 (-60.7, 28.4)	3,271.3	22.7	-53.6	6.32	1.91	9.58 %	8.41	2.54

[a]The alternative study-specific tail probability of live births falling below the public health definition of low birth weight based on normal distribution with intercept b as mean and standard deviation of 590.7 based on U.S. population.

[b]Smallest BMDL using the five individual studies.

ACE Biomonitoring on Perfluorochemicals also provides the median blood serum levels of PFHxS among women ages 16 to 49 in 1999–2000 (1.3 ng/mL), in 2003–2004 (1.4 ng/mL) and in 2011–2012 (0.8 ng/mL). PA performed a sensitivity analysis by estimating BMD and BMDL using these values as background exposures. The results for Bach et al. (2016b) presented in Table D-10, demonstrate the robustness of EPA's approaches with alternative assumptions on background exposures.

Table D-10. BMDs and BMDLs for effect of PFHxS on decreased birth weight by background exposure, using the exact percentage of the population (8.27%) of live births falling below the public health definition of low birth weight, or alternative study-specific tail

Study	Background exposure [a]	Intercept *b*	Exact percentage (P(0)= 8.27%)		Alternative tail probability [b]		
			BMD (ng/mL)	BMDL (ng/mL)	P(0)	BMD (ng/mL)	BMDL (ng/mL)
(Bach et al., 2016b)	0.60	3286.1	2.87	1.12	9.16%	3.44	1.34
	0.80	3294.2	3.07	1.20	8.94%	3.50	1.36
	1.30	3314.6	3.57	1.39	8.39%	3.65	1.42
	1.40	3318.7	3.67	1.43	8.29%	3.68	1.43

[a]Assumptions on background exposure for the estimation of intercept using Equation (3).
[b]The tail probability of live births falling below the public health definition of low birth weight based on normal distribution.

Uncertainty may be introduced by the re-expression of regression coefficients Linakis et al. . A sensitivity analysis was performed to compare BMD and BMDL with and without re-expression of β coefficients using Buck Louis et al. (2018). Buck Louis et al. (2018) reported a β coefficient of -17.1 g (95%CI: -40.7, 6.5) per 1 SD increase of ln(1+ng/mL), corresponding to a rescaled β coefficient of -53.1 g (95%CI: -126.4, 20.2) per ln(1+ng/mL) increase. The BMD of 8.12 ng/mL and BMDL 1.79 ng/mL was calculated using the general equation $y = m * \ln(1 + x) + b$ and the rescaled β coefficient per ln(1+ng/mL), while assuming the median blood serum levels of PFHxS of 0.60 ng/mL. This approach removed any uncertainty associated with the re-expression of regression coefficients in the modeling. Table D-11 shows the BMD/BMDL results at several background exposure levels using re-expressed β coefficient (g/ng/mL) or reported/rescaled β coefficient (g/ln(1+ng/mL)) for Buck Louis et al. (2018).

Table D-11. BMDs and BMDLs for effect of PFHxS on decreased birth weight by background exposure, using the exact percentage of the population (8.27%) of live births falling below the public health definition of low birth weight, with re-expressed β coefficient (g per ln(1+ng/mL))

Study	Background exposure [a]	Intercept *b*	Re-expressed β g/ng/mL		Reported/rescaled β g/ ln (1+ ng/mL)	
			BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
(Buck Louis et al., 2018)	0.60	3,279.6	3.70	1.71	8.12	1.79
	0.80	3,285.5	3.90	1.80	9.26	1.94
	1.30	3,300.5	4.40	2.04	12.11	2.30
	1.40	3,303.4	4.50	2.08	12.68	2.36

[a]Assumptions on background exposure for the estimation of intercept using Equation (3).

Modeling Results for Decreased Birth Weight Using Meta-analysis Results

In addition to the above five studies, epidemiologic data were also available on another 22 studies with different reported units of β coefficient for the association between birth weight and PFHxS concentrations as discussed in the Meta-Analysis Method section (see Appendix C). As noted above, EPA was able to convert the exposure-response functions quantifying the effects for these studies based on different units into natural log units (i.e., per ln(ng/mL)) according to Dzierlenga et al. (2020). Two studies, Lind et al. (2017) and Ashley-Martin et al. (2017) only reported separate estimates for boys and girls; before performing the overall meta-analysis, these estimates were pooled using inverse-variance weighting. The study by Maisonet et al. (2012) and Marks et al. (2019a) only reported sex-specific estimates for girls and boys from the same population. These two studies were also pooled to obtain an effect estimate in the overall population and included in the meta-analysis as Maisonet et al. (2012). Meta-analyses were performed using β coefficient per ln(ng/mL) of all 27 studies, since the majority of the studies reported results on log scale. Additionally, analyses were performed using subsets of the studies to evaluate whether the summary effect estimate varied by study confidence or by the timing of maternal serum sampling. The results were presented in Table D-12.

The meta-analysis conducted using β coefficient per ln(ng/mL) for all studies (n = 27) resulted in a β coefficient of –7.7 g (95%CI: -14.8, -0.5) mean birth weight per ln(ng/mL) PFHxS increase based on a random effect model with inverse-variance weights. This β coefficient can be re-expressed in terms of per ng/mL according to Dzierlenga et al. (2020). First, the distribution of exposure for each individual study was estimated by assuming the exposure follows a log-normal distribution. One hundred replicates of random samples (sample size was the same as the reported sample size in each study) were then simulated from the exposure distributions for each study included in the meta-analysis, and random samples from all studies were pooled for each replicate to get quantiles from the pooled random samples for each replicate. Lastly, the mean quantiles (median and IQR) from the 100 replicates were used to obtain the exposure distribution for all

studies using Equation (1) and (2), since the joint distribution of the exposures are also log normally distributed. The re-expressed β coefficient is -7.7 g (95%CI: -14.9, -0.5) per ng/mL.

The BMD of 12.55 ng/mL from all studies can be calculated using the same approach as above with the same values for the mean birth weight in the United States. To calculate the BMDL, the same procedure as above was used to calculate the 95% one-sided lower limit for the re-expressed β coefficient. Using the one-sided lower limit, a BMDL of 7.05 ng/mL is calculated.

The BMD and BMDL for the effect of PFHxS on decreased birth weight using meta-analysis results, conducted in log scale overall, and stratified by study confidence and by sample timing, are presented in Table D-12 below. As shown in Table D-12 (and Appendix C), the overall combined β coefficient of –7.7 g (95%CI: -14.8, -0.5) per ln(ng/mL) increase was robust and very comparable to that seen for only the twelve *high* studies (-6.8 g; 95%CI: -16.3, 2.8) or the 23 *medium* and *high* studies combined (-8.0 g; 95%CI: -15.2, -0.7). Similarly, the BMDLs for the earlier sampled study subsets (6.34) were very comparable to the overall full set of studies (7.05).

EPA also conducted the analysis with the alternative approach discussed above by considering an alternative control-group response distribution ($N(\mu_c, \sigma_c)$). The results from this alternative approach, presented in Table D-13 below, are very similar to the previous results.

Table D-12. BMDs and BMDLs for effect of PFHxS on decreased birth weight using meta-analysis results conducted in log scale overall, by study confidence and by sample timing, using the percentage (8.27%) of live births falling below the public health definition of low birth weight

Set of studies	Meta-analysis in log scale			
	β per ln(ng/mL) (95%CI)	Re-expressed β per ng/mL (95%CI)	BMD (ng/mL)	BMDL (ng/mL)
All studies (n = 27)	-7.7 (-14.8, -0.5)	-7.7 (-14.9, -0.5)	12.55	7.05
Study Confidence				
High (n = 12)	-6.8 (-16.3, 2.8)	-7.3 (-17.5, 3.0)	13.26	6.09
Medium (n = 11)	-9.6 (-20.8, 1.6)	-8.5 (-18.4, 1.4)	11.50	5.81
Low (n = 4)	-1.5 (-51.6, 48.7)	-3.7 (-127.1, 120.0)	25.61	0.88
High + Medium (n = 23)	-8.0 (-15.2, -0.7)	-7.9 (-15.0, -0.7)	12.32	7.00
Sample Timing[a]				
Early Pregnancy (n = 12)	-7.3 (-16.0, 1.4)	-7.7 (-16.8, 1.5)	12.68	6.34
Late Pregnancy (n = 10)	-3.9 (-17.7, 9.9)	-4.1 (-18.6, 10.4)	23.10	5.82
Post Pregnancy (n = 5)	-28.3 (-69.3, 12.7)	-11.7 (-28.7, 5.3)	8.48	3.83
Late + Post Pregnancy (n = 15)	-8.5 (-21.0, 4.1)	-7.3 (-18.1, 3.5)	13.19	5.89

[a]Sample time periods include early pregnancy (the 1st trimester, 1st or 2nd trimester), late pregnancy (2nd trimester, 2nd, or 3rd trimester), post pregnancy (birth and post-birth); n = number of studies; effect estimates, β, represent change in birthweight (grams) per unit change in ln (ng/mL) or ng/mL PFHxS exposure; CI = confidence interval.

Table D-13. BMDs and BMDLs for effect of PFHxS on decreased birth weight using meta-analysis results conducted in log scale overall, by study confidence and by sample timing, using the alternative study-specific tail probability of live births falling below the public health definition of low birth weight

Set of studies	Meta-analysis in log scale			
	β per ln(ng/mL) (95%CI)	Re-expressed β per ng/mL (95%CI)	BMD (ng/mL)	BMDL (ng/mL)
All studies (n = 27)	-7.7 (-14.8, -0.5)	-7.7 (-14.9, -0.5)	17.38	9.77
Study Confidence				
High (n = 12)	-6.8 (-16.3, 2.8)	-7.3 (-17.5, 3.0)	18.41	8.45
Medium (n = 11)	-9.6 (-20.8, 1.6)	-8.5 (-18.4, 1.4)	15.88	8.02
Low (n = 4)	-1.5 (-51.6, 48.7)	-3.7 (-127.1, 120.0)	36.21	1.25
High + Medium (n = 23)	-8.0 (-15.2, -0.7)	-7.9 (-15.0, -0.7)	17.06	9.69
Sample Timing[a]				
Early Pregnancy (n = 12)	-7.3 (-16.0, 1.4)	-7.7 (-16.8, 1.5)	17.58	8.79
Late Pregnancy (n = 10)	-3.9 (-17.7, 9.9)	-4.1 (-18.6, 10.4)	32.59	8.21
Post Pregnancy (n = 5)	-28.3 (-69.3, 12.7)	-11.7 (-28.7, 5.3)	11.52	5.20
Late + Post Pregnancy (n = 15)	-8.5 (-21.0, 4.1)	-7.3 (-18.1, 3.5)	18.31	8.18

[a]Sample time periods include early pregnancy (the 1st trimester, 1st or 2nd trimester), late pregnancy (2nd trimester, 2nd or 3rd trimester), post pregnancy (birth and post-birth); n = number of studies; effect estimates, β, represent change in birthweight (grams) per unit change in ln (ng/mL) or ng/mL PFHxS exposure; CI = confidence interval.

Summary of modeling results for decreased birth weight

For decreased birth weight associated with PFHxS exposure, the POD selected from the available epidemiologic literature (considering both individual studies and the results of meta-analyses using either *high* and *medium* confidence studies or focusing on early trimester sample timing) is 1.12 ng/mL maternal serum concentration, based on birth weight data from Bach et al. (2016a). The PODs from the meta-analyses of *high*, *medium*, or early sampling time studies were higher than the PODs from individual study PODs, and thus were not considered health-protective and were not considered further for POD selection. Of the five individual studies, (Buck Louis et al., 2018; Manzano-Salgado et al., 2017; Bach et al., 2016a) assessed maternal PFHxS serum concentrations either exclusively or predominately in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Additionally, use of the Bach et al. (2016a) results expressed in natural scale eliminated uncertainty associated with the re-expression of regression coefficients. Therefore, the POD from Bach et al. (2016a), which also was the lowest, was ultimately selected.

D.2. BENCHMARK DOSE MODELING RESULTS FROM ANIMAL STUDIES

D.2.1. Benchmark Dose Modeling Approaches

The endpoints selected for benchmark dose (BMD) modeling are listed in Table D-14. The animal doses in the study were used in the BMD modeling and then converted to human equivalent doses (HEDs) using the PK model described in Section 3.1 of the main document; the BMD modeling results are presented in this appendix.

Modeling Procedure for Dichotomous Noncancer Data

BMD modeling of dichotomous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.2). For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, Weibull, and Dichotomous Hill models available within the software were fit using a benchmark response (BMR) of 10% extra risk. The Multistage model is run for all polynomial degrees up to $n - 2$, where n is the number of dose groups including control. Adequacy of model fit was judged on the basis of χ^2 goodness -of -fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. Among all models providing *adequate* fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD.

Modeling Procedure for Continuous Noncancer Data

BMD modeling of continuous noncancer data was conducted using EPA's Benchmark Dose Software (BMDS, version 3.2). For these data, the Exponential, Hill, Polynomial, and Power models available within the software are fit using a BMR of 1 standard deviation (SD) when no toxicological information was available to determine an adverse level of response. When toxicological information was available, the BMR was based on relative deviation, as outlined in the Benchmark Dose Technical Guidance (U.S. EPA, 2012). An *adequate* fit is judged on the basis of χ^2 goodness of fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made on whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.05$), the model is run again, while modeling the variance as a power function of the mean to account for this nonhomogeneous variance.

Among all models providing *adequate* fit, the BMDL from the model with the lowest AIC was selected as a potential POD when BMDL estimates differed by less than threefold. When BMDL

estimates differed by greater than threefold, the model with the lowest BMDL was selected to account for model uncertainty.

Data Used for Modeling

The source of the data used for modeling endpoints from animal studies is provided in Table D-14. These data also are included in full in the tables below.

Table D-14. Sources of data used in benchmark dose modeling of PFHxS endpoints from animal studies

Endpoint/Reference	Reference	HAWC link
Endocrine effects		
↓ T4 Total – M	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100508242/
↓ T3 – M	NTP (2018)	https://hawcprd.epa.gov/ani/endpoint/100508240/
↓ T3 – C F1 PND 16	Ramhøj et al. (2020)	https://hawc.epa.gov/ani/endpoint/100515830/
↓ T4 Free – M F1 (PND16)	Ramhøj et al. (2018) RANGE FINDING	https://hawcprd.epa.gov/ani/endpoint/100508572/
↓ T4 Free – C F1 (PND16)	Ramhøj et al. (2018) RANGE FINDING	https://hawcprd.epa.gov/ani/endpoint/100508582/
↓ T4 Free – C F1 (PND22)	Ramhøj et al. (2018) MULTI-GEN	https://hawcprd.epa.gov/ani/endpoint/100508636/
↓ T4 Free – C F1 (PND16)	Ramhøj et al. (2018) MULTI-GEN	https://hawcprd.epa.gov/ani/endpoint/100508634/
↑ Thyroid hypertrophy/hypoplasia-M F0 (44day)	Butenhoff et al. (2009)	https://hawcprd.epa.gov/ani/endpoint/100507599/

Decreased free T4 — male rats NTP (2018)

Table D-15. Dose-response data for decreased free T4 in male rats NTP (2018)

Dose (mg/kg-d)	n	Mean (ng/dL)	SD
0	10	1.737	0.1
0.625	10	0.817	0.067
1.25	10	0.481	0.031
2.5	10	0.357	0.022
5	10	0.386	0.032
10	10	0.385	0.027

Table D-16. Benchmark dose results for decreased free T4 in male rats— non-constant variance, BMR = 1 standard deviation

Models[a]	Test 3 (*p* value)	1 standard deviation		Goodness of fit (*p*-Value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Exponential 2 (NCV—normal)	0.7202	0.0623	0.0470	<0.0001	-21.2050	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMD 10× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Exponential 3 (NCV—normal)	0.7202	0.0623	0.0470	<0.0001	-21.2050	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMD 10× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Exponential 4 (NCV—normal)	0.7202	0.0425	0.0307	0.0002	-191.1842	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMD 10× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Exponential 5 (NCV—normal)	0.7202	0.0707	0.0475	0.0096	-199.6740	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Hill (NCV—normal)	0.7202	0.1890	0.1498	0.0003	-192.8821	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose
Polynomial (5 degree) (NCV—normal)	0.7202	80.468	74.3240	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (4 degree) (NCV—normal)	0.7202	80.4680	24.7291	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1 BMD/BMDL ratio > 3 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (3 degree) (NCV—normal)	0.7202	80.4680	27.7219	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (2 degree) (NCV—normal)	0.7202	80.4680	27.8561	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power (NCV—	0.7202	80.4680	54.9996	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1

Models[a]	Test 3 (*p* value)	1 standard deviation		Goodness of fit (*p*-Value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
normal)							BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear (NCV—normal)	0.7202	80.4680	30.5047	<0.0001	-2.4126	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

[a]Non-constant models failed to model the data.
[b]"Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds.

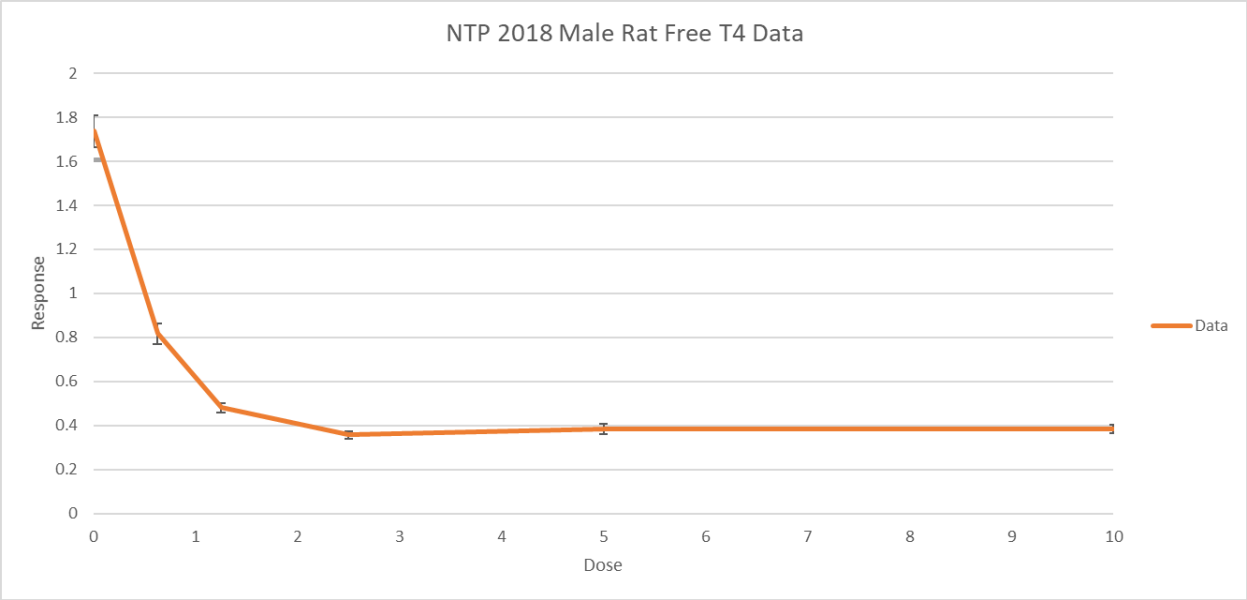

Figure D-3. Dose response data for male rat free T4 NTP (2018). X-axis is dose (mg/kg-d) and y-axis is level of free T4 (ng/dL).

Decreased total T4 — male rats (NTP, 2018)

Table D-17. Dose-response data for decreased T4 in male rats NTP (2018)

Dose (mg/kg-d)	n	Mean (µg/dL)	SD
0	10	4.24	0.229
0.625	10	2.39	0.078
1.25	10	1.7	0.058

Dose (mg/kg-d)	n	Mean (µg/dL)	SD
2.5	10	1.47	0.07
5	10	1.54	0.093
10	10	1.66	0.048

Table D-18. Benchmark dose results for decreased Total T4 in male rats— non-constant variance, BMR = 1 standard deviation

Models	Test 3 (*p*-Value)	1 standard deviation		Goodness of fit (*p*-value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Exponential 2 (NCV—normal)	0.0694	25.9593	20.9559	<0.0001	92.8124	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 3 (NCV—normal)	0.0694	25.9589	20.9558	<0.0001	92.8124	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 4 (NCV—normal)	0.0694	0.0410	0.0302	<0.0001	-69.8120	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMD 10× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Exponential 5 (NCV—normal)	0.0694	0.0802	0.0569	<0.0001	-85.4291	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose
Hill (NCV—normal)	0.0694	0.2273	0.1763	<0.0001	-80.5334	Questionable	Goodness of fit *p*-value < 0.1 BMDL 3× lower than lowest non-zero dose
Polynomial (5 degree) (NCV—normal)	0.0694	111.5387	69.6621	<0.0001	78.5088	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (4 degree) (NCV—normal)	0.0694	112.5746	91.2386	<0.0001	78.4897	Questionable	Goodness of fit *p*-value < 0.1

Models	Test 3 (*p*-Value)	1 standard deviation		Goodness of fit (*p*-value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (3 degree) (NCV—normal)	0.0694	111.6385	59.1142	<0.0001	78.5079	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (2 degree) (NCV—normal)	0.0694	112.3898	70.3271	<0.0001	78.4933	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power (NCV—normal)	0.0694	111.6423	57.6923	<0.0001	78.5078	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear (NCV—normal)	0.0694	115.0030	56.6839	<0.0001	78.4446	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

[a]Nonconstant models failed to model the data.

[b]"Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds.

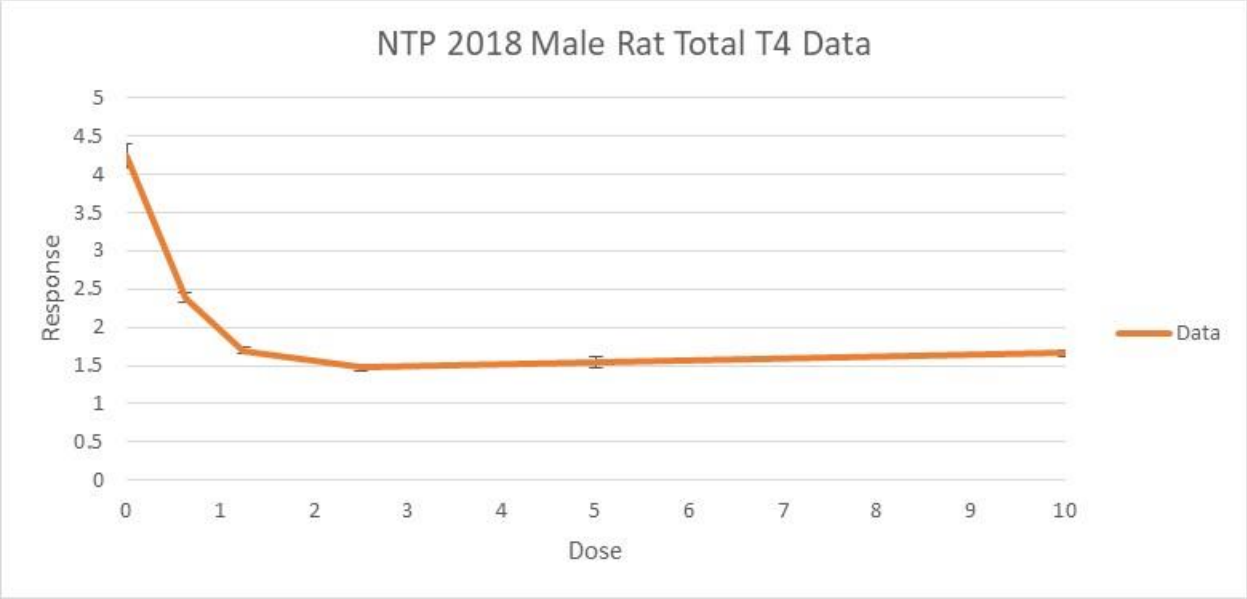

Figure D-4. Dose response data for male rat Total T4 NTP (2018). X-axis is dose (mg/kg-d) and y-axis is level of Total T4 (µg/dL).

Decreased total T4 — female rats (NTP, 2018)

Table D-19. Dose-response data for total T4 in female rats NTP (2018)

Dose (mg/kg-d)	*n*	Mean (µg/dL)	SD
0	10	3.99	0.186
3.12	10	3.53	0.196
6.25	10	3.37	0.165
12.5	10	2.97	0.108
25	10	2.96	0.194
50	10	2.69	0.145

1

Table D-20. Benchmark dose results for decreased total T4 in female rats— constant variance, BMR = 1 standard deviation

Models[a]	Test 2 (*p*-Value)	1 standard deviation		Goodness of fit (*p*-Value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Exponential 2 (CV—normal)	0.4699	10.6750	8.5600	<0.0001	20.0623	Questionable	Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 3 (CV—normal)	0.4699	10.6760	8.5600	<0.0001	20.0623	Questionable	Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 4 (CV—normal)	0.4699	1.2952	0.9782	0.0075	-29.7163	Questionable	Goodness of fit *p*-value < 0.1 BMDL 3× lower than lowest non-zero dose
Exponential 5 (CV—normal)	0.4699	1.2955	0.9784	0.0075	-29.7163	Questionable	Goodness of fit *p*-value < 0.1 BMDL 3× lower than lowest non-zero dose
Hill (NCV—normal)	0.4699	0.9571	0.6949	0.0358	-33.1163	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose
Polynomial (5 degree) (CV—normal)	0.4699	13.4343	11.3456	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (4 degree) (CV—normal)	0.4699	13.4343	10.9606	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

Models[a]	Test 2 (*p*-Value)	1 standard deviation		Goodness of fit (*p*-Value)	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Polynomial (3 degree) (CV—normal)	0.4699	13.4343	10.9605	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (2 degree) (CV—normal)	0.4699	13.4343	10.9605	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power (CV—normal)	0.4699	13.4343	10.9610	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear (CV—normal)	0.4699	13.4343	10.9605	<0.0001	25.0677	Questionable	Goodness of fit *p*-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

[a]Constant models failed to model the data.
[b]"Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds.

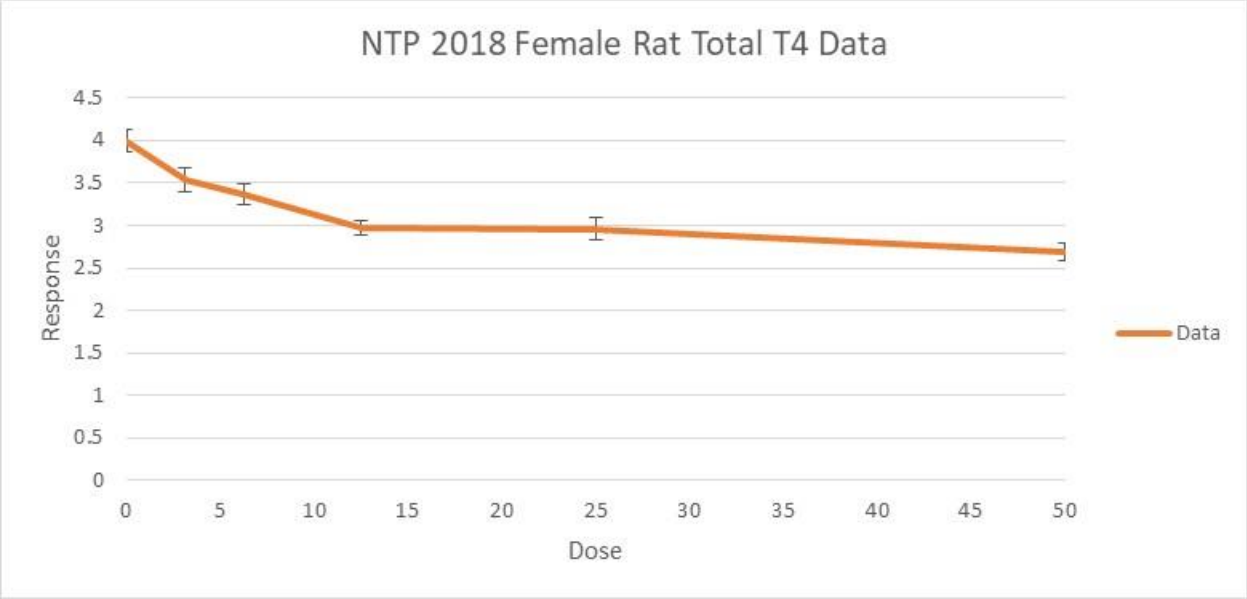

Figure D-5. Dose response data for female rat Total T4 NTP (2018). X-axis is dose (mg/kg-d) and y-axis is level of Total T4 (µg/dL).

Table D-21. Benchmark dose results for decreased T3 in male rats—nonconstant variance, BMR = 1 standard deviation

Models	Restriction[a]	1 standard deviation		*p*-Value	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
Exponential 2 (NCV—normal)	0.1150	7.7723	5.7661	<0.0001	441.4262	Questionable	Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 3 (NCV—normal)	0.1150	7.7700	5.7661	<0.0001	441.4262	Questionable	Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 4 (NCV—normal)	0.1150	0.1196	0.0825	0.0014	332.8680	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose
Exponential 5 (NCV—normal)	0.1150	0.1527	0.0849	0.0005	334.4245	Questionable	Goodness of fit *p*-value < 0.1 BMD 3× lower than lowest non-zero dose BMDL 3× lower than lowest non-zero dose
Hill (NCV—normal)	0.1150	0.2869	0.1542	0.0002	336.3586	Questionable	Goodness of fit *p*-value < 0.1 BMDL 3× lower than lowest non-zero dose
Polynomial (5 degree) (NCV—normal)	0.1150	19.8036	10.2767	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (4 degree) (NCV—normal)	0.1150	19.8036	10.2769	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2

Models	Restriction[a]	1 standard deviation		*p*-Value	AIC	BMDS classification[b]	BMDS notes
		BMD	BMDL				
							Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (3 degree) (NCV—normal)	0.1150	19.8036	10.2767	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial (2 degree) (NCV—normal)	0.1150	19.8036	10.2769	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power (NCV—normal)	0.1150	19.8036	10.2767	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear (NCV—normal)	0.1150	19.8036	10.2768	<0.0001	439.1893	Questionable	Goodness of fit *p*-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

[a]Nonconstant models failed to model the data.
[b]"Classification" column denotes whether a model can be considered for model selection purposes. See BMDS User Guide: https://www.epa.gov/bmds.

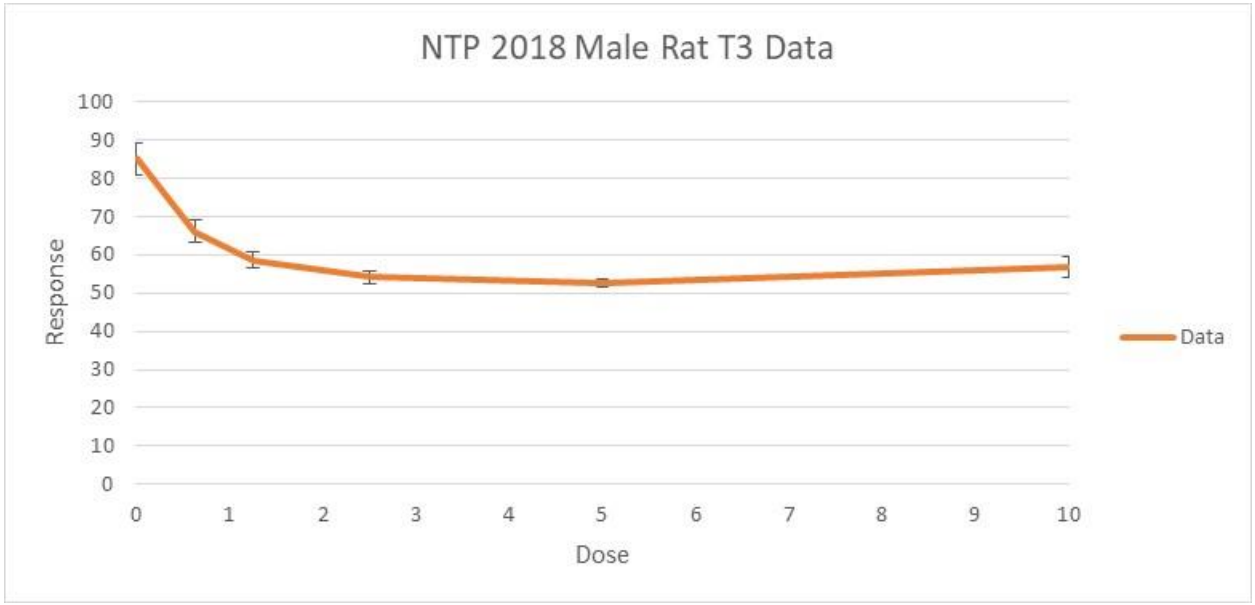

Figure D-6. Dose response data for male rat T3 NTP (2018).

X-axis is dose (mg/kg-d) and y-axis is level of T3 (ng/dL).

Decreased T3 — F1 rats PND 17 Ramhøj et al. (2020)

Table D-22. Dose response data for decreased free T3 in F1 combined PND16/17 rats Ramhøj et al. (2020)

Dose (mg/kg-d)	*n*	Mean (ng/dL)	SD
0	18	99.91023737	13.57584288
0.05	14	102.8114448	5.986381078
5	14	92.91322639	8.979571512
25	14	83.80963867	11.99271652

Table D-23. Benchmark dose results for decreased T3 in F1 PND16 male rats — constant variance, BMR = 1 standard deviation Ramhøj et al. (2020)

Model	1 standard deviation		Test 4 *P*-Value	AIC	BMDS recommendation	BMDS recommendation notes
	BMD	BMDL				
Exponential 2 (CV - normal)	4.670072	3.90788	<0.0001	323.9549629	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 3 (CV - normal)	4.669809	3.907839	<0.0001	323.9549627	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 4 (CV - normal)	1.408148	1.066629	0.0020435	303.9294537	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Exponential 5 (CV - normal)	3.260612	1.101338	NA	305.2949793	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill (CV - normal)	3.12622	0.961024	NA	305.2949187	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3 (CV - normal)	5.175471	4.358225	<0.0001	326.0612444	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Polynomial Degree 2 (CV - normal)	5.175471	4.358136	<0.0001	326.0612444	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Power (CV - normal)	5.175466	4.357586	<0.0001	326.0612444	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2
Linear (CV - normal)	5.175471	4.358409	<0.0001	326.0612444	Questionable	Constant variance test failed (Test 2 *p*-value < 0.05) Goodness of fit *p*-value < 0.1 |Residual for Dose Group Near BMD| > 2

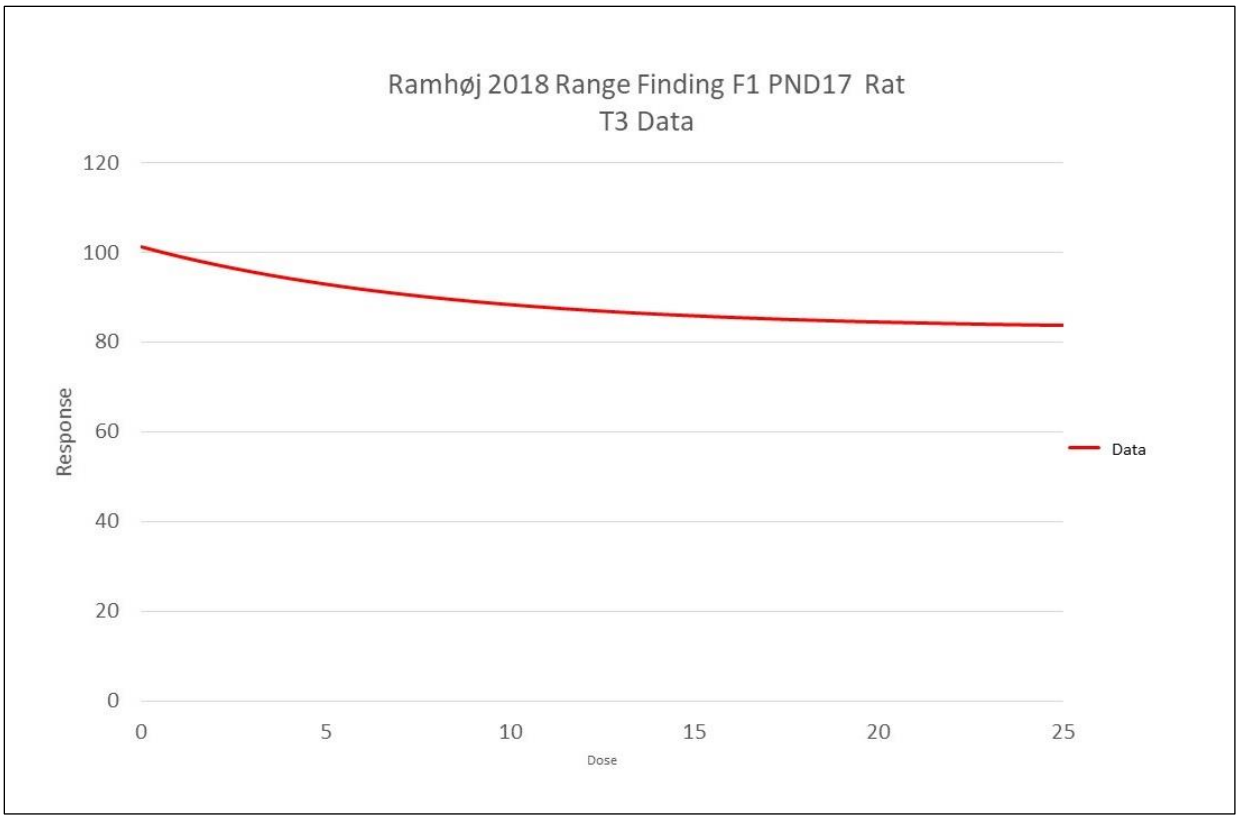

Figure D-7. Dose response data for decreased T3 in F1 PND17 rats Ramhøj et al. (2020). X-axis is dose (mg/kg-d), and y-axis is level of T3 (ng/dL).

APPENDIX E. DETAILED PHARMACOKINETIC ANALYSES

This appendix provides two detailed pharmacokinetic analyses. The first is a Bayesian analysis of perfluorohexanesulfonic acid (PFHxS) pharmacokinetics in laboratory animals to estimate key pharmacokinetic parameters. The second is the description and evaluation of a one-compartment pharmacokinetic (PK) modeling approach for estimating internal doses, evaluated against rat PFHxS PK data using the mean parameter values estimated for male rats in the Bayesian estimation.

E.1. BAYESIAN ANALYSIS OF PFHxS PHARMACOKINETICS IN RATS, MICE, AND MONKEYS

We estimated the sex-specific pharmacokinetic parameters (half-life, volume of distribution, and clearance) of PFHxS in rats, mice, and nonhuman primates (cynomolgus monkeys) by fitting one- and two-compartment models to the available concentration versus time data. A Bayesian hierarchical methodology was developed to fit these models because of the need to pool time-course concentration data across numerous studies with varying exposure scenarios within each study. This approach allowed for each concentration-versus-time data set to be fit to each model in which fitted parameters for each data set are sampled from a population-level distribution that models the similarities between each data set. In addition, the Bayesian analysis allowed for the generation of central estimates and credible intervals for the pharmacokinetic parameter of interest (e.g., half-life, volume of distribution and clearance) using posterior distributions from the estimated variables. Finally, the Bayesian methodology allowed for hypothesis testing of the one- and two-compartment formulations to decide which model more appropriately fit the data.

E.1.1. Pharmacokinetic Model

To determine pharmacokinetic parameters for PFHxS, we estimated constants for both one- and two-compartment model assumptions. For a one-compartment model assumption, the following exponential decay functions were fit to the available data:

$$C_{1-cmpt}^{IV}(t) = \frac{D}{V} e^{-k_e t}$$

$$C_{1-cmpt}^{oral}(t) = \frac{D}{V}\left(\frac{k_a}{k_a - k_e}\right)\left(e^{-k_e t} - e^{-k_a}\right)$$

where D represents the administered dose and V, k_e, and k_a represent the central compartment volume, elimination constant, and absorption constant (for oral only) to be fit. From these fitted constants, pharmacokinetic parameters are derived:

$$V_d = \frac{V}{BW}$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_e}$$

$$CLC = V_d * k_e$$

where V_d, $t_{1/2}$, and CLC represent the volume of distribution, terminal half-life, and clearance respectively and BW represents the animal body weight.

For the two-compartment model assumption, the following exponential decay functions were fit to available data:

$$A^{IV} = \frac{\alpha - k_{dc}}{\alpha - \beta};\ A^{oral} = k_a\left(\frac{k_{dc} - \alpha}{(k_a - \alpha)(\beta - \alpha)}\right)$$

$$B^{IV} = \frac{\beta - k_{dc}}{\beta - \alpha};\ B^{oral} = k_a\left(\frac{k_{dc} - \beta}{(k_a - \beta)(\alpha - \beta)}\right)$$

$$C^{IV}_{2-cmpt}(t) = \frac{D}{V}\left(A^{IV}e^{-\alpha t} + B^{IV}e^{-\beta t}\right)$$

$$C^{oral}_{2-cmpt}(t) = \frac{D}{V}\left(A^{oral}e^{-\alpha t} + B^{oral}e^{-\beta t} - \left(A^{oral} + B^{oral}\right)e^{-k_a t}\right)$$

where D represents the administered dose and V, α, β, k_{dc}, and k_a represent central compartment volume, alpha-phase elimination constant, beta-phase elimination constant, deep-to-central compartment rate constant, and absorption constant (for oral only) to be fit. From these fitted constants, the remaining two-compartment constants (k_{cd}: central-to-deep compartment rate constant and k_e: elimination constant) and the deep compartment volume (V_{deep}) are derived by solving:

$$\alpha + \beta = k_{cd} + k_{dc} + k_e$$

$$\alpha * \beta = k_{dc} * k_e$$

$$V_{deep} = V\frac{k_{cd}}{k_{dc}}$$

which allows for the desired pharmacokinetic parameters to be derived using the following equations:

$$V_{d-ss} = \frac{V + V_{deep}}{BW} = \frac{V}{BW}\left(\frac{k_{cd} + k_{dc}}{k_{dc}}\right)$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{\beta}$$

$$CLC = \frac{V}{BW} * k_e$$

where $V_{d\text{-}ss}$, $t_{1/2}$, and CLC represent the steady-state volume of distribution, terminal half-life, and clearance respectively and BW represents the animal body weight.

Bayesian Inference

The fitted constants for each model structure (described above) were determined using available time-course concentration data reported in mice and rats with the parameters for each model estimated using a Bayesian calibration approach. As described in the main text, owing to the discrepancy between oral and IV dosing bioavailability, only mice and rat time-course concentration data following oral gavage dosing was used for rodent-specific fits, while only IV dosing was available for nonhuman primate fits. In addition, for mice and nonhuman primates, time-course data from only one study (Sundström et al., 2012) were available and all sex-specific data were pooled into a single data set and fit to the one- and two-compartment models described above. However, a hierarchical Bayesian calibration approach was used to fit the observed time-course concentration data for male and female rats using data reported from multiple studies (Huang et al., 2019; Kim et al., 2018; Kim et al., 2016). For the two-compartment model, to ensure parameter identifiability, α and β were constrained to be ordered such that $\alpha > \beta$. This constraint ensures the exponential terms are identifiable and do not "flip" while exploring the parameter space during Markov-chain Monte-Carlo (MCMC) sampling. Finally, priors for each pharmacokinetic parameter were chosen to be "weakly informative" based on prior knowledge of PFAS pharmacokinetics (ATSDR ref) with 95% equal-tailed intervals spanning multiple order of magnitude.

Priors for pharmacokinetic parameters are presented in Table E-1 with corresponding model-specific parameter prior distributions presented below. Finally, a sensitivity analysis on the model priors is shown in the Prior Sensitivity Analysis section.

Table E-1. Weakly informed prior distributions for pharmacokinetic parameters used in the Bayesian analysis

	Median	Mad	Eti_3%	Eti_97%
Half-life (d)	15	12	0.88	250
Clearance (ml/kg-d)	50	49	0.32	6000
$V_{d\text{-}ss}$ (ml/kg)	900	811	9.3	32822

Median, mean absolute deviation (mad), lower (eti_3%), and upper (eti_97%) of the equal-tailed interval prior for each pharmacokinetic parameter.

For the mouse and nonhuman primate data, the following model was implemented to fit all data reported in Sundström et al. (2012):

$$\ln k_a \sim N(0,1)$$
$$\ln V \sim N(0,1)$$
$$\ln k_e \sim N(-3,1.5)\ one\ compartment\ model$$
$$\ln k_{dc} \sim N(0,1)\ two\ compartment\ model$$

$$\ln \alpha, \beta \sim N(-3, 1.5), \beta < \alpha \; two\; compartment\; model$$
$$C_i = \begin{cases} C_{1-cmpt}^{route} & \text{for 1-compartment model,} \\ C_{2-cmpt}^{route} & \text{for 2-compartment model} \end{cases}$$
$$\breve{\sigma} \sim Exp(1)$$
$$C_i \sim LN(\bar{x}_i, \tilde{\sigma})$$

where $\bar{x}_i$ is the sample mean of the observed concentrations at time t_i for all times reported. Model parameter priors are derived from the pharmacokinetic parameter priors defined earlier.

For the hierarchical approach, the concentration versus time data comprised a population- and data set-level for which model parameters were estimated. Here, each data set represented each study/sex/dose concentration versus time data set extracted from the literature and were fit using the model:

$$C_{ij} = \begin{cases} C_{1-cmpt}^{route} & \text{for 1-compartment model,} \\ C_{2-cmpt}^{route} & \text{for 2-compartment model} \end{cases}$$
$$C_{ik} \sim LN(\bar{x}_{ij}, \tilde{\sigma}_k)$$

where $\bar{x}_{ij}$ is the sample mean of the observed concentrations at time t_{ij} for data set j and $\tilde{\sigma}_k$ is study-level log-transformed standard deviation for the relative errors based on study k. Study-level priors for $\tilde{\sigma}_k$ were determined using the average log-transformed standard deviations:

$$\bar{\sigma}_{i,j}^2 = \ln\left(1 + \frac{s_{i,j}^2}{\bar{x}_{i,j}^2}\right)$$
$$\gamma_k = \frac{\sum_i \bar{\sigma}_{i,j \in k}}{n_k}$$

where $s_{i,j}$ is the sample standard deviation on the observed concentrations at time $t_{i,j}$ for study k. If s_{ij} was available, $\bar{\sigma}_{i,j}$ is the log-transformed standard deviation using the sample mean and standard deviation. For studies in which sample standard deviations could not be extracted, an average of all log-transformed standard deviations was used, which allowed for study-level prior distributions on the error model log-transformed standard deviation:

$$\tilde{\sigma}_k \sim \begin{cases} \text{Exp}(1/\gamma_k) & \text{if } \gamma_k \text{ available,} \\ \text{Exp}(1/\gamma) & \text{otherwise.} \end{cases}$$

Using this model, data set-level fitted constants were assigned priors based on a noncentered parameterization of a population-level distribution. This reparameterization of a typical hierarchical Bayesian model allows for increased sampling efficiency and can be more efficient for sampling when there is limited data (Betancourt and Girolami, 2013). Finally, nonelimination rate constants (k_a and k_{dc}) were assigned a unit normal, weakly informative prior to aid parameter identifiability (Gelman et al., 2015):

$$\ln \mu_{k_a} \sim N(0,1)$$
$$\ln \mu_V \sim N(0,1)$$
$$\ln \mu_{k_e} \sim N(-3,1.5)\ one\ compartment\ model$$
$$\ln \mu_{k_{dc}} \sim N(0,1)\ two\ compartment\ model$$
$$\ln \mu_{\alpha,\beta} \sim N(-3,1.5), \mu_\beta < \mu_\alpha\ two\ compartment\ model$$
$$\sigma_{k_a,V,k_e,\alpha,\beta,k_{dc}} \sim \mathrm{Exp}(1)$$
$$\ln(k_a, V, k_e, \alpha, \beta, k_{dc})_j \sim N\left(\mu_{k_a,V,k_e,\alpha,\beta,k_{dc}}, \sigma_{k_a,V,k_e,\alpha,\beta,k_{dc}}\right)$$

For both the single-level and hierarchical approaches, one- and two-compartment model goodness of fits were compared using the widely applicable information criteria (WAIC, (Watanabe, 2010)). Pharmacokinetic parameters from the most appropriate model, as judged by the WAIC comparison, were reported. To estimate the resulting pharmacokinetic parameters, we examined posterior probability densities of the parameters from the WAIC-determined model and calculated distributional estimates of the half-life, volume of distribution, and clearance using the equations described above. The parameter space was sampled using PyMC (Salvatier et al., 2016) using four independent Markov chains run for 10,000 iterations per chain. Posterior parameter distributions were determined using the final 5,000 iterations of each chain ensuring an effective sample size (ESS) greater than 10,000 (Kruschke, 2021). Convergence was assessed using a potential scale reduction factor with a maximum threshold of $\hat{R} = 1.05$ (Kruschke, 2021).

Prior Sensitivity Analysis

To investigate the impact of prior selection on posterior pharmacokinetic parameter estimation, we conducted a sensitivity analysis on the priors used in the Bayesian analysis. Priors were classified into three categories: weakly informed, broad, and uninformed. Weakly informed priors are defined using the half-life, clearance, and volume of distribution described above based on reported ranges of PFHxS pharmacokinetics with a prior predictive check demonstrating available data for fitting fall within the prior 90% credible interval.

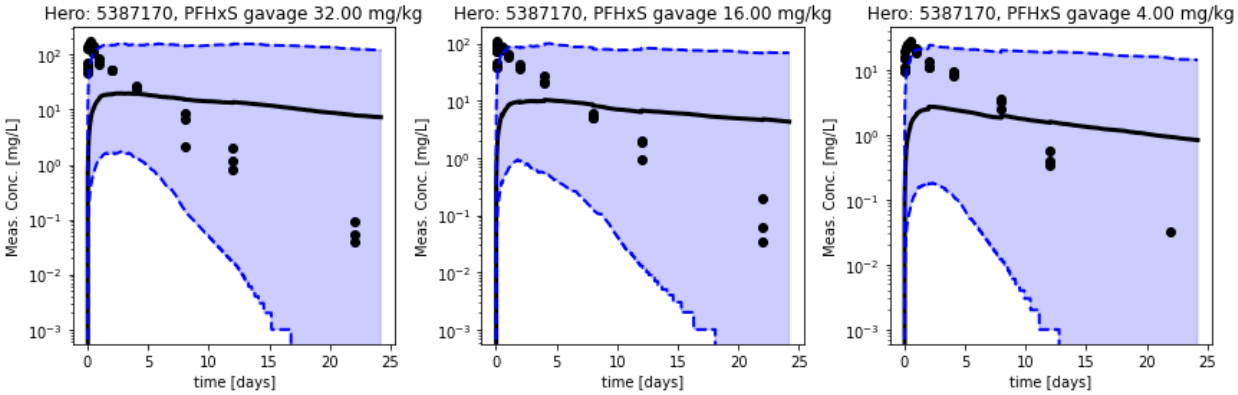

Figure E-1. Prior predictive check to ensure equal-tailed interval from prior distributions encompass the available time-course concentration data for fitting. Observed data from Kim et al. (2016).

Broad priors are defined as uniform distributions spanning the 3% and 97% ETI defined from the weakly informed priors and uninformed priors represent uniform priors spanning multiple orders of magnitude and are essentially flat priors. The following figure compares these three classes of priors and their impact on the posterior pharmacokinetic parameter distributions.

Table E-2. Results from prior sensitivity analysis for the three classes of priors (weakly informed, broad, and uninformed). For each pharmacokinetic parameters, mean, standard deviation (SD), lower HDI (HDI 5%), and upper HDI (95%) are presented.

Prior	Half-life (d)			Clearance (ml/kg-d)			$V_{d\text{-}ss}$ (mL/kg)		
	Mean	SD	HDI 5%, 95%	Mean	SD	HDI 5%, 95%	Mean	SD	HDI 5%, 95%
Weakly informed	1.86	0.16	1.62, 2.11	84.1	12.7	64.7, 103.8	224.1	28.1	182.7, 266.4
Broad	1.85	0.16	1.61, 2.10	82.6	12.4	63.4, 101.8	218.6	26.6	177.7, 259.7
Uninformed	1.85	0.16	1.60, 2.08	82.9	12.4	63.4, 101.6	219.3	27.1	176.5, 258.6

Informed by these findings, EPA used the weakly informed pharmacokinetic priors for fitting available time-course concentration data.

Study-specific clearance values and model fits

As described above, three data sets were used for the female rat-specific parameter estimation for which only the oral gavage data were used for fitting (Huang et al., 2019; Kim et al., 2018; Kim et al., 2016). In addition to these three, a fourth data set (Benskin et al., 2009) was used for male rats. The sex-specific clearance value distribution obtained from fitting the three data sets

together had means and 90% credible intervals of 61.36 (55.5 –67.17) mL/kg-day in female rats and 7.15 (3.73–10.26) mL/kg-day in male rats. Comparatively, sex-specific clearances in mice, determined from Sundström et al. (2012), had means and 90% credible intervals of 3.18 (2.83–3.52) mL/kg-day in female mice and 3.86 (2.83–3.52) mL/kg-day in male mice. For rat and mice data, a one-compartment PK model was deemed superior for mice and rats based on the WAIC and visual inspection of the plots indicating a lack of distribution and excretion phase. For nonhuman primates, a clear distribution and excretion phase is observed in the data with WAIC indicating a two-compartment model for fitting. Data from Sundström et al. (2012) had means and 90% credible intervals of 2.12 (1.81–2.44) mL/kg-day in female cynomolgus monkeys and 1.39 (0.94–1.83) mL/kg-day in male cynomolgus monkeys.

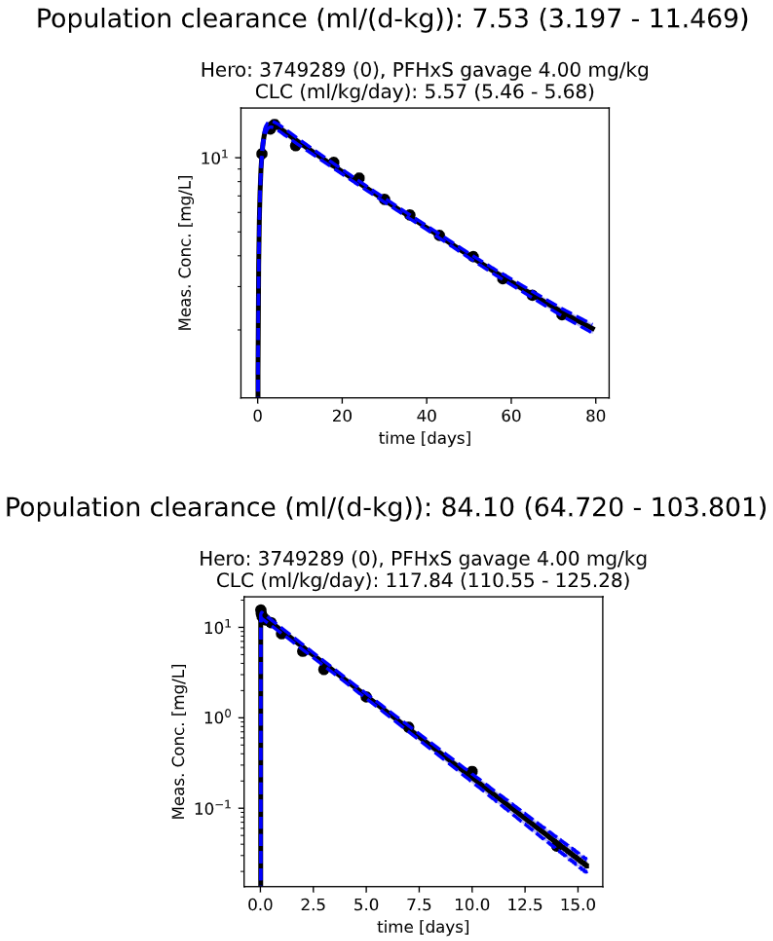

Figure E-2. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male (top panel) and female (bottom panel) rats after a 4 mg/kg gavage PFHxS. Observed data from Kim et al. (2016).

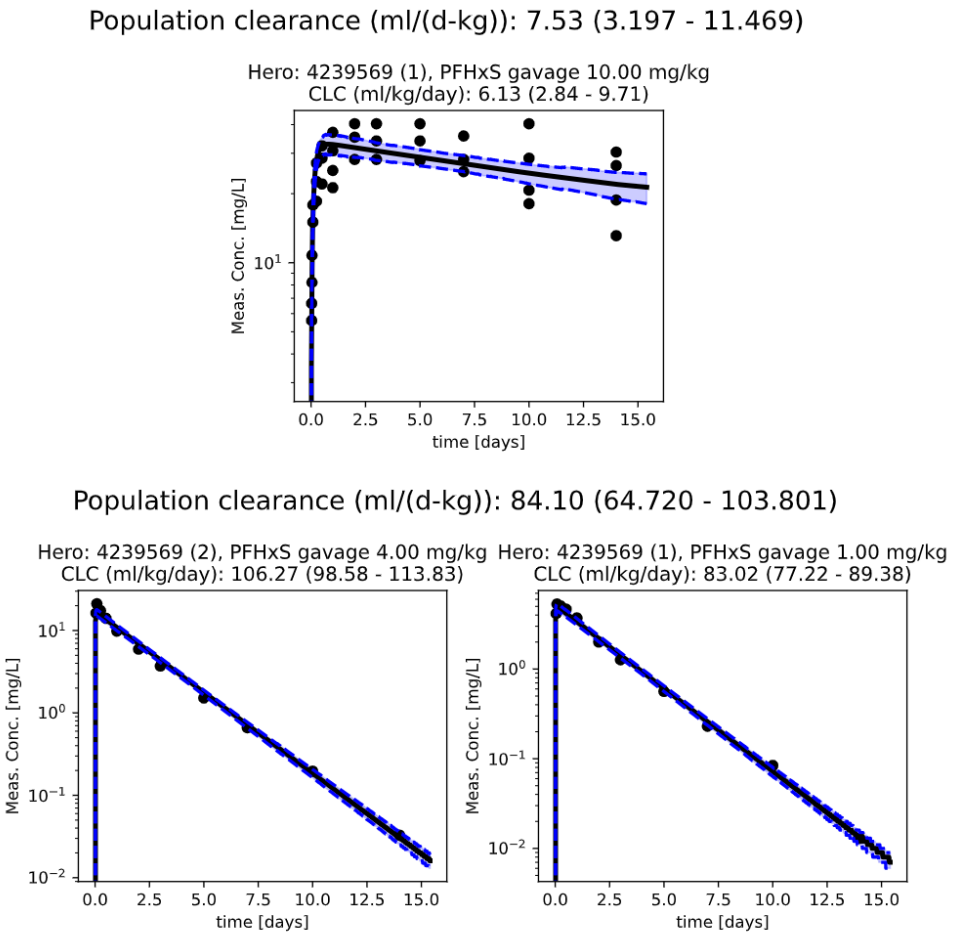

Figure E-3. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male (top panel) and female (bottom two panels) rats after a 10 mg/kg gavage (both male and female) and 4 mg/kg gavage (female only) PFHxS. Data from **Kim et al. (2018)**.

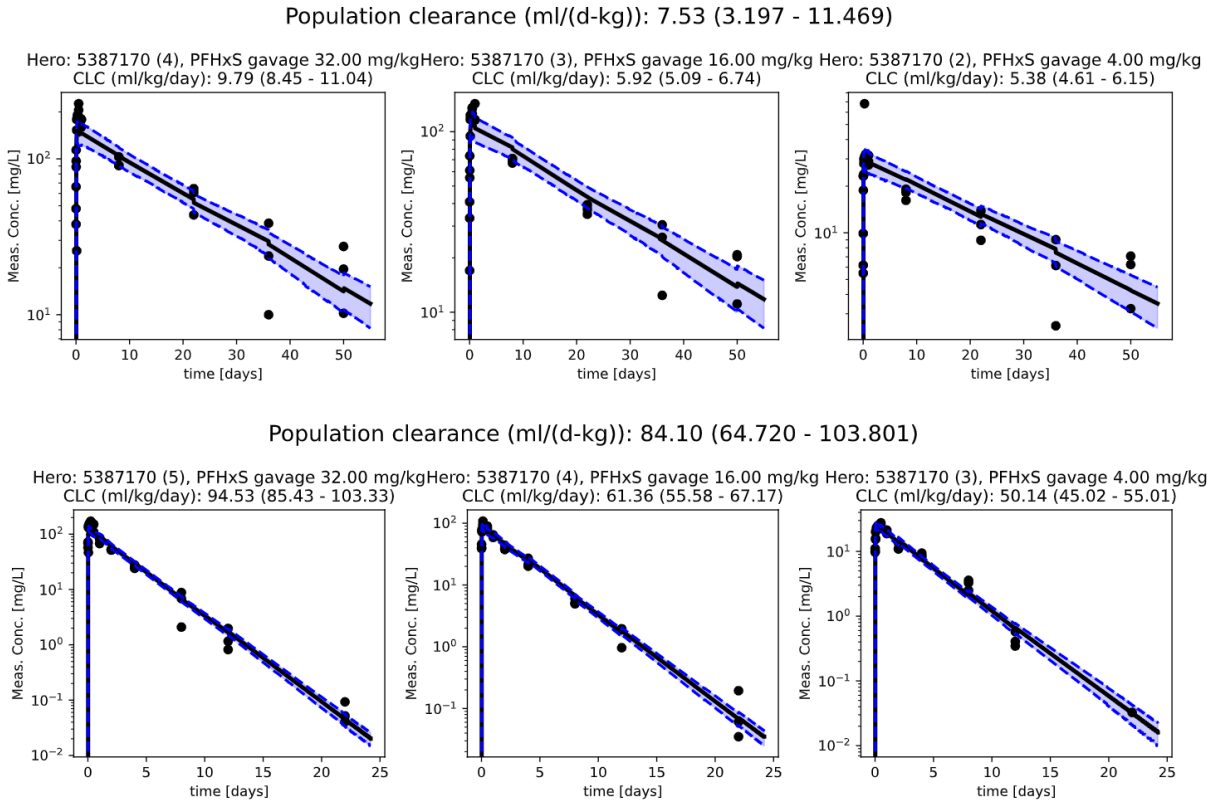

Figure E-4. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male (top panel) and female (bottom panel) rats after a 4, 16, or 32 mg/kg gavage PFHxS. Data from **Huang et al. (2019)**.

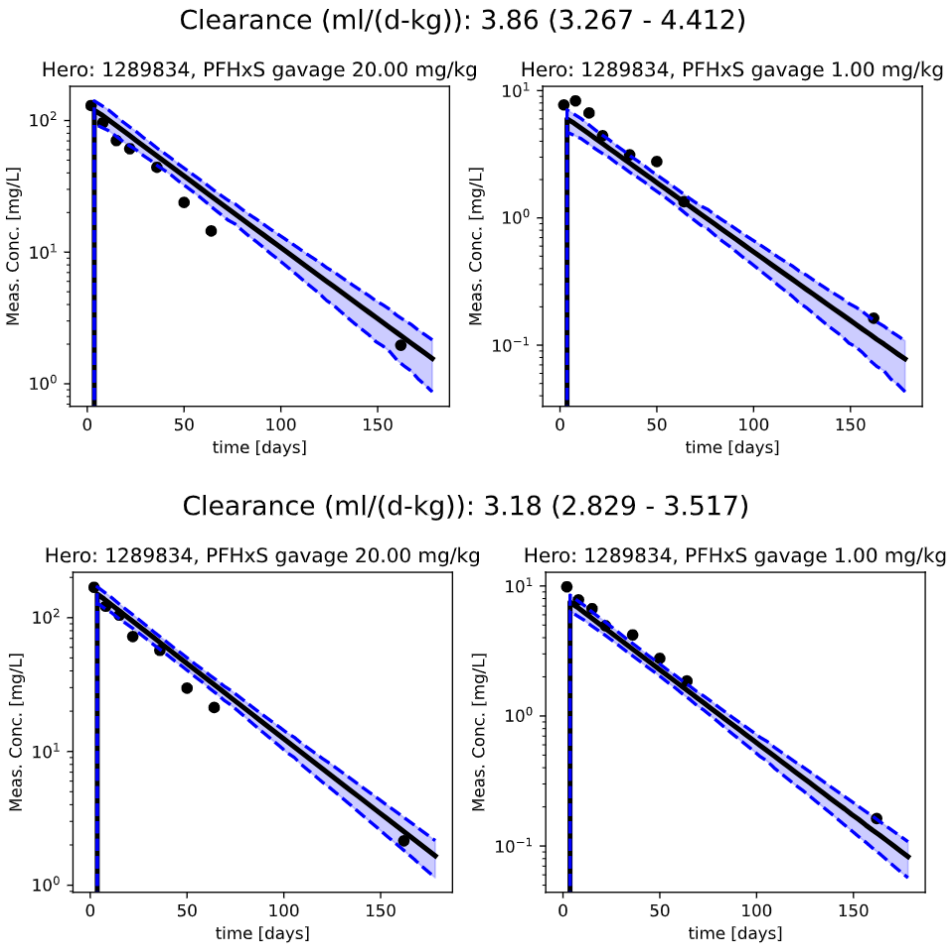

Figure E-5. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male (top panel) and female (bottom panel) mice after a 1 or 20 mg/kg gavage PFHxS. Data from **Sundström et al. (2012)**.

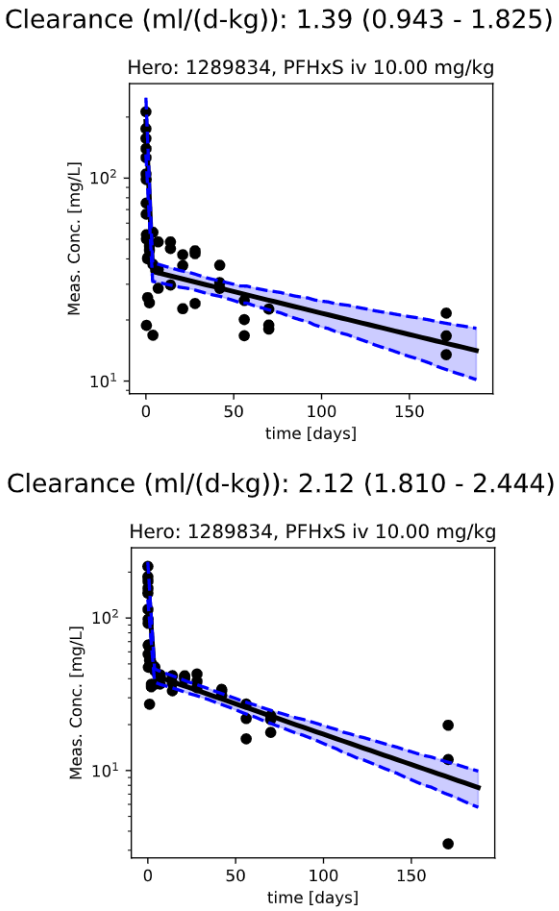

Figure E-6. Predicted (black line with blue 90% credible interval) and observed (black circles) serum time-courses for male (top panel) and female (bottom panel) nonhuman primates following a 10 mg/kg IV PFHxS dose. Data from **Sundström et al. (2012)**.

E.2. DESCRIPTION AND EVALUATION OF A SINGLE-COMPARTMENT PK APPROACH

A single-compartment PK model based on that described by Verner et al. (2016) was implemented. Verner et al. (2016) described linked one-compartment models for a mother and fetus or child. For this analysis the sub-model for the mother was used with distribution to the offspring set to zero and the model parameter inputs adjusted to use clearance (CL) rather than half-life as the input parameter but given the change in parameters the model is otherwise mathematically identical. The resulting differential equation for the amount of a substance in an individual after oral dosing is:

$$\frac{dA}{dt} = F_{abs} \times d \times BW - CL \times r_{m:mf} \times A/Vd$$

where A is the amount in the individual (mg), d is the dose (mg/kg-day), BW the body weight (kg), CL is the clearance (L/kg-day), Vd is the volume of distribution (L/kg), and $r_{m:mf}$ is a factor to account for nonuniform distribution between a pregnant individual and her fetus(es), in the event that one wishes to simulate dosimetry during gestation. For the following simulations of dosimetry in nonpregnant animals $r_{m:mf}$ is set to 1. While data are not available to indicate that distribution differs in pregnancy versus nonpregnant adult animals, the term is still included to allow for this possibility. For this analysis CL and Vd are assumed to be constant at the values determined from PK studies in young adult animals, as described above. The concentration in an individual's blood is then $C = A \cdot r_{m:mf}/(Vd_m \cdot BW)$.

The changes in male and female rat BW observed in the NTP bioassay (28-day exposure (NTP, 2019)) are shown in Figure E-7. Internal doses of PFHxS predicted by the PK model as a function of exposure day, using the population mean male rat parameters from Table 3-1 and Table 3-3, are shown in Figure E-8. The dose is assumed to be adjusted for changes in BW each day. Because the animals were necropsied on day 29, 1 day after the final dose, the model simulations include a final day with zero exposure. Mean plasma PFHxS concentrations from the NTP study, collected at time of necropsy, are shown for comparison. Very little accumulation was predicted in female rats after exposure day 10, whereas male rats accumulated PFHxS throughout the study.

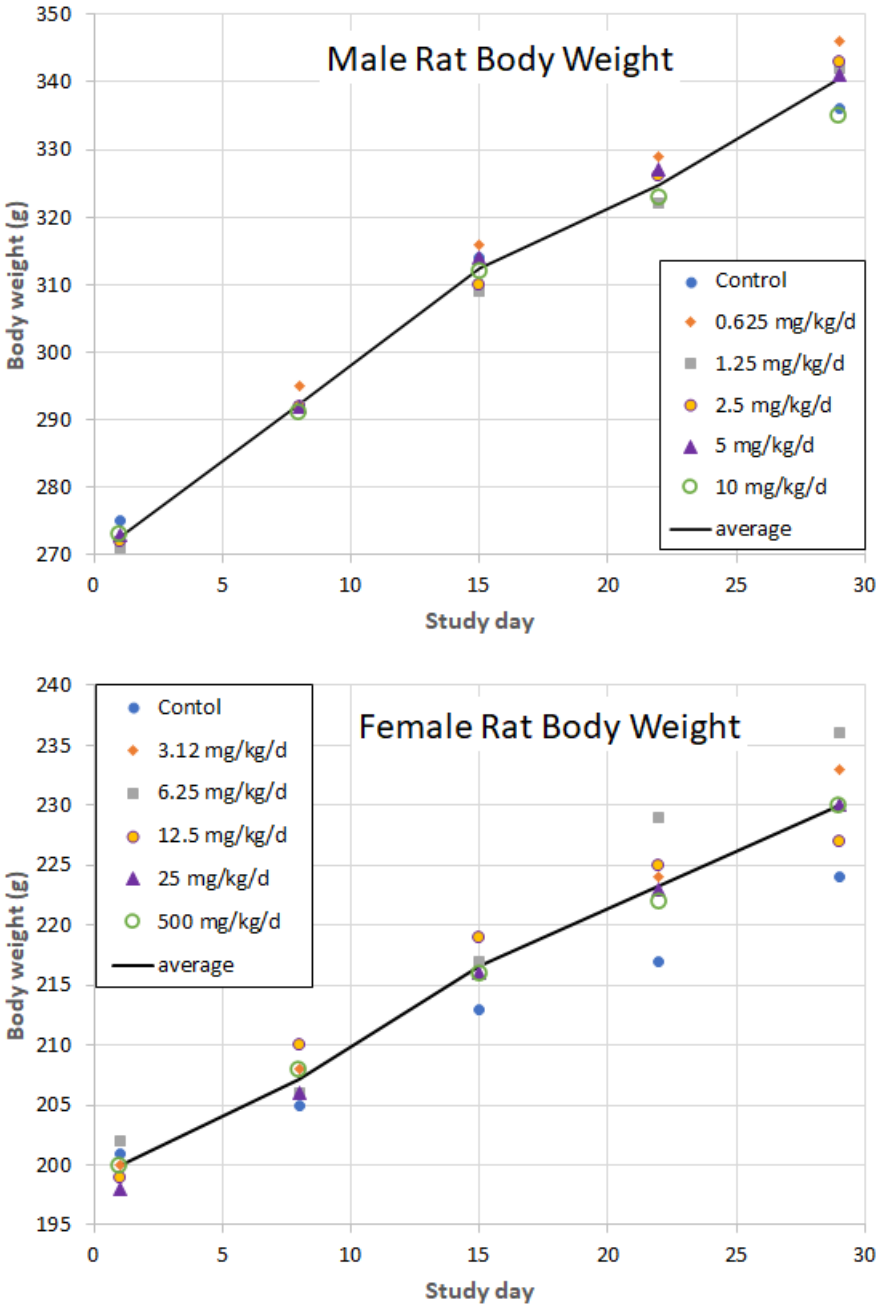

Figure E-7. Male and female rat body weight changes during 28-day PFHxS bioassay. Data sets from NTP (2019) are identified by the dose (mg/kg-d).

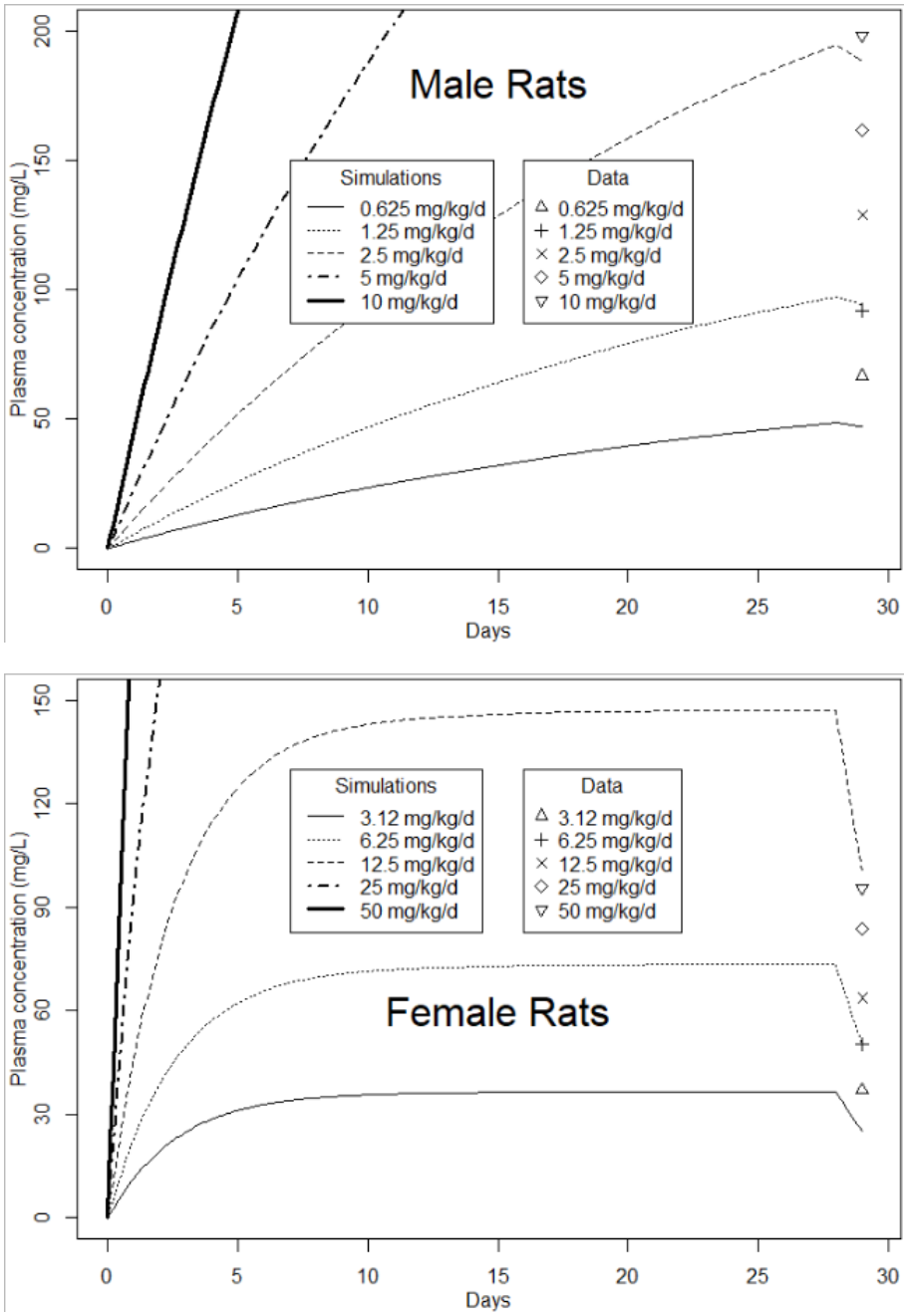

Figure E-8. Predicted accumulation and observed end-of-study of PFHxS in male rats as a function of dose. The plasma concentrations, observed in the NTP (2019) bioassay, were measured one day after the final dose, hence are plotted on day 29. Exposure is treated as continuous for 28 days.

The y-axis scales in Figure E-8 are set to focus on the range of the experimental data and because of nonlinearity in that data (discussed in more detail in the Pharmacokinetics section) the upper portions of the higher concentration curves from the PK model are outside that range. For example, when the dose to male rats was increased from 5 to 10 mg/kg-day, the resulting mean plasma concentrations only increased from 162 to 198 mg/L, or 22%. This nonlinearity was

suggested by the PK data, described in the introduction to this Pharmacokinetics section, for which clearance appeared to be higher at higher administered doses in rats, although the difference in the PK parameters may not have been statistically significant. Because the PK model evaluated is based on first-order kinetics, it predicts that the plasma concentration doubles when the dose is doubled. A likely mechanism for the observed nonlinearity is saturable renal resorption, allowing for faster elimination of PFHxS at higher internal concentrations. It is also possible that absorption is less efficient at higher dose levels. While the linear PK model is thereby shown to provide reasonable predictions of internal dose at lower exposures, the results are inadequate for 2.5 mg/kg-day and higher in male rats and for 12.5 mg/kg-day and higher in female rats.

APPENDIX F. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF PERFLUOROHEXANESULFONIC ACID AND RELATED SALTS

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.3) and follows the specifications outlined in EPA Order CIO 2105.3.

As required by CIO 2105.1, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using Guidance for Developing Quality Systems for Environmental Programs (QA/G-1). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of perfluorohexanesulfonic acid (PFHxS) is designated as Highly Influential Scientific Information (HISA)/Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA Guidance for Quality Assurance Project Plans (QA/G-5). All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document number	Date
Umbrella Quality Assurance Project Plan for CPHEA PFAS Toxicity Assessments	L-CPAD-0031652-QP-1-5	February 2023
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-6	June 2023
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBPK)	L-CPAD-0032188-QP-1-3	May 2023
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-3	June 2023

During assessment development, this project undergoes five quality audits during assessment development including:

Date	Type of audit	Major findings	Actions taken
August 2019	Technical system audit	None	None
August 2020	Technical system audit	None	None
July 2021	Technical system audit	None	None
August 2022	Technical system audit	None	None
June 2023	Technical system audit	None	None

APPENDIX G. SUMMARY OF PUBLIC AND EXTERNAL PEER REVIEW COMMENTS AND EPA'S DISPOSITION

REFERENCES

3M (3M Company). (1999). The science of organic fluorochemistry. (EPA-HQ-OPPT-2002-0043-0006). https://www.regulations.gov/document/EPA-HQ-OPPT-2002-0043-0006.

ACOG (American College of Obstetricians and Gynecologists). (2020). How your fetus grows during pregnancy. Available online at https://www.acog.org/womens-health/faqs/how-your-fetus-grows-during-pregnancy (accessed

Alkhalawi, E; Kasper-Sonnenberg, M; Wilhelm, M; Völkel, W; Wittsiepe, J. (2016). Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the Duisburg Birth Cohort. J Toxicol Environ Health A 79: 1041-1049. http://dx.doi.org/10.1080/15287394.2016.1219552.

Ammitzbøll, C; Börnsen, L; Petersen, ER; Oturai, AB; Søndergaard, HB; Grandjean, P; Sellebjerg, F. (2019). Perfluorinated substances, risk factors for multiple sclerosis and cellular immune activation. J Neuroimmunol 330: 90-95. http://dx.doi.org/10.1016/j.jneuroim.2019.03.002.

Angrish, MM; Kaiser, JP; Mcqueen, CA; Chorley, BN. (2016). Tipping the balance: Hepatotoxicity and the 4 apical key events of hepatic steatosis [Review]. Toxicol Sci 150: 261-268. http://dx.doi.org/10.1093/toxsci/kfw018.

Arrebola, JP; Ramos, JJ; Bartolomé, M; Esteban, M; Huetos, O; Cañas, AI; López-Herranz, A; Calvo, E; Pérez-Gómez, B; Castaño, A; BIOAMBIENT.ES. (2019). Associations of multiple exposures to persistent toxic substances with the risk of hyperuricemia and subclinical uric acid levels in BIOAMBIENT.ES study. Environ Int 123: 512-521. http://dx.doi.org/10.1016/j.envint.2018.12.030.

Ashley-Martin, J; Dodds, L; Arbuckle, TE; Bouchard, MF; Fisher, M; Morriset, AS; Monnier, P; Shapiro, GD; Ettinger, AS; Dallaire, R; Taback, S; Fraser, W; Platt, RW. (2017). Maternal concentrations of perfluoroalkyl substances and fetal markers of metabolic function and birth weight. Am J Epidemiol 185: 185-193. http://dx.doi.org/10.1093/aje/kww213.

ATSDR (Agency for Toxic Substances and Disease Registry). (2018). Toxicological profile for perfluoroalkyls. Draft for public comment [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

Averina, M; Brox, J; Huber, S; Furberg, AS; Sørensen, M. (2019). Serum perfluoroalkyl substances (PFAS) and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern Norway. Environ Res 169: 114-121. http://dx.doi.org/10.1016/j.envres.2018.11.005.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bonefeld-Jørgensen, EC; Bossi, R; Henriksen, TB. (2016a). Perfluoroalkyl acids in maternal serum and indices of fetal growth: The Aarhus Birth Cohort. Environ Health Perspect 124: 848-854. http://dx.doi.org/10.1289/ehp.1510046.

Bach, CC; Vested, A; Jørgensen, KT; Bonde, JP; Henriksen, TB; Toft, G. (2016b). Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review [Review]. Crit Rev Toxicol 46: 735-755. http://dx.doi.org/10.1080/10408444.2016.1182117.

Batzella, E; Zare Jeddi, M; Pitter, G; Russo, F; Fletcher, T; Canova, C. (2022). Associations between Mixture of Perfluoroalkyl Substances and Lipid Profile in a Highly Exposed Adult Community in the Veneto Region. Int J Environ Res Public Health 19. http://dx.doi.org/10.3390/ijerph191912421.

Benskin, JP; De Silva, AO; Martin, LJ; Arsenault, G; Mccrindle, R; Riddell, N; Mabury, SA; Martin, JW. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. Environ Toxicol Chem 28: 542-554. http://dx.doi.org/10.1897/08-239.1.

Bessone, F; Razori, MV; Roma, MG. (2019). Molecular pathways of nonalcoholic fatty liver disease development and progression. Cell Mol Life Sci 76: 99-128. http://dx.doi.org/10.1007/s00018-018-2947-0.

Betancourt, MJ; Girolami, M. (2013). Hamiltonian monte carlo for hierarchical models. Betancourt, MJ; Girolami, M. http://dx.doi.org/10.48550/arXiv.1312.0906.

Bjerregaard-Olesen, C; Bach, CC; Long, M; Wielsøe, M; Bech, BH; Henriksen, TB; Olsen, J; Bonefeld-Jørgensen, EC. (2019). Associations of Fetal Growth Outcomes with Measures of the Combined Xenoestrogenic Activity of Maternal Serum Perfluorinated Alkyl Acids in Danish Pregnant Women. Environ Health Perspect 127: 17006. http://dx.doi.org/10.1289/EHP1884.

Bonefeld-Jørgensen, EC. (2022). RE: Hjermitslev et al. Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. Available online at (accessed

Boobis, AR; Doe, JE; Heinrich-Hirsch, B; Meek, ME; Munn, S; Ruchirawat, M; Schlatter, J; Seed, J; Vickers, C. (2008). IPCS framework for analyzing the relevance of a noncancer mode of action for humans [Review]. Crit Rev Toxicol 38: 87-96. http://dx.doi.org/10.1080/10408440701749421.

Borenstein, M; Hedges, LV; Higgins, JPT; Rothstein, HR. (2009). Introduction to meta-analysis. Chichester, UK: John Wiley & Sons.

Borghese, MM; Liang, CL; Owen, J; Fisher, M. (2022). Individual and mixture associations of perfluoroalkyl substances on liver function biomarkers in the Canadian Health Measures Survey. Environ Health 21: 85. http://dx.doi.org/10.1186/s12940-022-00892-6.

Boucher, JM; Cousins, I; Scheringer, M; Hungerbuhler, K; Wang, Z. (2019). Toward a comprehensive global emission inventory of C_4-C_10 perfluoroalkanesulfonic acids (PFSAS) and related precursors: Focus on the life cycle of C_6- and C_10-based products [Letter]. Environ Sci Technol Lett 6: 1-7. http://dx.doi.org/10.1021/acs.estlett.8b00531.

Brosset, E; Ngueta, G. (2022). Exposure to per- and polyfluoroalkyl substances and glycemic control in older US adults with type 2 diabetes mellitus. Environ Res 216: 114697. http://dx.doi.org/10.1016/j.envres.2022.114697.

Buck, CO; Eliot, MN; Kelsey, KT; Calafat, AM; Chen, A; Ehrlich, S; Lanphear, BP; Braun, JM. (2018). Prenatal exposure to perfluoroalkyl substances and adipocytokines: the HOME Study. Pediatr Res 84: 854-860. http://dx.doi.org/10.1038/s41390-018-0170-1.

Buck Louis, GM; Zhai, S; Smarr, MM; Grewal, J; Zhang, C; Grantz, KL; Hinkle, SN; Sundaram, R; Lee, S; Honda, M; Oh, J; Kannan, K. (2018). Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environ Int 119: 515-526. http://dx.doi.org/10.1016/j.envint.2018.07.024.

Budtz-Jørgensen, E; Grandjean, P. (2018a). Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS ONE 13: e0205388. http://dx.doi.org/10.1371/journal.pone.0205388.

Budtz-Jørgensen, E; Grandjean, P. (2018b). Computational details for the paper "Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity".

Butenhoff, JL; Chang, SC; Ehresman, DJ; York, RG. (2009). Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol 27: 331-341. http://dx.doi.org/10.1016/j.reprotox.2009.01.004.

Callan, AC; Rotander, A; Thompson, K; Heyworth, J; Mueller, JF; Odland, JØ; Hinwood, AL. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in

offspring. Sci Total Environ 569-570: 1107-1113. http://dx.doi.org/10.1016/j.scitotenv.2016.06.177.

Cao, L; Quan, XB; Zeng, WJ; Yang, XO; Wang, MJ. (2016). Mechanism of hepatocyte apoptosis [Review]. Journal of Cell Death 9: 19-29. http://dx.doi.org/10.4137/JCD.S39824.

Cao, W; Liu, X; Liu, X; Zhou, Y; Zhang, X; Tian, H; Wang, J; Feng, S; Wu, Y; Bhatti, P; Wen, S; Sun, X. (2018). Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a Chinese birth cohort. Environ Int 116: 197-205. http://dx.doi.org/10.1016/j.envint.2018.04.015.

Cellesi, C; Michelangeli, C; Rossolini, GM; Giovannoni, F; Rossolini, A. (1989). Immunity to diphtheria, six to 15 years after a basic three-dose immunization schedule. Journal of Biological Standardization 17: 29-34. http://dx.doi.org/10.1016/0092-1157(89)90025-5.

Chang, CJ; Barr, DB; Ryan, PB; Panuwet, P; Smarr, MM; Liu, K; Kannan, K; Yakimavets, V; Tan, Y; Ly, V; Marsit, CJ; Jones, DP; Corwin, EJ; Dunlop, AL; Liang, D. (2022). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. Environ Int 158: 106964. http://dx.doi.org/10.1016/j.envint.2021.106964.

Chapman, AB; Abraham, WT; Zamudio, S; Coffin, C; Merouani, A; Young, D; Johnson, A; Osorio, F; Goldberg, C; Moore, LG; Dahms, T; Schrier, RW. (1998). Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. Kidney Int 54: 2056-2063. http://dx.doi.org/10.1046/j.1523-1755.1998.00217.x.

Chen, L; Tong, C; Huo, X; Zhang, J; Tian, Y. (2021). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and birth outcomes: A longitudinal cohort with repeated measurements. Chemosphere 267: 128899. http://dx.doi.org/10.1016/j.chemosphere.2020.128899.

Chen, M; Zhu, L; Wang, Q; Shan, G. (2020). Tissue distribution and bioaccumulation of legacy and emerging per-and polyfluoroalkyl substances (PFASs) in edible fishes from Taihu Lake, China. Environ Pollut 268: 115887. http://dx.doi.org/10.1016/j.envpol.2020.115887.

Chiu, WA; Lynch, MT; Lay, CR; Antezana, A; Malek, P; Sokolinski, S; Rogers, RD. (2022). Bayesian estimation of human population toxicokinetics of PFOA, PFOS, PFHxS, and PFNA from studies of contaminated drinking water. Environ Health Perspect 130: 127001. http://dx.doi.org/10.1289/EHP10103.

Christensen, KY; Raymond, MR; Thompson, BA; Anderson, HA. (2016). Fish consumption, levels of nutrients and contaminants, and endocrine-related health outcomes among older male anglers in Wisconsin. J Occup Environ Med 58: 668-675. http://dx.doi.org/10.1097/JOM.0000000000000758.

Christenson, B; Böttiger, M. (1986). Serological immunity to diphtheria in Sweden in 1978 and 1984. Scand J Infect Dis 18: 227-233. http://dx.doi.org/10.3109/00365548609032331.

Cohen, NJ; Yao, M; Midya, V; India-Aldana, S; Mouzica, T; Andra, SS; Narasimhan, S; Meher, AK; Arora, M; Chan, JKY; Chan, SY; Loy, SL; Minguez-Alarcon, L; Oulhote, Y; Huang, J; Valvi, D. (2023). Exposure to perfluoroalkyl substances and women's fertility outcomes in a Singaporean population-based preconception cohort. Sci Total Environ 873: 162267. http://dx.doi.org/10.1016/j.scitotenv.2023.162267.

Colicino, E; Pedretti, NF; Busgang, SA; Gennings, C. (2020). Per- and poly-fluoroalkyl substances and bone mineral density: Results from the Bayesian weighted quantile sum regression. Environmental Epidemiology 4: 1. http://dx.doi.org/10.1097/EE9.0000000000000092.

Collier, RJ. (1975). Diphtheria toxin: Mode of action and structure [Review]. Bacteriol Rev 39: 54-85. http://dx.doi.org/10.1128/br.39.1.54-85.1975.

Cui, F; Liu, H; Li, Y; Zheng, TZ; Xu, S; Xia, W; Sheng, X. (2022). Association of exposure to per- and polyfluoroalkyl substances with hemoglobin and hematocrit during pregnancy. Ecotoxicol Environ Saf 248: 114319. http://dx.doi.org/10.1016/j.ecoenv.2022.114319.

Derakhshan, A; Kortenkamp, A; Shu, H; Broeren, MAC; Lindh, CH; Peeters, RP; Bornehag, CG; Demeneix, B; Korevaar, TIM. (2022). Association of per- and polyfluoroalkyl substances with thyroid homeostasis during pregnancy in the SELMA study. Environ Int 167: 107420. http://dx.doi.org/10.1016/j.envint.2022.107420.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Kiviranta, H; Koponen, J; Rolandsson, O; Åkesson, A. (2019a). Perfluoroalkyl substances and risk of type II diabetes: A prospective nested case-control study. Environ Int 123: 390-398. http://dx.doi.org/10.1016/j.envint.2018.12.026.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Koponen, J; Kiviranta, H; Åkesson, A. (2019b). Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environ Int 124: 58-65. http://dx.doi.org/10.1016/j.envint.2019.01.007.

Dufour, P; Pirard, C; Petrossians, P; Beckers, A; Charlier, C. (2020). Association between mixture of persistent organic pollutants and thyroid pathologies in a Belgian population. Environ Res 181: 108922. http://dx.doi.org/10.1016/j.envres.2019.108922.

Dunder, L; Salihovic, S; Elmståhl, S; Lind, PM; Lind, L. (2023). Associations between per- and polyfluoroalkyl substances (PFAS) and diabetes in two population-based cohort studies from Sweden. J Expo Sci Environ Epidemiol. http://dx.doi.org/10.1038/s41370-023-00529-x.

Dzierlenga, M, .W.; Crawford, L, .; Longnecker, M, .P. (2020). Birth weight and perfluorooctane sulfonic acid: a random-effects meta-regression analysis. Environmental Epidemiology 4: e095. http://dx.doi.org/10.1097/EE9.0000000000000095.

E, L; Zhang, S; Jiang, X. (2023). Association between perfluoroalkyl substances exposure and the prevalence of nonalcoholic fatty liver disease in the different sexes: a study from the National Health and Nutrition Examination Survey 2005-2018. Environ Sci Pollut Res Int 30: 44292-44303. http://dx.doi.org/10.1007/s11356-023-25258-4.

Edwards, SW; Tan, YM; Villeneuve, DL; Meek, ME; McQueen, CA. (2016). Adverse outcome pathways—Organizing toxicological information to improve decision making [Review]. J Pharmacol Exp Ther 356: 170-181. http://dx.doi.org/10.1124/jpet.115.228239.

Eick, SM; Demicco, E; Valeri, L; Woodruff, TJ; Morello-Frosch, R; Hom Thepaksorn, EK; Izano, MA; Cushing, LJ; Wang, Y; Smith, SC; Gao, S; Park, JS; Padula, AM. (2020). Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. Environ Health 19: 100-100. http://dx.doi.org/10.1186/s12940-020-00654-2.

Fan, S; Wu, Y; Bloom, MS; Lv, J; Chen, L; Wang, W; Li, Z; Jiang, Q; Bu, L; Shi, J; Shi, T; Zeng, X; Zhang, L; Zhang, Z; Yang, B; Dong, G; Feng, W. (2023). Associations of per- and polyfluoroalkyl substances and their alternatives with bone mineral density levels and osteoporosis prevalence: A community-based population study in Guangzhou, Southern China. Sci Total Environ 862: 160617-160617. http://dx.doi.org/10.1016/j.scitotenv.2022.160617.

Feng, Y; Bai, Y; Lu, Y; Chen, M; Fu, M; Guan, X; Cao, Q; Yuan, F; Jie, J; Li, M; Meng, H; Wang, C; Hong, S; Zhou, Y; Zhang, X; He, M; Guo, H. (2022a). Plasma perfluoroalkyl substance exposure and incidence risk of breast cancer: A case-cohort study in the Dongfeng-Tongji cohort. Environ Pollut 306: 119345. http://dx.doi.org/10.1016/j.envpol.2022.119345.

Feng, Y; Fu, M; Guan, X; Wang, C; Meng, H; Zhou, Y; He, M; Guo, H. (2022b). Associations of exposure to perfluoroalkyl substances with serum uric acid change and hyperuricemia among Chinese

women: Results from a longitudinal study. Chemosphere 308: 136438. http://dx.doi.org/10.1016/j.chemosphere.2022.136438.

Galazka, A; Kardymowicz, B. (1989). Immunity against diphtheria in adults in Poland. Epidemiol Infect 103: 587-593. http://dx.doi.org/10.1017/s0950268800030983.

Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. (1993). The immunological basis for immunization module 2 : Diphtheria. (WHO/EPI/Gen/93.11-18). Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. http://apps.who.int/iris/bitstream/handle/10665/58891/WHO-EPI-GEN-93.12-mod2-eng.pdf?sequence=38&isAllowed=y.

Gao, K, e; Zhuang, T; Liu, X; Fu, J; Zhang, J; Fu, J, ie; Wang, L; Zhang, A; Liang, Y; Song, M; Jiang, G. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. Environ Sci Technol 53: 6529-6538. http://dx.doi.org/10.1021/acs.est.9b00715.

Gao, K; Fu, J; Xue, Q; Li, Y; Liang, Y; Pan, Y; Zhang, A; Jiang, G. (2018). An integrated method for simultaneously determining 10 classes of per- and polyfluoroalkyl substances in one drop of human serum. Anal Chim Acta 999: 76-86. http://dx.doi.org/10.1016/j.aca.2017.10.038.

Gaylord, A; Berger, KI; Naidu, M; Attina, TM; Gilbert, J; Koshy, TT; Han, X; Marmor, M; Shao, Y; Giusti, R; Goldring, RM; Kannan, K; Trasande, L. (2019). Serum perfluoroalkyl substances and lung function in adolescents exposed to the World Trade Center disaster. Environ Res 172: 266-272. http://dx.doi.org/10.1016/j.envres.2019.02.024.

Gaylord, A; Trasande, L; Kannan, K; Thomas, KM; Lee, S; Liu, M; Levine, J. (2020). Persistent organic pollutant exposure and celiac disease: A pilot study. Environ Res 186: 109439. http://dx.doi.org/10.1016/j.envres.2020.109439.

Gelman, A; Lee, D; Guo, L. (2015). Stan: a probabilistic programming language for bayesian inference and optimization. American Educational Research Journal 40. http://dx.doi.org/10.3102/1076998615606113.

Gibson, H, .M. (1973). Plasma volume and glomerular filtration rate in pregnancy and their relation to differences in fetal growth. Br J Obstet Gynaecol 80: 1067-1074. http://dx.doi.org/10.1111/j.1471-0528.1973.tb02981.x.

Gleason, J, essie A. Cooper, K.,eith R. Klotz, J.,udith B. Post, G.,loria B. Van Orden, G.,eorge New Jersey Drinking Water Quality Institute (NJDWQI). (2017). Health-based maximum contaminant level support document: Perfluorooctanoic acid (PFOA): Appendix A. New Jersey Drinking Water Quality Institute (NJDWQI), Health Effects Subcommittee. https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf.

Glynn, A; Berger, U; Bignert, A; Ullah, S; Aune, M; Lignell, S; Darnerud, PO. (2012). Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environ Sci Technol 46: 9071-9079. http://dx.doi.org/10.1021/es301168c.

Goodrich, JA; Walker, D; Lin, X; Wang, H; Lim, T; McConnell, R; Conti, DV; Chatzi, L; Setiawan, VW. (2022). Exposure to perfluoroalkyl substances and risk of hepatocellular carcinoma in a multiethnic cohort. JHEP Rep 4: 100550. http://dx.doi.org/10.1016/j.jhepr.2022.100550.

Grandjean, P; Andersen, EW; Budtz-Jørgensen, E; Nielsen, F; Mølbak, K; Weihe, P; Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307: 391-397. http://dx.doi.org/10.1001/jama.2011.2034.

Grandjean, P; Bateson, T. (2021). RE: Benchmark analysis for PFAS immunotoxicity. Available online at (accessed

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Timmermann, A; Budtz-Jørgensen, E. (2017). Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine

antibody concentrations at age 5-years. J Immunotoxicol 14: 188-195. http://dx.doi.org/10.1080/1547691X.2017.1360968.

Guo, J; Huang, S; Yang, L; Zhou, J; Xu, X; Lin, S; Li, H; Xie, X; Wu, S. (2023). Association between polyfluoroalkyl substances exposure and sex steroids in adolescents: The mediating role of serum albumin. Ecotoxicol Environ Saf 253: 114687. http://dx.doi.org/10.1016/j.ecoenv.2023.114687.

Gyllenhammar, I; Benskin, JP; Sandblom, O; Berger, U; Ahrens, L; Lignell, S; Wiberg, K; Glynn, A. (2018a). Perfluoroalkyl acids (PFAAs) in serum from 2-4-month-old infants: Influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. Environ Sci Technol 52: 7101-7110. http://dx.doi.org/10.1021/acs.est.8b00770.

Gyllenhammar, I; Diderholm, B; Gustafsson, J; Berger, U; Ridefelt, P; Benskin, JP; Lignell, S; Lampa, E; Glynn, A. (2018b). Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int 111: 191-199. http://dx.doi.org/10.1016/j.envint.2017.12.002.

Hack, M; Klein, NK; Taylor, HG. (1995). Long-term developmental outcomes of low birth weight infants [Review]. Future Child 5: 176-196. http://dx.doi.org/10.2307/1602514.

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A; Küttler, K; Malarkey, DE; Maronpot, RR; Nishikawa, A; Nolte, T; Schulte, A; Strauss, V; York, MJ. (2012). Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes—Conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicol Pathol 40: 971-994. http://dx.doi.org/10.1177/0192623312448935.

Hamm, MP; Cherry, NM; Chan, E; Martin, JW; Burstyn, I. (2010). Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20: 589-597. http://dx.doi.org/10.1038/jes.2009.57.

Harris, MH; Rifas-Shiman, SL; Calafat, AM; Ye, X; Mora, AM; Webster, TF; Oken, E; Sagiv, SK. (2017). Predictors of per- and polyfluoroalkyl substance (PFAS) plasma concentrations in 6-10 year old American children. Environ Sci Technol 51: 5193-5204. http://dx.doi.org/10.1021/acs.est.6b05811.

Haug, M; Dunder, L; Lind, PM; Lind, L; Salihovic, S. (2023). Associations of perfluoroalkyl substances (PFAS) with lipid and lipoprotein profiles. J Expo Sci Environ Epidemiol. http://dx.doi.org/10.1038/s41370-023-00545-x.

Higgins, JPT; Thomas, J; Chandler, J; Cumpston, M; Li, T; Page, MJ; Welch, VA. (2022). Cochrane handbook for systematic reviews of interventions version 6.3. Higgins, JPT; Thomas, J; Chandler, J; Cumpston, M; Li, T; Page, MJ; Welch, VA. http://www.training.cochrane.org/handbook.

Hjermitslev, MH; Long, M; Wielsøe, M; Bonefeld-Jørgensen, EC. (2020). Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. Sci Total Environ 698: 134118. http://dx.doi.org/10.1016/j.scitotenv.2019.134118.

Højsager, FD; Andersen, M; Juul, A; Nielsen, F; Möller, S; Christensen, HT; Grøntved, A; Grandjean, P; Jensen, TK. (2022). Prenatal and early postnatal exposure to perfluoroalkyl substances and bone mineral content and density in the Odense child cohort. Environ Int 167: 107417. http://dx.doi.org/10.1016/j.envint.2022.107417.

Hong, A; Zhuang, L; Cui, W; Lu, Q; Yang, P; Su, S; Wang, B; Zhang, G; Chen, D. (2022). Per- and polyfluoroalkyl substances (PFAS) exposure in women seeking in vitro fertilization-embryo transfer treatment (IVF-ET) in China: Blood-follicular transfer and associations with IVF-ET outcomes. Sci Total Environ 838: 156323. http://dx.doi.org/10.1016/j.scitotenv.2022.156323.

Huang, MC; Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA; Granville, CA; Gibbs, ST; Blystone, CR. (2019). Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female

Hsd:Sprague Dawley SD rats after intravenous and gavage administration. Toxicol Rep 6: 645-655. http://dx.doi.org/10.1016/j.toxrep.2019.06.016.

Huedo-Medina, TB; Sánchez-Meca, J; Marín-Martínez, F; Botella, J. (2006). Assessing heterogeneity in meta-analysis: Q statistic or I2 index? Psychol Methods 11: 193-206. http://dx.doi.org/10.1037/1082-989X.11.2.193.

Huo, X; Huang, R; Gan, Y; Luo, K; Aimuzi, R; Nian, M; Ao, J; Feng, L; Tian, Y; Wang, W; Ye, W; Zhang, J. (2020). Perfluoroalkyl substances in early pregnancy and risk of hypertensive disorders of pregnancy: A prospective cohort study. Environ Int 138: 105656. http://dx.doi.org/10.1016/j.envint.2020.105656.

IPCS (International Programme on Chemical Safety). (2007). Harmonization project document no. 4: Part 2: IPCS framework for analysing the relevance of a non-cancer mode of action for humans. Geneva, Switzerland: World Health Organization. http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1.

Ipsen, J. (1946). Circulating antitoxin at the onset of diphtheria in 425 patients. J Immunol 54: 325-347.

Jain, RB; Ducatman, A. (2019). Perfluoroalkyl acids and thyroid hormones across stages of kidney function. Sci Total Environ 696: 133994. http://dx.doi.org/10.1016/j.scitotenv.2019.133994.

Jain, RB; Ducatman, A. (2022). Serum concentrations of selected perfluoroalkyl substances for US females compared to males as they age. Sci Total Environ 842: 156891. http://dx.doi.org/10.1016/j.scitotenv.2022.156891.

Jensen, RC; Glintborg, D; Timmermann, CAG; Nielsen, F; Boye, H; Madsen, JB; Bilenberg, N; Grandjean, P; Jensen, TK; Andersen, MS. (2022). Higher free thyroxine associated with PFAS exposure in first trimester. The Odense Child Cohort. Environ Res 212: 113492. http://dx.doi.org/10.1016/j.envres.2022.113492.

Ji, K; Kim, S; Kho, Y; Sakong, J; Paek, D; Choi, K. (2012). Major perfluoroalkyl acid (PFAA) concentrations and influence of food consumption among the general population of Daegu, Korea. Sci Total Environ 438: 42-48. http://dx.doi.org/10.1016/j.scitotenv.2012.08.007.

Joshi-Barve, S; Kirpich, I; Cave, MC; Marsano, LS; McClain, CJ. (2015). Alcoholic, nonalcoholic, and toxicant-associated steatohepatitis: Mechanistic similarities and differences [Review]. CMGH 1: 356-367. http://dx.doi.org/10.1016/j.jcmgh.2015.05.006.

Kashino, I; Sasaki, S; Okada, E; Matsuura, H; Goudarzi, H; Miyashita, C; Okada, E; Ito, YM; Araki, A; Kishi, R. (2020). Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environ Int 136: 105355. http://dx.doi.org/10.1016/j.envint.2019.105355.

Kaur, K; Lesseur, C; Chen, L; Andra, SS; Narasimhan, S; Pulivarthi, D; Midya, V; Ma, Y; Ibroci, E; Gigase, F; Lieber, M; Lieb, W; Janevic, T; De Witte, LD; Bergink, V; Rommel, AS; Chen, J. (2023). Cross-sectional associations of maternal PFAS exposure on SARS-CoV-2 IgG antibody levels during pregnancy. Environ Res 219: 115067. http://dx.doi.org/10.1016/j.envres.2022.115067.

Kim, JH; Park, HY; Jeon, JD; Kho, Y; Kim, SK; Park, MS; Hong, YC. (2015). The modifying effect of vitamin C on the association between perfluorinated compounds and insulin resistance in the Korean elderly: a double-blind, randomized, placebo-controlled crossover trial. Eur J Nutr 55: 1011-1020. http://dx.doi.org/10.1007/s00394-015-0915-0.

Kim, JI; Kim, BN; Lee, YA; Shin, CH; Hong, YC; Døssing, LD; Hildebrandt, G; Lim, YH. (2023a). Association between early-childhood exposure to perfluoroalkyl substances and ADHD symptoms: A prospective cohort study. Sci Total Environ 879: 163081. http://dx.doi.org/10.1016/j.scitotenv.2023.163081.

Kim, OJ; Kim, S; Park, EY; Oh, JK; Jung, SK; Park, S; Hong, S; Jeon, HL; Kim, HJ; Park, B; Park, B; Kim, S; Kim, B. (2023b). Exposure to serum perfluoroalkyl substances and biomarkers of liver function: The

Korean national environmental health survey 2015-2017. Chemosphere 322: 138208. http://dx.doi.org/10.1016/j.chemosphere.2023.138208.

Kim, SJ; Heo, SH; Lee, DS; Hwang, IG; Lee, YB; Cho, HY. (2016). Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. Food Chem Toxicol 97: 243-255. http://dx.doi.org/10.1016/j.fct.2016.09.017.

Kim, SJ; Shin, H; Lee, YB; Cho, HY. (2018). Sex-specific risk assessment of PFHxS using a physiologically based pharmacokinetic model. Arch Toxicol 92: 1113-1131. http://dx.doi.org/10.1007/s00204-017-2116-5.

Kruschke, JK. (2021). Bayesian analysis reporting guidelines. Nat Hum Behav 5: 1282-1291. http://dx.doi.org/10.1038/s41562-021-01177-7.

Kwon, EJ; Shin, JS; Kim, BM; Shah-Kulkarni, S; Park, H; Kho, YL; Park, EA; Kim, YJ; Ha, EH. (2016). Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med 58: e198-e205. http://dx.doi.org/10.1097/JOM.0000000000000739.

Larsen, A. (2022). R markdown code for the meta-analysis of PFHxS on birthweight [Computer Program].

Lee, E; Kinninger, A; Ursin, G; Tseng, C; Hurley, S; Wang, M; Wang, Y; Park, JS; Petreas, M; Deapen, D; Reynolds, P. (2020). Serum levels of commonly detected persistent organic pollutants and per- and polyfluoroalkyl substances (PFASs) and mammographic density in postmenopausal women. Int J Environ Res Public Health 17: 606. http://dx.doi.org/10.3390/ijerph17020606.

Lee, I; Viberg, H. (2013). A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology 37: 190-196. http://dx.doi.org/10.1016/j.neuro.2013.05.007.

Lenters, V; Portengen, L; Rignell-Hydbom, A; Jönsson, BA; Lindh, CH; Piersma, AH; Toft, G; Bonde, JP; Heederik, D; Rylander, L; Vermeulen, R. (2016). Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect 124: 365-372. http://dx.doi.org/10.1289/ehp.1408933.

Li, A; Hou, J; Fu, J; Wang, Y; Hu, Y; Zhuang, T; Li, M; Song, M; Jiang, G. (2023a). Association between serum levels of TSH and free T4 and per- and polyfluoroalkyl compounds concentrations in pregnant women. J Environ Sci 124: 11-18. http://dx.doi.org/10.1016/j.jes.2021.10.026.

Li, B; Bao, Y; Xu, Y; Xie, S; Huang, J. (2017a). Vertical distribution of microbial communities in soils contaminated by chromium and perfluoroalkyl substances. Sci Total Environ 599-600: 156-164. http://dx.doi.org/10.1016/j.scitotenv.2017.04.241.

Li, H; Chen, J; Jingchao, L; Yang, J; Tan, Z; Li, L; Xiao, F; An, Z; Ma, C; Liu, Y; Wang, L; Zhang, X; Guo, H. (2023b). Association of exposure to perfluoroalkyl substances and risk of the acute coronary syndrome: A case-control study in Shijiazhuang Hebei Province. Chemosphere 313: 137464. http://dx.doi.org/10.1016/j.chemosphere.2022.137464.

Li, J; Yang, L; He, G; Wang, B; Miao, M; Ji, H; Wen, S; Cao, W; Yuan, W; Liang, H. (2022a). Association between prenatal exposure to perfluoroalkyl substances and anogenital distance in female neonates. Ecotoxicol Environ Saf 245: 114130. http://dx.doi.org/10.1016/j.ecoenv.2022.114130.

Li, J; Yao, J; Xia, W; Dai, J; Liu, H; Pan, Y; Xu, S; Lu, S; Jin, S; Li, Y; Sun, X; Zhang, B; Zheng, T; Jiang, Y; Jing, T. (2020). Association between exposure to per- and polyfluoroalkyl substances and blood glucose in pregnant women. Int J Hyg Environ Health 230: 113596. http://dx.doi.org/10.1016/j.ijheh.2020.113596.

Li, K; Gao, P; Xiang, P; Zhang, X; Cui, X; Ma, LQ. (2017b). Molecular mechanisms of PFOA-induced toxicity in animals and humans: Implications for health risks [Review]. Environ Int 99: 43-54. http://dx.doi.org/10.1016/j.envint.2016.11.014.

Li, M; Zeng, XW; Qian, ZM; Vaughn, MG; Sauvé, S; Paul, G; Lin, S; Lu, L; Hu, LW; Yang, BY; Zhou, Y; Qin, XD; Xu, SL; Bao, WW; Zhang, YZ; Yuan, P; Wang, J; Zhang, C; Tian, YP; Nian, M; Xiao, X; Fu, C;

Dong, GH. (2017c). Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environ Int 102: 1-8. http://dx.doi.org/10.1016/j.envint.2017.03.006.

Li, QQ; Huang, J; Cai, D; Chou, WC; Zeeshan, M; Chu, C; Zhou, Y; Lin, L; Ma, HM; Tang, C; Kong, M; Xie, Y; Dong, GH; Zeng, XW. (2023c). Prenatal exposure to legacy and alternative per- and polyfluoroalkyl substances and neuropsychological development trajectories over the first 3 years of life. Environ Sci Technol 57: 3746-3757. http://dx.doi.org/10.1021/acs.est.2c07807.

Li, X; Song, F; Liu, X; Shan, A; Huang, Y; Yang, Z; Li, H; Yang, Q; Yu, Y; Zheng, H; Cao, XC; Chen, D; Chen, KX; Chen, X; Tang, NJ. (2022b). Perfluoroalkyl substances (PFASs) as risk factors for breast cancer: a case-control study in Chinese population. Environ Health 21: 83. http://dx.doi.org/10.1186/s12940-022-00895-3.

Li, Y; Andersson, A; Xu, Y; Pineda, D; Nilsson, CA; Lindh, CH; Jakobsson, K; Fletcher, T. (2022c). Determinants of serum half-lives for linear and branched perfluoroalkyl substances after long-term high exposure—A study in Ronneby, Sweden. Environ Int 163: 107198. http://dx.doi.org/10.1016/j.envint.2022.107198.

Liang, JL; Tiwari, T; Moro, P; Messonnier, NE; Reingold, A; Sawyer, M; Clark, TA. (2018). Prevention of pertussis, tetanus, and diphtheria with vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 67: 1-44. http://dx.doi.org/10.15585/mmwr.rr6702a1.

Liang, Y; Zhou, H; Zhang, J; Li, S; Shen, W; Lei, L. (2023). Exposure to perfluoroalkyl and polyfluoroalkyl substances and estimated glomerular filtration rate in adults: a cross-sectional study based on NHANES (2017-2018). Environ Sci Pollut Res Int. http://dx.doi.org/10.1007/s11356-023-26384-9.

Liao, Q; Tang, P; Fan, H; Song, Y; Liang, J; Huang, H; Pan, D; Mo, M; Leilei; Lin, M; Chen, J; Wei, H; Long, J; Shao, Y; Zeng, X; Liu, S; Huang, D; Qiu, X. (2023). Association between maternal exposure to per- and polyfluoroalkyl substances and serum markers of liver function during pregnancy in China: A mixture-based approach. Environ Pollut 323: 121348. http://dx.doi.org/10.1016/j.envpol.2023.121348.

Liao, Q; Tang, P; Pan, D; Song, Y; Lei, L; Liang, J; Liu, B; Lin, M; Huang, H; Mo, M; Huang, C; Wei, M; Liu, S; Huang, D; Qiu, X. (2022a). Association of serum per- and polyfluoroalkyl substances and gestational anemia during different trimesters in Zhuang ethnic pregnancy women of Guangxi, China. Chemosphere 309: 136798. http://dx.doi.org/10.1016/j.chemosphere.2022.136798.

Liao, Q; Tang, P; Song, Y; Liu, B; Huang, H; Liang, J; Lin, M; Shao, Y; Liu, S; Pan, D; Huang, D; Qiu, X. (2022b). Association of single and multiple prefluoroalkyl substances exposure with preterm birth: Results from a Chinese birth cohort study. Chemosphere 307: 135741. http://dx.doi.org/10.1016/j.chemosphere.2022.135741.

Liew, Z; Luo, J; Nohr, EA; Bech, BH; Bossi, R; Arah, OA; Olsen, J. (2020). Maternal plasma perfluoroalkyl substances and miscarriage: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect 128: 47007. http://dx.doi.org/10.1289/EHP6202.

Lin, M; Liao, Q; Tang, P; Song, Y; Liang, J, un; Li, J; Mu, C; Liu, S; Qiu, X; Yi, R, ui; Pang, Q; Pan, D; Zeng, X; Huang, D. (2022). Association of maternal perfluoroalkyl substance exposure with postpartum haemorrhage in Guangxi, China. Ecotoxicol Environ Saf 245: 114078. http://dx.doi.org/10.1016/j.ecoenv.2022.114078.

Linakis, MW; Landingham, CW; Longnecker, MP. Re-expressing coefficients from regression models for inclusion in a meta-analysis. Linakis, MW; Landingham, CW; Longnecker, MP. http://dx.doi.org/10.1101/2021.11.02.466931.

Lind, DV; Priskorn, L; Lassen, TH; Nielsen, F; Kyhl, HB; Kristensen, DM; Christesen, HT; Jørgensen, JS; Grandjean, P; Jensen, TK. (2017). Prenatal exposure to perfluoroalkyl substances and anogenital

distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 68: 200-206. http://dx.doi.org/10.1016/j.reprotox.2016.08.019.

Lind, PM; Salihovic, S; Stubleski, J; Kärrman, A; Lind, L. (2018). Changes in plasma levels of perfluoroalkyl substances (PFASs) are related to increase in carotid intima-media thickness over 10 years - a longitudinal study. Environ Health 17: 59. http://dx.doi.org/10.1186/s12940-018-0403-0.

Liu, H; Pan, Y; Jin, S; Sun, X; Jiang, Y; Wang, Y; Ghassabian, A; Li, Y; Xia, W; Cui, Q; Zhang, B; Zhou, A; Dai, J; Xu, S. (2020a). Associations between six common per- and polyfluoroalkyl substances and estrogens in neonates of China. J Hazard Mater 407: 124378. http://dx.doi.org/10.1016/j.jhazmat.2020.124378.

Liu, J; Gao, X; Wang, Y; Leng, J; Li, J; Zhao, Y; Wu, Y. (2020b). Profiling of emerging and legacy per-/polyfluoroalkyl substances in serum among pregnant women in China. Environ Pollut 271: 116376. http://dx.doi.org/10.1016/j.envpol.2020.116376.

Liu, Y; Zhang, Z; Han, D; Zhao, Y; Yan, X; Cui, S. (2022). Association between environmental chemicals co-exposure and peripheral blood immune-inflammatory indicators. Front Public Health 10: 980987. http://dx.doi.org/10.3389/fpubh.2022.980987.

Luo, D; Wu, WX; Pan, YA; Du, BB; Shen, MJ; Zeng, LX. (2021). Associations of prenatal exposure to per- and polyfluoroalkyl substances with the neonatal birth size and hormones in the growth hormone/insulin-like growth factor axis. Environ Sci Technol 55: 11859-11873. http://dx.doi.org/10.1021/acs.est.1c02670.

Luo, F; Chen, Q; Yu, G; Huo, X; Wang, H; Nian, M; Tian, Y; Xu, J; Zhang, J; Zhang, J. (2022a). Exposure to perfluoroalkyl substances and neurodevelopment in 2-year-old children: A prospective cohort study. Environ Int 166: 107384. http://dx.doi.org/10.1016/j.envint.2022.107384.

Luo, K; Huang, W; Zhang, Q; Liu, X; Nian, M; Wei, M; Wang, Y; Chen, D; Chen, X; Zhang, J. (2022b). Environmental exposure to legacy poly/perfluoroalkyl substances, emerging alternatives and isomers and semen quality in men: A mixture analysis. Sci Total Environ 833: 155158. http://dx.doi.org/10.1016/j.scitotenv.2022.155158.

Luo, K; Liu, X; Zhou, W; Nian, M; Qiu, W; Yang, Y; Zhang, J. (2022c). Preconception exposure to perfluoroalkyl and polyfluoroalkyl substances and couple fecundity: A couple-based exploration. Environ Int 170: 107567. http://dx.doi.org/10.1016/j.envint.2022.107567.

Ma, X; Cui, L; Chen, L; Zhang, J; Zhang, X; Kang, Q; Jin, F; Ye, Y. (2021). Parental plasma concentrations of perfluoroalkyl substances and In Vitro fertilization outcomes. Environ Pollut 269: 116159. http://dx.doi.org/10.1016/j.envpol.2020.116159.

Maekawa, R; Ito, R; Iwasaki, Y; Saito, K; Akutsu, K; Takatori, S; Ishii, R; Kondo, F; Arai, Y; Ohgane, J; Shiota, K; Makino, T; Sugino, N. (2017). Evidence of exposure to chemicals and heavy metals during pregnancy in Japanese women. Reproductive Medicine and Biology 16: 337-348. http://dx.doi.org/10.1002/rmb2.12049.

Maisonet, M; Terrell, ML; Mcgeehin, MA; Christensen, KY; Holmes, A; Calafat, AM; Marcus, M. (2012). Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect 120: 1432-1437. http://dx.doi.org/10.1289/ehp.1003096.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D; Costa, O; Santa-Marina, L; Pereda-Pereda, E; Schettgen, T; Sunyer, J; Vrijheid, M. (2017). Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environ Int 108: 278-284. http://dx.doi.org/10.1016/j.envint.2017.09.006.

Manzano-Salgado, CB; Granum, B; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Gascón, M; Martínez, D; Guxens, M; Basterretxea, M; Zabaleta, C; Schettgen, T; Sunyer, J; Vrijheid, M; Casas, M. (2019). Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in

children from a Spanish birth cohort study. Int J Hyg Environ Health 222: 945-954. http://dx.doi.org/10.1016/j.ijheh.2019.06.005.

Marks, KJ; Cutler, AJ; Jeddy, Z; Northstone, K; Kato, K; Hartman, TJ. (2019a). Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. Int J Hyg Environ Health 222: 889-895. http://dx.doi.org/10.1016/j.ijheh.2019.03.008.

Marks, KJ; Jeddy, Z; Flanders, WD; Northstone, K; Fraser, A; Calafat, AM; Kato, K; Hartman, TJ. (2019b). Maternal serum concentrations of perfluoroalkyl substances during pregnancy and gestational weight gain: The Avon Longitudinal Study of Parents and Children. Reprod Toxicol 90: 8-14. http://dx.doi.org/10.1016/j.reprotox.2019.08.003.

Meek, ME; Boobis, A; Cote, I; Dellarco, V; Fotakis, G; Munn, S; Seed, J; Vickers, C. (2014). New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis [Review]. J Appl Toxicol 34: 1-18. http://dx.doi.org/10.1002/jat.2949.

Mehta, SS; James-Todd, T; Applebaum, KM; Bellavia, A; Coleman-Phox, K; Adler, N; Laraia, B; Epel, E; Parry, E; Wang, M; Park, JS; Zota, AR. (2021). Persistent organic pollutants and maternal glycemic outcomes in a diverse pregnancy cohort of overweight women. Environ Res 193: 110551. http://dx.doi.org/10.1016/j.envres.2020.110551.

Mellor, CL; Steinmetz, FP; Cronin, MT. (2016). The identification of nuclear receptors associated with hepatic steatosis to develop and extend adverse outcome pathways [Review]. Crit Rev Toxicol 46: 138-152. http://dx.doi.org/10.3109/10408444.2015.1089471.

Meng, Q; Inoue, K; Ritz, B; Olsen, J; Liew, Z. (2018). Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the danish national birth cohort. Int J Environ Res Public Health 15: 1832. http://dx.doi.org/10.3390/ijerph15091832.

Mitro, SD; Sagiv, SK; Fleisch, AF; Jaacks, LM; Williams, PL; Rifas-Shiman, SL; Calafat, AM; Hivert, MF; Oken, E; James-Todd, TM. (2020). Pregnancy per- and polyfluoroalkyl substance concentrations and postpartum health in project viva: A prospective cohort. J Clin Endocrinol Metab 105: e3415–e3426. http://dx.doi.org/10.1210/clinem/dgaa431.

Monroy, R; Morrison, K; Teo, K; Atkinson, S; Kubwabo, C; Stewart, B; Foster, WG. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res 108: 56-62. http://dx.doi.org/10.1016/j.envres.2008.06.001.

Morgan, S; Mottaleb, MA; Kraemer, MP; Moser, DK; Worley, J; Morris, AJ; Petriello, MC. (2023). Effect of lifestyle-based lipid lowering interventions on the relationship between circulating levels of per- and polyfluoroalkyl substances and serum cholesterol. Environ Toxicol Pharmacol 98: 104062. http://dx.doi.org/10.1016/j.etap.2023.104062.

Morken, NH; Travlos, GS; Wilson, RE; Eggesbø, M; Longnecker, MP. (2014). Maternal glomerular filtration rate in pregnancy and fetal size. PLoS ONE 9: e101897. http://dx.doi.org/10.1371/journal.pone.0101897.

Nian, M; Luo, K; Luo, F; Aimuzi, R; Huo, X; Chen, Q; Tian, Y; Zhang, J. (2020). Association between prenatal exposure to PFAS and fetal sex hormones: Are the short-chain PFAS safer? Environ Sci Technol 54: 8291-8299. http://dx.doi.org/10.1021/acs.est.0c02444.

Nilsson, S; Smurthwaite, K; Aylward, LL; Kay, M; Toms, LM; King, L; Marrington, S; Barnes, C; Kirk, MD; Mueller, JF; Bräunig, J. (2022a). Serum concentration trends and apparent half-lives of per- and polyfluoroalkyl substances (PFAS) in Australian firefighters. Int J Hyg Environ Health 246: 114040. http://dx.doi.org/10.1016/j.ijheh.2022.114040.

Nilsson, S; Smurthwaite, K; Aylward, LL; Kay, M; Toms, LM; King, L; Marrington, S; Kirk, MD; Mueller, JF; Bräunig, J. (2022b). Associations between serum perfluoroalkyl acid (PFAA) concentrations and health related biomarkers in firefighters. Environ Res 215: 114370. http://dx.doi.org/10.1016/j.envres.2022.114370.

NJDWQI (New Jersey Drinking Water Quality Institute). (2017). Public review draft: Health-based maximum contaminant level support document: Perfluorooctane sulfonate (PFOS) (CAS #: 1763-23-1; Chemical Formula: C8HF17O3S). NJDWQI Health Effects Subcommittee. https://cswab.org/wp-content/uploads/2018/09/New-Jersey-Scientific-Documentation-Supporting-PFOS-13-ppt.pdf.

NTP (National Toxicology Program). (2018). 28-day evaluation of the toxicity (C06100) of perfluorohexane sulfonate potassium salt (PFHSKslt) (3871-99-6) on Harlan Sprague-Dawley rats exposed via gavage. Available online at http://doi.org/10.22427/NTP-DATA-002-03267-0002-0000-2 (accessed October 13, 2020).

NTP (National Toxicology Program). (2019). NTP technical report on the toxicity studies of perfluoroalkyl sulfonates (perfluorobutane sulfonic acid, perfluorohexane sulfonate potassium salt, and perfluorooctane sulfonic acid) administered by gavage to Sprague Dawley (Hsd:Sprague Dawley SD) rats. (Toxicity Report 96). Research Triangle Park, NC. https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox096_508.pdf.

Oh, J; Bennett, DH; Tancredi, DJ; Calafat, AM; Schmidt, RJ; Hertz-Picciotto, I; Shin, HM. (2022a). Longitudinal Changes in Maternal Serum Concentrations of Per- and Polyfluoroalkyl Substances from Pregnancy to Two Years Postpartum. Environ Sci Technol 56: 11449-11459. http://dx.doi.org/10.1021/acs.est.1c07970.

Oh, J; Shin, HM; Kannan, K; Busgang, SA; Schmidt, RJ; Schweitzer, JB; Hertz-Picciotto, I; Bennett, DH. (2022b). Childhood exposure to per- and polyfluoroalkyl substances and neurodevelopment in the CHARGE case-control study. Environ Res 215: 114322. http://dx.doi.org/10.1016/j.envres.2022.114322.

Ouidir, M; Buck Louis, GM; Kanner, J; Grantz, KL; Zhang, C; Sundaram, R; Rahman, ML; Lee, S; Kannan, K; Tekola-Ayele, F; Mendola, P. (2020). Association of maternal exposure to persistent organic pollutants in early pregnancy with fetal growth. JAMA Pediatr 174: 149-161. http://dx.doi.org/10.1001/jamapediatrics.2019.5104.

Oulhote, Y; Coull, B; Bind, MA; Debes, F; Nielsen, F; Tamayo, I; Weihe, P; Grandjean, P. (2019). Joint and independent neurotoxic effects of early life exposures to a chemical mixture: A multi-pollutant approach combining ensemble learning and g-computation. Environmental Epidemiology 3: e063. http://dx.doi.org/10.1097/ee9.0000000000000063.

Padula, AM; Ning, X; Bakre, S; Barrett, ES; Bastain, T; Bennett, DH; Bloom, MS; Breton, CV; Dunlop, AL; Eick, SM; Ferrara, A; Fleisch, A; Geiger, S; Goin, DE; Kannan, K; Karagas, MR; Korrick, S; Meeker, JD; Morello-Frosch, R. (2023). Birth outcomes in relation to prenatal exposure to per-and polyfluoroalkyl substances and stress in the environmental influences on child health outcomes (echo) program [Supplemental Data]. Environ Health Perspect 131: (037006) 037001-037011. http://dx.doi.org/10.1289/EHP10723.

Pan, Z; Guo, Y; Zhou, Q; Wang, Q; Pan, S; Xu, S; Li, L. (2023). Perfluoroalkyl substance exposure is associated with asthma and innate immune cell count in US adolescents stratified by sex. Environ Sci Pollut Res Int 30: 52535-52548. http://dx.doi.org/10.1007/s11356-023-26065-7.

Park, SK; Wang, X; Ding, N; Karvonen-Gutierrez, CA; Calafat, AM; Herman, WH; Mukherjee, B; Harlow, SD. (2022). Per- and polyfluoroalkyl substances and incident diabetes in midlife women: the Study of Women's Health Across the Nation (SWAN). Diabetologia 65: 1157-1168. http://dx.doi.org/10.1007/s00125-022-05695-5.

Passen, EL; Andersen, BR. (1986). Clinical tetanus despite a protective level of toxin-neutralizing antibody [Case Report]. JAMA 255: 1171-1173. http://dx.doi.org/10.1001/jama.1986.03370090093029.

Patel, JC; Mehta, BC. (1999). Tetanus: Study of 8,697 cases. Indian J Med Sci 53: 393-401.

Peterson, AK; Eckel, SP; Habre, R; Yang, T; Faham, D; Amin, M; Grubbs, BH; Farzan, SF; Kannan, K; Robinson, M; Lerner, D; Al-Marayati, LA; Walker, DK; Grant, EG; Breton, CV; Bastain, TM. (2022). Detected prenatal perfluorooctanoic acid (PFOA) exposure is associated with decreased fetal head biometric parameters in participants experiencing higher perceived stress during pregnancy in the MADRES cohort. 9. http://dx.doi.org/10.1016/j.envadv.2022.100286.

Petroff, RL; Cavalcante, RG; Langen, ES; Dolinoy, DC; Padmanabhan, V; Goodrich, JM. (2023). Mediation effects of DNA methylation and hydroxymethylation on birth outcomes after prenatal per- and polyfluoroalkyl substances (PFAS) exposure in the Michigan mother-infant Pairs cohort. Clinical Epigenetics 15: 49. http://dx.doi.org/10.1186/s13148-023-01461-5.

Pirard, C; Dufour, P; Charlier, C. (2020). Background contamination of perfluoralkyl substances in a Belgian general population. Toxicol Lett 333: 13-21. http://dx.doi.org/10.1016/j.toxlet.2020.07.015.

Porter, AK; Kleinschmidt, SE; Andres, KL; Reusch, CN; Krisko, RM; Taiwo, OA; Olsen, GW; Longnecker, MP. (2022). Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances. Environ Int 169: 107537. http://dx.doi.org/10.1016/j.envint.2022.107537.

Ramhøj, L; Hass, U; Boberg, J; Scholze, M; Christiansen, S; Nielsen, F; Axelstad, M. (2018). Perfluorohexane sulfonate (PFHxS) and a mixture of endocrine disrupters reduce thyroxine levels and cause anti-androgenic effects in rats. Toxicol Sci 163: 579-591. http://dx.doi.org/10.1093/toxsci/kfy055.

Ramhøj, L; Hass, U; Gilbert, ME; Wood, C; Svingen, T; Usai, D; Vinggaard, AM; Mandrup, K; Axelstad, M. (2020). Evaluating thyroid hormone disruption: investigations of long-term neurodevelopmental effects in rats after perinatal exposure to perfluorohexane sulfonate (PFHxS). Sci Rep 10: 2672. http://dx.doi.org/10.1038/s41598-020-59354-z.

Rantakokko, P; Männistö, V; Airaksinen, R; Koponen, J; Viluksela, M; Kiviranta, H; Pihlajamäki, J. (2015). Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: A cohort study. Environ Health 14: 79. http://dx.doi.org/10.1186/s12940-015-0066-z.

Reyes, L; Mañalich, R. (2005). Long-term consequences of low birth weight [Review]. Kidney Int Suppl 68: S107-S111. http://dx.doi.org/10.1111/j.1523-1755.2005.09718.x.

Rivera-Núñez, Z; Kinkade, CW; Khoury, L; Brunner, J; Murphy, H; Wang, C; Kannan, K; Miller, RK; O'Connor, TG; Barrett, ES. (2023). Prenatal perfluoroalkyl substances exposure and maternal sex steroid hormones across pregnancy. Environ Res 220: 115233. http://dx.doi.org/10.1016/j.envres.2023.115233.

Sagiv, SK; Rifas-Shiman, SL; Fleisch, AF; Webster, TF; Calafat, AM; Ye, X; Gillman, MW; Oken, E. (2018). Early Pregnancy Perfluoroalkyl Substance Plasma Concentrations and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? Am J Epidemiol 187: 793-802. http://dx.doi.org/10.1093/aje/kwx332.

Salvatier, J; Wiecki, TV; Fonnesbeck, C. (2016). Probabilistic programming in Python using PyMC3. PeerJ Computer Science 2: e55. http://dx.doi.org/10.7717/peerj-cs.55.

Sanghavi, M; Rutherford, JD. (2014). Cardiovascular physiology of pregnancy. Circulation 130: 1003-1008. http://dx.doi.org/10.1161/CIRCULATIONAHA.114.009029.

Schillemans, T; Iszatt, N; Remy, S; Schoeters, G; Fernández, MF; D'Cruz, SC; Desalegn, A; Haug, LS; Lignell, S; Lindroos, AK; Fábelová, L; Murinova, LP; Kosjek, T; Tkalec, Ž; Gabriel, C; Sarigiannis, D; Pedraza-Díaz, S; Esteban-López, M; Castaño, A; ... Åkesson, A. (2022). Cross-sectional associations between exposure to per- and polyfluoroalkyl substances and body mass index among European teenagers in the HBM4EU aligned studies. Environ Pollut 316: 120566. http://dx.doi.org/10.1016/j.envpol.2022.120566.

Selgrade, MK. (2007). Immunotoxicity: The risk is real [Review]. Toxicol Sci 100: 328-332. http://dx.doi.org/10.1093/toxsci/kfm244.

Shearer, JJ; Callahan, CL; Calafat, AM; Huang, WY; Jones, RR; Sabbisetti, VS; Freedman, ND; Sampson, JN; Silverman, DT; Purdue, MP; Hofmann, JN. (2021). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. J Natl Cancer Inst 113: 580-587. http://dx.doi.org/10.1093/jnci/djaa143.

Shi, S; Ding, Y; Wu, B; Hu, P; Chen, M; Dong, N; Vinturache, A; Gu, H; Dong, X; Ding, G. (2023). Association of perfluoroalkyl substances with pulmonary function in adolescents (NHANES 2007-2012). Environ Sci Pollut Res Int. http://dx.doi.org/10.1007/s11356-023-26119-w.

Shi, Y; Yang, L; Li, J; Lai, J; Wang, Y; Zhao, Y; Wu, Y. (2017). Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 169: 396-402. http://dx.doi.org/10.1016/j.chemosphere.2016.11.050.

Shiue, I. (2015a). Arsenic, heavy metals, phthalates, pesticides, hydrocarbons and polyfluorinated compounds but not parabens or phenols are associated with adult remembering condition: US NHANES, 2011-2012. Environ Sci Pollut Res Int 22: 6381-6386. http://dx.doi.org/10.1007/s11356-015-4261-9.

Shiue, I. (2015b). Urinary heavy metals, phthalates and polyaromatic hydrocarbons independent of health events are associated with adult depression: USA NHANES, 2011-2012. Environ Sci Pollut Res Int 22: 17095-17103. http://dx.doi.org/10.1007/s11356-015-4944-2.

Shiue, I. (2015c). Urinary heavy metals, phthalates, perchlorate, nitrate, thiocyanate, hydrocarbons, and polyfluorinated compounds are associated with adult hearing disturbance: USA NHANES, 2011-2012. Environ Sci Pollut Res Int 22: 20306-20311. http://dx.doi.org/10.1007/s11356-015-5546-8.

Shiue, I. (2015d). Urinary heavy metals, phthalates, phenols, thiocyanate, parabens, pesticides, polyaromatic hydrocarbons but not arsenic or polyfluorinated compounds are associated with adult oral health: USA NHANES, 2011-2012. Environ Sci Pollut Res Int 22: 15636-15645. http://dx.doi.org/10.1007/s11356-015-4749-3.

Shoaff, J; Papandonatos, GD; Calafat, AM; Chen, A; Lanphear, BP; Ehrlich, S; Kelsey, KT; Braun, JM. (2018). Prenatal exposure to perfluoroalkyl substances: Infant birth weight and early life growth. Environmental Epidemiology 2: e010. http://dx.doi.org/10.1097/EE9.0000000000000010.

Shoaff, JR. (2018). [Email to Michael Wright in response to a question about Shoaff et al. 2018]. Available online at (accessed

Sood, S; Ojo, AO; Adu, D; Kannan, K; Ghassabian, A; Koshy, T; Vento, SM; Pehrson, LJ; Gilbert, JF; Arogundade, FA; Ademola, AD; Salako, BO; Raji, Y; Osafo, C; Antwi, S; Trachtman, H; Trasande, L; Ajayi, S; Burke, D; Cooper, R; Gbadegesin, R; Ilori, T; Mamven, M; Olanrewaju, T; Parekh, R; Rhule, J; Salako, T; Tayo, B; Ulasi, I; Investigators, HAKDRN. (2019). Association Between Perfluoroalkyl Substance Exposure and Renal Function in Children With CKD Enrolled in H3Africa Kidney Disease Research Network. 4: 1641-1645. http://dx.doi.org/10.1016/j.ekir.2019.07.017.

Starling, AP; Adgate, JL; Hamman, RF; Kechris, K; Calafat, AM; Ye, X; Dabelea, D. (2017). Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: Examining mediation by maternal fasting glucose in the healthy start study. Environ Health Perspect 125: 067016. http://dx.doi.org/10.1289/EHP641.

Steenland, K; Barry, V; Savitz, D. (2018). Serum perfluorooctanoic acid and birthweight: an updated meta-analysis with bias analysis. Epidemiology 29: 765-776. http://dx.doi.org/10.1097/EDE.0000000000000903.

Sundström, M; Chang, SC; Noker, PE; Gorman, GS; Hart, JA; Ehresman, DJ; Bergman, A; Butenhoff, JL. (2012). Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and monkeys. Reprod Toxicol 33: 441-451. http://dx.doi.org/10.1016/j.reprotox.2011.07.004.

Takacs, ML; Abbott, BD. (2007). Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicol Sci 95: 108-117. http://dx.doi.org/10.1093/toxsci/kfl135.

Tan, Y; Zeng, Z; Liang, H; Weng, X; Yao, H; Fu, Y; Li, Y; Chen, J; Wei, X; Jing, C. (2022). Association between Perfluoroalkyl and Polyfluoroalkyl Substances and Women's Infertility, NHANES 2013-2016. Int J Environ Res Public Health 19. http://dx.doi.org/10.3390/ijerph192215348.

Tian, M, .; Reichetzeder, C, .; Li, J, .; Hocher, B, . (2019). Low birth weight, a risk factor for diseases in later life, is a surrogate of insulin resistance at birth. J Hypertens 37: 2123-2134. http://dx.doi.org/10.1097/HJH.0000000000002156.

Tillaut, H; Monfort, C; Giton, F; Warembourg, C; Rouget, F; Cordier, S; Laine, F; Gaudreau, E; Garlantezec, R; Saint-Amour, D; Chevrier, C. (2022). Persistent organic pollutant exposure and thyroid function among 12-year-old children. Neuroendocrinology. http://dx.doi.org/10.1159/000528631.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2012). Benchmark dose technical guidance [EPA Report]. (EPA100R12001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance.

U.S. EPA (U.S. Environmental Protection Agency). (2016a). Health effects support document for perfluorooctane sulfonate (PFOS) [EPA Report]. (EPA 822-R-16-002). Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division. https://www.epa.gov/sites/production/files/2016-05/documents/pfos_hesd_final_508.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2016b). Health effects support document for perfluorooctanoic acid (PFOA) [EPA Report]. (EPA 822-R-16-003). Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2019). CompTox Chemicals Dashboard [Database]. Research Triangle Park, NC. Retrieved from https://comptox.epa.gov/dashboard

Valvi, D; Oulhote, Y; Weihe, P; Dalgård, C; Bjerve, KS; Steuerwald, U; Grandjean, P. (2017). Gestational diabetes and offspring birth size at elevated environmental pollutant exposures. Environ Int 107: 205-215. http://dx.doi.org/10.1016/j.envint.2017.07.016.

van Larebeke, N; Koppen, G; Decraemer, S, am; Colles, A, nn; Bruckers, L; Den Hond, E; Govarts, E, va; Morrens, B; Schettgen, T; Remy, S; Coertjens, D; Nawrot, T, im; Nelen, V; Baeyens, W; Schoeters, G. (2022). Per- and polyfluoroalkyl substances (PFAS) and neurobehavioral function and cognition in adolescents (2010-2011) and elderly people (2014): results from the Flanders Environment and Health Studies (FLEHS). Environ Sci Eur 34. http://dx.doi.org/10.1186/s12302-022-00675-3.

Verner, MA; Ngueta, G; Jensen, ET; Fromme, H; Vöelkel, W; Nygaard, UC; Granum, B; Longnecker, MP. (2016). A simple pharmacokinetic model of prenatal and postnatal exposure to perfluoroalkyl substances (PFASs). Environ Sci Technol 50: 978-986. http://dx.doi.org/10.1021/acs.est.5b04399.

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. J Stat Softw 36: 1-48.

Wahlang, B; Beier, JI; Clair, HB; Bellis-Jones, HJ; Falkner, K; Mcclain, CJ; Cave, MC. (2013). Toxicant-associated steatohepatitis [Review]. Toxicol Pathol 41: 343-360. http://dx.doi.org/10.1177/0192623312468517.

Wang, B; Chen, Q; Shen, L; Zhao, S; Pang, W; Zhang, J. (2016). Perfluoroalkyl and polyfluoroalkyl substances in cord blood of newborns in Shanghai, China: Implications for risk assessment [Review]. Environ Int 97: 7-14. http://dx.doi.org/10.1016/j.envint.2016.10.008.

Wang, H; Li, W; Yang, J; Wang, Y; Du, H; Han, M; Xu, L; Liu, S; Yi, J; Chen, Y; Jiang, Q; He, G. (2022). Gestational exposure to perfluoroalkyl substances is associated with placental DNA methylation and birth size. Sci Total Environ 858: 159747. http://dx.doi.org/10.1016/j.scitotenv.2022.159747.

Wang, W; Zhou, W; Wu, S; Liang, F; Li, Y; Zhang, J; Cui, L; Feng, Y; Wang, Y. (2019). Perfluoroalkyl substances exposure and risk of polycystic ovarian syndrome related infertility in Chinese women. Environ Pollut 247: 824-831. http://dx.doi.org/10.1016/j.envpol.2019.01.039.

Watanabe, S. (2010). Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. J Mach Learn Res 11: 3571-3594.

Weisskopf, MG; Seals, RM; Webster, TF. (2018). Bias amplification in epidemiologic analysis of exposure to mixtures. Environ Health Perspect 126. http://dx.doi.org/10.1289/EHP2450.

Weisskopf, MG; Webster, TF. (2017). Trade-offs of personal versus more proxy exposure measures in environmental epidemiology. Epidemiology 28: 635-643. http://dx.doi.org/10.1097/EDE.0000000000000686.

Whitworth, KW; Haug, LS; Baird, DD; Becher, G; Hoppin, JA; Eggesbo, M; Skjaerven, R; Thomsen, C; Longnecker, MP. (2012). P60—Preliminary results on subfecundity and plasma concentrations of perfluorinated compounds during pregnancy in the Norwegian Mother and Child Cohort Study, 2002–2004. Reprod Toxicol 33: 620-621. http://dx.doi.org/10.1016/j.reprotox.2011.11.094.

Whitworth, KW; Haug, LS; Sabaredzovic, A; Eggesbo, M; Longnecker, MP. (2016). Brief Report: Plasma Concentrations of Perfluorooctane Sulfonamide and Time-to-pregnancy Among Primiparous Women. Epidemiology 27: 712-715. http://dx.doi.org/10.1097/EDE.0000000000000524.

Wielsøe, M; Eiberg, H; Ghisari, M; Kern, P; Lind, O; Bonefeld-Jørgensen, EC. (2018). Genetic variations, exposure to persistent organic pollutants and breast cancer risk - a greenlandic case-control study. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 123: 335-346. http://dx.doi.org/10.1111/bcpt.13002.

Wielsøe, M; Kern, P; Bonefeld-Jørgensen, EC. (2017). Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health 16: 56. http://dx.doi.org/10.1186/s12940-017-0269-6.

Wikström, S; Lin, PI; Lindh, CH; Shu, H; Bornehag, CG. (2020). Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87: 1093-1099. http://dx.doi.org/10.1038/s41390-019-0720-1.

Woods, MM; Lanphear, BP; Braun, JM; McCandless, LC. (2017). Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: A Bayesian analysis of the HOME Study. Environ Health 16: 115. http://dx.doi.org/10.1186/s12940-017-0332-3.

Workman, CE; Becker, AB; Azad, MB; Moraes, TJ; Mandhane, PJ; Turvey, SE; Subbarao, P; Brook, JR; Sears, MR; Wong, CS. (2019). Associations between concentrations of perfluoroalkyl substances in human plasma and maternal, infant, and home characteristics in Winnipeg, Canada. Environ Pollut 249: 758-766. http://dx.doi.org/10.1016/j.envpol.2019.03.054.

Wright, JM; Larsen, A; Rappazzo, K; Ru, H; Radke, EG; Bateson, TF. (2023). Systematic review and meta-analysis of birthweight and PFNA exposures. Environ Res115357. http://dx.doi.org/10.1016/j.envres.2023.115357.

Wright, M; Larsen, A. (2022). Email correspondence with study author, Cyntia Manzano-Salgado. Available online at (accessed

Xiong, X; Chen, B; Wang, Z; Ma, L; Li, S; Gao, Y. (2022). Association between perfluoroalkyl substances concentration and bone mineral density in the US adolescents aged 12-19 years in NHANES

2005-2010. Front Endocrinol (Lausanne) 13: 980608. http://dx.doi.org/10.3389/fendo.2022.980608.

Xu, C; Yin, S; Liu, Y; Chen, F; Zhong, Z; Li, F; Liu, K; Liu, W. (2019). Prenatal exposure to chlorinated polyfluoroalkyl ether sulfonic acids and perfluoroalkyl acids: Potential role of maternal determinants and associations with birth outcomes. J Hazard Mater 380: 120867. http://dx.doi.org/10.1016/j.jhazmat.2019.120867.

Xu, C; Zhang, L; Zhou, Q; Ding, J; Yin, S; Shang, X; Tian, Y. (2022). Exposure to per- and polyfluoroalkyl substances as a risk factor for gestational diabetes mellitus through interference with glucose homeostasis. Sci Total Environ 838: 156561. http://dx.doi.org/10.1016/j.scitotenv.2022.156561.

Xu, H; Zhou, Q; Zhang, J; Chen, X; Zhao, H; Lu, H; Ma, B; Wang, Z; Wu, C; Ying, C; Xiong, Y; Zhou, Z; Li, X. (2020). Exposure to elevated per- and polyfluoroalkyl substances in early pregnancy is related to increased risk of gestational diabetes mellitus: A nested case-control study in Shanghai, China. Environ Int 143: 105952. http://dx.doi.org/10.1016/j.envint.2020.105952.

Yang, L; Ji, H; Liang, H; Yuan, W; Song, X; Li, X; Niu, J; Shi, H; Wen, S; Miao, M. (2022). Associations of perfluoroalkyl and polyfluoroalkyl substances with gestational hypertension and blood pressure during pregnancy: A cohort study. Environ Res 215: 114284. http://dx.doi.org/10.1016/j.envres.2022.114284.

Yao, J; Pan, Y; Sheng, N; Su, Z; Guo, Y; Wang, J; Dai, J. (2020). Novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs): Occurrence and association with serum biochemical parameters in residents living near a fluorochemical plant in China. Environ Sci Technol 54: 13389-13398. http://dx.doi.org/10.1021/acs.est.0c02888.

Yao, Q; Gao, Y; Zhang, Y; Qin, K; Liew, Z; Tian, Y. (2021). Associations of paternal and maternal per- and polyfluoroalkyl substances exposure with cord serum reproductive hormones, placental steroidogenic enzyme and birth weight. Chemosphere 285: 131521. http://dx.doi.org/10.1016/j.chemosphere.2021.131521.

Ye, WL; Chen, ZX; Xie, YQ; Kong, ML; Li, QQ; Yu, S; Chu, C; Dong, GH; Zeng, XW. (2021). Associations between serum isomers of perfluoroalkyl acids and metabolic syndrome in adults: Isomers of C8 Health Project in China. Environ Res 196: 110430. http://dx.doi.org/10.1016/j.envres.2020.110430.

Yu, Y; Qin, XD; Bloom, MS; Chu, C; Dai, X; Li, QQ; Chen, ZX; Kong, ML; Xie, YQ; Meng, WJ; Yang, BY; Hu, LW; Zeng, XW; Zhao, XM; Zhou, Y; Dong, GH. (2022). Associations of prenatal exposure to perfluoroalkyl substances with preterm birth: A family-based birth cohort study. Environ Res 214: 113803. http://dx.doi.org/10.1016/j.envres.2022.113803.

Zeng, X; Chen, T; Cui, Y; Zhao, J; Chen, Q; Yu, Z; Zhang, Y; Han, L; Chen, Y; Zhang, J. (2023). In utero exposure to perfluoroalkyl substances and early childhood BMI trajectories: A mediation analysis with neonatal metabolic profiles. Sci Total Environ 867: 161504. http://dx.doi.org/10.1016/j.scitotenv.2023.161504.

Zhang, JJ. (2022). RE: Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and birth outcomes: A longitudinal cohort with repeated measurements. Available online at (accessed

Zhang, Y; Chen, R; Gao, Y; Qu, J; Wang, Z; Zhao, M; Bai, X; Jin, H. (2023a). Human serum poly- and perfluoroalkyl substance concentrations and their associations with gestational diabetes mellitus. Environ Pollut 317: 120833. http://dx.doi.org/10.1016/j.envpol.2022.120833.

Zhang, Y; Mustieles, V; Sun, Y; Oulhote, Y; Wang, YX; Messerlian, C. (2022). Association between serum per- and polyfluoroalkyl substances concentrations and common cold among children and adolescents in the United States. Environ Int 164: 107239. http://dx.doi.org/10.1016/j.envint.2022.107239.

Zhang, Y; Mustieles, V; Wang, YX; Sun, Q; Coull, B; Sun, Y; Slitt, A; Messerlian, C. (2023b). Red blood cell folate modifies the association between serum per- and polyfluoroalkyl substances and

antibody concentrations in U.S. adolescents. Environ Sci Technol 57: 2445-2456. http://dx.doi.org/10.1021/acs.est.2c07152.

Zhao, X; Lin, JY; Dong, WW; Tang, ML; Yan, SG. (2022). Per- and polyfluoroalkyl substances exposure and bone mineral density in the U.S. population from NHANES 2005-2014. J Expo Sci Environ Epidemiol. http://dx.doi.org/10.1038/s41370-022-00452-7.

Zhou, Y; Li, Q; Wang, P; Li, J; Zhao, W; Zhang, L; Wang, H; Cheng, Y; Shi, H; Li, J; Zhang, Y. (2023). Associations of prenatal PFAS exposure and early childhood neurodevelopment: Evidence from the Shanghai Maternal-Child Pairs Cohort. Environ Int 173: 107850. http://dx.doi.org/10.1016/j.envint.2023.107850.

Zhu, Y; Bartell, SM. (2022). Per- and polyfluoroalkyl substances in drinking water and hypertensive disorders of pregnancy in the United States during 2013-2015. Environmental Epidemiology 6: e209. http://dx.doi.org/10.1097/EE9.0000000000000209.