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FINAL
APPENDIX: Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts

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Acronyms and Abbreviations

17-OHP	17-hydroxyprogesterone
ABC	ATP-binding cassette transporter
aBMD	areal bone mineral density
ACD	anterior chamber depth
ACE	America's Children and the Environment
ACTH	adrenocorticotropic hormone
ADHD	attention deficit hyperactivity disorder
ADME	absorption, distribution, metabolism, and excretion
AF:CB	amniotic fluid and cord blood ratio
AFFF	aqueous film forming foam
AGD	anogenital distance
AIC	Akaike information criterion
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	alanine aminotransferase
AMH	anti-Müllerian hormone
ASBT	apical sodium-dependent bile acid transporter
ASD	autism spectrum disorder
ASQ	Ages and Stages Questionnaire
ATP III	Adult Treatment Panel III
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
AUMC	area under the first moment curve
AZI	azithromycin-dihydrate
β	regression coefficients
BALF	bronchoalveolar lavage fluid
BBB	blood-brain barrier
BCERP	Breast Cancer and Environment Research Program
BCRP	breast cancer resistance protein
BD	bolus dose
BDI	Beck Depression Inventory
BDI-II	Beck Depression Inventory-II
BMC	bone mineral content
BMD	benchmark dose
BMDL	lower limit of benchmark dose
$BMDL_{0.5SD}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean
$BMDL_{1SD}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean
$BMDL_5$	lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level
$BMDL_{10}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change
BMDS	Benchmark Dose Software

BMI	body mass index
BMR	benchmark response
BRIEF	Behavior Rating Inventory of Executive Function
BUN	blood urea nitrogen
BW	body weight
$C_{7,avg}$	average concentration over final week of study
CalEPA	California Environmental Protection Agency
$C_{avg,pup,gest}$	area under the curve normalized per day during gestation
$C_{avg,pup,gest,lact}$	area under the curve normalized dose per day during gestation/lactation
$C_{avg,pup,lact}$	area under the curve normalized per day during lactation
$C_{avg\text{-}pup\text{-}diet}$	average concentration during the post-weaning phase
CDI	Comprehensive Developmental Inventory
C-F	carbon-fluorine
CHCA	α-Cyano-4-hydroxycinnamic acid
CHECK	Children's Health and Environmental Chemicals in Korea
CHEF	Children's Health and the Environment in the Faroes
CHO	Chinese hamster ovary
CI	confidence interval
CKD	chronic kidney disease
C_{max}	maximum blood concentration
$C_{max,dam}$	maximum maternal concentration during gestation
$C_{max,pup,gest}$	maximum fetal concentration during gestation
$C_{max,pup,lact}$	maximum pup concentration during lactation
CNS	central nervous system
CRH	corticotropin releasing hormone
CSF	cancer slope factor
CSM	cholestyramine
CTX	type I collagen
CVD	cardiovascular disease
DFI	deoxyribonucleic acid fragmentation index
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DPP	Diabetes Prevention Program
dU	diurnal urinary
E2	estradiol
EFSA	European Food Safety Authority
GLP	good laboratory practice
eGFR	estimated glomerular filtration rate
eNT	equilibrative nucleoside transporter
EPA	U.S. Environmental Protection Agency
EYHS	European Youth Heart Study
F_1	first generation
FDA	U.S. Food and Drug Administration
FEV1	forced expiratory volume in one second
FR	folate receptor

FSH	follicle stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
FTI	free thyroxine index
FVC	forced vital capacity
GD	gestation day
GI	gastrointestinal
GM	geometric mean
Hb	hemoglobin
HDL	high-density-lipoprotein
HED	human equivalent dose
HEK 293	human embryonic kidney cells
HERO	Health and Environmental Research Online
HESD	health effects support document
HHRA	human health risk assessment
HOMA-B	Homeostatic Model Assessment of Beta-Cell Function
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HOME	Health Outcome Measures of the Environment
HUMIS	Norwegian Human Milk Study
IBD	inflammatory bowel disease
IC_{50}	median inhibiting concentration
ID	intellectual disability
IMS	imaging mass spectrometry
INUENDO	Biopersistent Organochlorines in Diet and Human Fertility
IQ	intelligence quotient
IQR	interquartile range
IRIS	Integrated Risk Information System
IUFD	intrauterine fetal death
IV	intravenous
K_d	disassociation constant
$K_{mem/w}$	membrane/water partition coefficients
K_ps	tissue-to-plasma partition coefficients
LC_{50}	median lethal concentration
LD	lactation day
LDL	low-density lipoprotein
L-FABP	liver fatty acid binding protein
LH	luteinizing hormone
LIFE	Longitudinal Investigation of Fertility and the Environment Study
LINC	Linking Maternal Nutrition to Child Health
LLOQ	lower limit of quantification
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
MALDI	matrix-assisted laser desorption/ionization
MCDI	MacArthur Communicative Development Inventories for Infants
MCLG	Maximum Contaminant Level Goal
MDH	Minnesota Department of Health
MDI	Mental Development Index
MDL	minimum detection limit
MDR1	p-glycoprotein

MeSH	medical subject headings
Mg/kg-day	milligrams per kilogram per day
MIREC	Maternal-Infant Research on Environmental Chemicals
MLR	mixed linear regression
MPAH	N-methyl-PFOSA
mPL-II	mouse placental lactogen
mPLP	prolactin-like protein
MRL	minimum reporting level
mRNA	messenger ribonucleic acid
MRP	multi-drug resistance-associated protein
MOA	mode of action
MoBA	Norwegian Mother, Father, and Child Cohort Study
MPAH	2-(N-methyl-PFOSA) acetate
NHANES	National Health and Examination Survey
NICHD	U.S. National Institute of Child Health and Human Development
NJDEP	New Jersey Department of Environmental Protection
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no-observed-adverse-effect level
NOAEC	no observed adverse effect concentration
NPDWR	national primary drinking water regulation
NTCP	sodium-taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OATs	organic anion transporters
OATPs	organic anion transporting polypeptides
OCC	Odense Child Cohort
OCT	organic cation/carnitine transporter
OECD	Organisation for Economic Co-operation and Development
OR	Odds Ratio
ORD	Office of Research and Development
OVA	ovalbumin
P_0	parental generation
PBPK	physiologically-based pharmacokinetic
P_c	partition coefficient
PC	phosphatidylcholine
PCOS	polycystic ovary syndrome
PDI	Psychomotor Development Index
PECO	Populations, Exposures, Comparator, and Outcomes
PEF	peak expiratory flow rate
PFAA	perfluorinated alkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFC	plaque forming cell
PFCA	perfluorocarboxylates
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFNA	perfluorononanoic acid
PFSA	perfluoroalkanesulfonic acid

PHQ-9	Patient Health Questionnaire
P_{ion}	passive anionic permeability
PFUnDA	perfluoroundecanoic acid
PK	pharmacokinetic
PND	postnatal day
PNW	postnatal week
POD	point-of-departure
POD_{HED}	point-of-departure human equivalent dose
POI	premature ovarian insufficiency
POPUP	Persistent Organic Pollutants in Uppsala Primiparas
PPARα	proliferator-activated receptor alpha
Q_1	quantile 1
Q_2	quantile 2
Q_3	quantile 3
Q_4	quantile 4
QA	quality assurance
QUICKI	Quantitative Insulin Sensitivity Check Index
RBC	red blood cell
RCM	ratio of cord blood to maternal blood concentrations
RFC	reduce folate carrier
RfD	reference dose
RIS	Research Information System
ROBINS-I	Risk of Bias in Nonrandomized Studies of Interventions
R_{PM}	ratio of placental:maternal concentrations
RSC	relative source contribution
rT3	reverse triiodothyronine
SAB	Science Advisory Board
SE	standard errors
SERT	serotonin transporter
SES	socioeconomic status
SD	standard deviation
SDQ	Strengths and Difficulties Questionnaire
SDWA	Safe Drinking Water Act
SHBG	sex hormone binding globulin
SMBCS	Shanghai Minhang Birth Cohort Study
SWAN	Study of Women's Health Across the Nation
T3	triiodothyronine
T4	thyroxine
TA	thyroid antibody
TC	total cholesterol
TDS	Total Diet Study
TgAB	thyroblobulin antibodies
TiAb	title-abstract
T_{max}	maximum plasma concentration
TPO	anti-thyroid peroxidase
TPoAb	thyroid peroxidase antibody
TSH	thyroid stimulating hormone
TT3	total triiodothyronine
TTE	transplacental transfer efficiencies
UCMR 3	third Unregulated Contaminant Monitoring Rule
V_1	volume of central distribution
V_2	volume of peripheral distribution
V_d	volume of distribution
VI	visual impairment
VLDL	very low-density lipoproteins

VMWM	Virtual Morris Water Maze
WBHGB	whole blood hemoglobin
WHO	World Health Organization
ww	wet weight

Appendix A. Systematic Review Protocol for Updated PFOS Toxicity Assessment

Per- and polyfluoroalkyl substances (PFAS) refers to a large group of fluorinated anthropogenic chemicals that includes perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and thousands of other chemicals. The universe of environmentally relevant PFAS, including parent chemicals, metabolites, and degradants, is greater than 12,000 compounds (https://comptox.epa.gov/dashboard/chemical-lists/PFASMASTER). The Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)* includes over 4,700 PFAS (OECD, 2018). The number of PFAS used globally in commercial products at the time of the drafting of this document is approximately 250 substances (Buck et al., 2021).

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States since the 1950's. PFAS have strong, stable, carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Buck et al., 2011; Beach et al., 2006). There are many families or classes of PFAS, each containing many individual structural homologues that can exist as either branched-chain or straight-chain isomers (Buck et al., 2011). The chemical structures of PFAS enable them to repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties; these properties make PFAS useful for commercial and industrial applications and make some PFAS extremely persistent in the human body and the environment (Calafat et al., 2019; Calafat et al., 2007). Because of their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many different PFAS co-occur in environmental media (e.g., air, water, ice, sediment) and in tissues and blood of aquatic and terrestrial organisms, including humans.

To understand and address the complexities associated with PFAS, the U.S. Environmental Protection Agency (EPA) is developing human health toxicity assessments for individual PFAS, in addition to other components of the broader PFAS action plan underway at EPA (https://www.epa.gov/pfas/epas-pfas-action-plan). The updated toxicity assessment that was developed for PFOS according to the scope and methods outlined in this protocol builds upon several other assessments, including the *Health Effects Support Document for PFOS* (U.S. EPA, 2016c) (hereafter referred to as the 2016 PFOS HESD) and *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c), which was released to the public for review by the Science Advisory Board (SAB) in November 2021.

This protocol describes the methods used for conducting the systematic review and dose-response analyses for the assessment of PFOS (*Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts*) and has been updated to address comments from the SAB. It should be noted that PFOA and PFOS underwent some steps of systematic review (e.g., literature searches) concurrently.

A.1 Overview of Background Information and Systematic Review Protocol

The methods used to conduct the systematic review for PFOS are consistent with the methods described in the draft and final *EPA ORD Staff Handbook for Developing IRIS Assessments* (U.S. EPA, 2022b, 2020a) (hereafter referred to as the Integrated Risk Information System (IRIS) Handbook) and a companion publication (Thayer et al., 2022). EPA's IRIS Handbook has incorporated feedback from the National Academy of Sciences (NAS) at workshops held in 2018 and 2019 and was well regarded by the NAS review panel for reflecting "significant improvements made by EPA to the IRIS assessment process, including systematic review methods for identifying chemical hazards" (NASEM, 2021). Furthermore, EPA's IRIS program has used the IRIS Handbook to develop toxicological reviews for numerous chemicals, including some PFAS (U.S. EPA, 2023b, 2022a). Though the IRIS Handbook was finalized concurrently with the development of this assessment, the revisions in the final IRIS Handbook compared to the draft version do not conflict with the methods used in this assessment. The assessment team concluded that implementing these minor changes in study quality evaluation between the draft and final IRIS Handbook versions would not change the assessment conclusions. Therefore, EPA considers the methods described herein to be consistent with the final IRIS Handbook and cites this version accordingly. Additionally, the methods used to conduct the systematic review are also consistent with and largely mirror the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

The Safe Drinking Water Act (SDWA) regulatory process enables EPA to receive comments and feedback on the systematic review protocol, including through SAB early input and via the public comment period associated with rule proposal. This protocol has been updated based on SAB recommendations to improve the clarity and transparency of the methods descriptions. It now includes information about additional data sources and how they were evaluated and expands the application of systematic review through dose-response analysis.

A.1.1 Summary of Background Information

This section summarizes more detailed sections on these topics from the *Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* (hereafter referred to as the Toxicity Assessment, (U.S. EPA, 2024)) and is provided for context. Please refer to the Toxicity Assessment (U.S. EPA, 2024) for more detailed information about chemical identity, physical-chemical properties, and occurrence.

A.1.1.1 Chemical Identity

PFOS is a PFAA that was used as an aqueous dispersion agent and emulsifier in a variety of water-, oil-, and stain-repellent products (e.g., agricultural chemicals, alkaline cleaners, carpets, firefighting foam, floor polish, textiles) (NLM, 2022). It can exist in linear- or branched-chain isomeric form. PFOS is a strong acid that is generally present as the sulfonate anion at typical environmental pH values. Therefore, this assessment applies to all isomers of PFOS, as well as nonmetal salts of PFOS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body). PFOS is stable in environmental media because it is resistant to environmental degradation processes such as biodegradation, photolysis, and

hydrolysis. In water, no natural degradation has been demonstrated, and dissipation is by advection, dispersion, and sorption to particulate matter.

A.1.1.2 Occurrence Summary

Key PFOS occurrence information is summarized below. More detail is provided in Chapter 1 of the Toxicity Assessment (U.S. EPA, 2024).

A.1.1.2.1 Biomonitoring

The CDC NHANES has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOS has been detected in up to 98% of serum samples taken in biomonitoring studies that are representative of the U.S. general population; however, blood levels have dropped 60% to 80% between 1999 and 2014, presumably due to restrictions on its commercial usage in the United States.

A.1.1.2.2 Occurrence in Water

PFOS is one of the dominant PFAS compounds detected in ambient water, along with PFOA (Remucal, 2019; Dinglasan-Panlilio et al., 2014; Zareitalabad et al., 2013; Benskin et al., 2012; Ahrens, 2011; Nakayama et al., 2007).

Data from the third Unregulated Contaminant Monitoring Rule (UCMR 3), collected from 2013–2015, are currently the best available national occurrence data for PFOA and PFOS (U.S. EPA, 2023a, 2021a, 2017). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed for PFOA and PFOS. The minimum reporting level (MRL)[1] for PFOA was 0.02 µg/L and the MRL for PFOS was 0.04 µg/L. A total of 1.37% of samples had reported detections (≥MRL) of at least one of the two compounds.

A.1.2 Problem Formulation

As described in the Toxicity Assessment (U.S. EPA, 2024), EPA conducted this updated assessment of PFOS (including all isomers as well as nonmetal salts of PFOS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body)) to support derivation of chronic cancer and noncancer toxicity values for PFOS. This problem formulation section will describe the key considerations and scope of the assessment, which were informed in part by EPA's past human health assessments of PFOS (2016 PFOS HESD and 2021 *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water*) (U.S. EPA, 2021c) as well as ongoing and final EPA assessments of other PFAS (e.g., perfluorobutanoic acid (PFBA) and draft perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) IRIS assessments (U.S. EPA, 2020b)).

The 2016 PFOS HESD identified several adverse health outcomes associated with PFOS exposure based on results from animal toxicological and epidemiological studies, including

[1] The reporting level is the threshold at or above which a contaminant's presence or concentration is officially quantitated. In the case of many of EPA's nationwide drinking water studies, the selected reporting level is known officially as the MRL. The MRL for each contaminant in each study is set at a level that EPA believes can be achieved with specified confidence by a broad spectrum of capable laboratories across the nation (U.S. EPA, 2021d).

developmental effects (e.g., decreased birth weight, accelerated puberty, skeletal variations), cancer (e.g., liver), liver effects (e.g., tissue damage), immune effects (e.g., antibody production and immunity), thyroid effects (e.g., hypothyroidism), and other effects (e.g., cholesterol changes). It concluded that there was "suggestive evidence of carcinogenic potential" for PFOS. EPA's 2021 *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water*) (U.S. EPA, 2021c) evaluated associations between PFOS and all cancer and noncancer health outcomes. After reviewing that draft scoping assessment, the SAB recommended that the scope be narrowed to focus on the five priority health outcomes that have the strongest weight of evidence (immune, developmental, hepatic, cardiovascular, and cancer), most of which were also identified in the conclusions from the 2016 PFOS HESD. Therefore, the current assessment provides a comprehensive systematic review of all health effects literature published through February 2022 for these five health outcomes. Mechanistic data for these health outcomes were also synthesized. For other health outcomes beyond the five priority outcomes, the current assessment summarizes the health effects literature published prior to 2016 and includes a systematic review of the health effects literature published from 2016–2020.

The *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* outlines key science issues related to PFAS in general (U.S. EPA, 2020b), many of which are relevant to PFOS. They include: toxicokinetic differences across species and sexes; human relevance of effects in animals that involve peroxisome proliferator-activated receptor alpha (PPARα); potential confounding by other PFAS exposures in epidemiology studies; and toxicological relevance of changes in certain hepatic endpoints in rodents. Differences in PFOS toxicokinetics across species and sexes were accounted for in the PFOS-specific animal and human pharmacokinetic models (see Toxicity Assessment, (U.S. EPA, 2024)). The human relevance of effects in animals that involve PPARα was investigated in the mechanistic syntheses of the five priority health outcomes (see Toxicity Assessment, (U.S. EPA, 2024)). Potential confounding by other PFAS (and other co-occurring contaminants) in epidemiology studies was considered as part of the confounding domain during study quality evaluations. Specifically, if a study did not account for potential confounding with other co-occurring PFAS in its statistical analyses, then the maximum quality rating this domain could receive was *adequate*. Concerns about potential confounding by other PFAS were limited when there was evidence that exposure was predominantly PFOS-based (such as in certain occupational or high-exposure studies) and the potential for co-exposure was minimal, or the correlations between co-exposures were small. The toxicological relevance of changes in certain hepatic endpoints in rodents was accounted for by incorporating the Hall (2012) criteria into the animal hepatic synthesis and hazard conclusions.

The *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* outlines key science issues related to PFAS in general (U.S. EPA, 2020b), many of which are relevant to PFOS. They include: toxicokinetic differences across species; human relevance of effects in animals that involve peroxisome proliferator-activated receptor alpha (PPARα); potential confounding by other PFAS exposures in epidemiology studies; and toxicological relevance of changes in certain hepatic endpoints in rodents. Differences in PFOS toxicokinetics across species were accounted for in the PFOS-specific animal and human toxicokinetic pharmacokinetic models (see Toxicity Assessment, (U.S. EPA, 2024)). The human relevance of effects in animals that involve PPARα was investigated in the

mechanistic syntheses of the five priority health outcomes (see Toxicity Assessment, (U.S. EPA, 2024)). Potential confounding by other PFAS (and other co-occurring contaminants) in epidemiology studies was considered as part of the confounding domain during study quality evaluations and is further discussed in Section 5 of the Toxicity Assessment (U.S. EPA, 2024). Specifically, if a study did not account for potential confounding with other co-occurring PFAS in its statistical analyses, then the maximum quality rating this domain could receive was *adequate*. Concerns about potential confounding by other PFAS were limited when there was evidence that exposure was predominantly PFOS-based (such as in certain occupational or high-exposure studies) and the potential for co-exposure was minimal, or the correlations between co-exposures were small. The toxicological relevance of changes in certain hepatic endpoints in rodents was accounted for by incorporating the Hall (2012) criteria into the animal hepatic synthesis and hazard conclusions.

An additional key science issue that EPA has encountered for PFAS toxicity assessments is a general lack of data on human and ecological toxicity. For PFOS, this is less of an issue as there has been substantial research and publication of both epidemiological and animal toxicological studies.

A.1.3 Overall Objective and Specific Aims

A.1.3.1 Objective

The primary objective of this toxicity assessment for PFOS is to support derivation of chronic cancer and noncancer toxicity values for PFOS, as well as update the cancer descriptor for PFOS, if warranted. EPA also considered potential pathways of exposure and derived a relative source contribution (RSC) specific to the final RfD for PFOS. The toxicity values, cancer classification, and RSC derived in this assessment build upon the work completed in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021c) and in the 2016 PFOS HESD (U.S. EPA, 2016c).

A.1.3.2 Specific Aims

The specific aims of the PFOS toxicity assessment document are to:

- Describe and document transparently the literature searches conducted and systematic review methods used to identify health effects information (epidemiological and animal toxicological studies and physiologically based pharmacokinetic models) in the literature (Sections 2 and 3 of the Toxicity Assessment (U.S. EPA, 2024); Appendix A and Appendix B).
- Describe and document literature screening methods, including use of the Populations, Exposures, Comparators, and Outcomes (PECO) criteria and the process for tracking studies throughout the literature screening (Section 2 of the Toxicity Assessment (U.S. EPA, 2024); Appendix A).
- Identify epidemiological and animal toxicological literature that reports health effects after exposure to PFOS (and its related salts) as outlined in the PECO criteria (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).

- Describe and document the study quality evaluations conducted on epidemiological and animal toxicological studies considered potentially useful for point-of-departure (POD) derivation (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Describe and document the data from all epidemiological studies and animal toxicological studies that were considered for POD derivation (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Synthesize and document the adverse health effects evidence across studies. The assessment focuses on synthesizing the available evidence for five priority health outcomes that were found to have the strongest weight of evidence, as recommended by the SAB – developmental, hepatic, immune, and cardiovascular effects, and cancer (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)) – and also provides supplemental syntheses of evidence for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects, reproductive effects in males or females, and general toxicity (Appendix C).
- Evaluate and document the available mechanistic information (including toxicokinetic understanding) associated with PFOS exposure to inform interpretation of findings related to potential health effects in studies of humans and animals, with a focus on five priority health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer) (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Develop and document strength of evidence judgments across studies (or subsets of studies) separately for epidemiological, animal toxicological, and mechanistic lines of evidence for the five priority health outcomes (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Develop and document integrated expert judgments across evidence streams (i.e., epidemiological, animal toxicological, and mechanistic streams) as to whether and to what extent the evidence supports that exposure to PFOS has the potential to be hazardous to humans (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Determine the cancer classification for PFOS using a weight-of-evidence approach (Section 3 of the Toxicity Assessment (U.S. EPA, 2024)).
- Describe and document the attributes used to evaluate and select studies for derivation of toxicity values. These attributes are considered in addition to the study confidence evaluation domains and enable extrapolation to relevant exposure levels (e.g., studies with exposure levels near the range of typical environmental human exposures, broad exposure range, or multiple exposure levels) (Section 4 of the Toxicity Assessment (U.S. EPA, 2024)).
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation (Section 4 of the Toxicity Assessment (U.S. EPA, 2024)).
- Derive candidate RfDs (Section 4.1 (U.S. EPA, 2024)) and CSFs (Section 4.2 of the Toxicity Assessment (U.S. EPA, 2024)), select the final RfD (Section 4.1.6 of the Toxicity Assessment (U.S. EPA, 2024)) and CSF (Section 4.2.3 of the Toxicity Assessment (U.S. EPA, 2024)) for PFOS, and describe the rationale.
- Characterize hazards (e.g., uncertainties, data gaps) (Sections 3, 4, and 5 of the Toxicity Assessment (U.S. EPA, 2024)).

A.1.4 Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

This section describes the PECO criteria that were developed and used for this assessment.[2] As described in the IRIS Handbook (U.S. EPA, 2022c), the PECO criteria provide the framework for literature search strategies and are the inclusion/exclusion criteria by which literature search results will be screened for relevancy to identify epidemiological and animal toxicological evidence that addresses the aims of the assessment. For the PFOS assessment, the PECO criteria were used to screen results of the literature searches to identify and prioritize the dose-response literature and studies containing pharmacokinetic (PK) or PBPK models. For studies captured in the 2019 and 2020 literature searches, the PECO criteria were used to screen and categorize ("tag") studies of PFOS absorption, distribution, metabolism, and excretion (ADME) and studies with mechanistic data for further evaluation using ADME- and mechanistic-specific PECO criteria. ADME, mechanistic, and other supplemental studies captured in the 2022 and 2023 literature searches were not tagged or considered further in this assessment.

Table A-1 describes the PECO criteria used to screen the results of the literature search (the literature search is described in Section A.1.5 of this appendix). ADME- and mechanistic-specific PECO criteria are outlined in Table A-2 and Table A-3, respectively.

Table A-1. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects From Exposure to PFOA and PFOS

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). In vitro/cell studies or in silico/modeling toxicity studies should be tagged as supplemental.
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including but not limited to:** PFOA (*Chemical Abstracts Service (CAS) number 335-67-1*). Other names: perfluorooctanoate; perfluorooctanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic-acid, pentadecafluoro- Relevant Salts of PFOA: ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluorooctanoate PFOS (*CAS number 1763-23-1*). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid Relevant Salts of PFOS: lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate **Human:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, or unknown/multiple routes will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information."

[2] Note: Although this appendix and its accompanying Toxicity Assessment (U.S. EPA, 2024) pertain to PFOS, the PECO criteria also cover PFOA because the literature searching and screening were performed concurrently for PFOA and PFOS.

PECO Element	Inclusion Criteria
	Animal: Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, injection or unknown/multiple routes, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." Studies involving exposures to mixtures will be included only if they include exposure to PFOA or PFOS alone. Studies with less than 28 d of dosing, with the exception of reproductive, developmental, immune and neurological health outcome studies, should be tagged as supplemental.
Comparator	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcome	All health outcomes (both cancer and noncancer).
PBPK Models	Studies describing physiologically based pharmacokinetic (PBPK) models will be included.

Epidemiological, animal toxicological, and *in vitro* studies tagged as containing potentially relevant ADME data were further screened using ADME-focused PECO criteria (Table A-2). Key information from each study meeting the ADME-focused PECO criteria was extracted using ICF's litstream™ software.

Table A-2. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Absorption, Distribution, Metabolism, and/or Excretion (ADME) Studies

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations): whole organism, tissues, individual cells, or biomolecules. **Animal:** Select nonhuman mammalian animal species: only nonhuman primates, rats, and mice (whole organism, tissues, individual cells, or biomolecules) of any lifestage (preconception, in utero, lactation, peripubertal, and adult stages).
Exposure	Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including in vitro, in vivo (by various routes of exposure), and ex vivo. In silico studies will also be included if the model system can be linked to a PECO-relevant species. PFOA (CAS number 335-67-1). Other names: perfluorooctanoate, perfluorooctanoic acid, perfluoroctanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroheptanecarboxylic acid, octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluoroctanoate PFOS (CAS number 1763-23-1). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctane sulfonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid, heptadecafluorooctanesulfonic acid, lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate, ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate
Comparator	Any comparison that informs PFOA or PFOS (1) absorption by the oral, inhalation, or dermal route of exposure, (2) distribution across biological compartments, (3) metabolism, and/or (4) excretion.
Outcome	Any examination of PFOA and/or PFOS (1) absorption of dose through gastrointestinal (GI) tract, lungs, or skin, (2) distribution across biological compartments, (3) metabolism, and/or (4) excretion. Studies describing PK models for PFOA and/or PFOS will be included.

PECO Element	Inclusion Criteria
	Information and terms that are typically found in relevant ADME/PK modeling studies include the following: **Absorption:** Bioavailability; absorption rate(s); uptake rates; tissue location of absorption (e.g., stomach versus intestine, nasal versus lung); blood:air partition coefficient (PC); irritant/respiratory depression; overall mass transfer coefficient; gas-phase diffusivity; gas-phase mass transfer coefficient; liquid- (or tissue-) phase mass transfer coefficient; deposition fraction; retained fractions; computational fluid (airway) dynamics. **Distribution:** Volume of distribution (V_d) and parameters that determine V_d, including blood: tissue PCs (especially for the target or a surrogate tissue) or lipophilicity; tissue burdens; storage tissues or tissue components (e.g., serum binding proteins) and the binding coefficients; transporters (active and passive). Note: PFOA/PFOS are not metabolized so we are not expecting studies that focus on metabolites. The terms below are general terms associated with metabolism. **Metabolism:** Metabolic/biotransformation pathway(s); enzymes involved; metabolic rate; maximum rate of transport (V_{max}), Michaelis constant (K_m); ; metabolic induction; metabolic inhibition, Ki; metabolic saturation/non-linearity; key organs involved in metabolism; key metabolites (if any)/pathways; metabolites measured; species-, inter-individual-, and/or age-related differences in enzyme activity or expression ("ontogeny"); site-specific activation (may be toxicologically significant, but little systemic impact); cofactor (e.g., glutathione) depletion. **Excretion:** Route(s)/pathway(s) of excretion for parent and metabolites; urine, fecal, exhalation, hair, sweat, lactation; elimination rate(s); mechanism(s) of excretion (e.g., passive diffusion, active transport).

Notes: CAS = Chemical Abstracts Service; PK = pharmacokinetic ADME = absorption, distribution, metabolism, and/or excretion.

Epidemiological and animal toxicological studies that were tagged as containing potentially relevant mechanistic data were further screened using mechanistic-focused PECO criteria (Table A-3). Studies meeting the mechanistic-focused PECO criteria underwent a light extraction of key study information using ICF's litstream™ software.

Table A-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Mechanistic Studies

PECO Element	Evidence
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Select mammals (i.e., nonhuman primates and rodents (i.e., rats, mice, rabbits, guinea pigs, other rodent models) and fish (i.e., zebrafish) of any lifestage (preconception, in utero, lactation, peripubertal, and adult stages). **Ex vivo, in vitro, in silico:** Cultures of human or animal cells from relevant animal models (primary, immortalized, transformed), organ slices, organotypic culture, in vitro molecular or biochemical assay systems. In silico modeling data if it informs PFOA/PFOS MOA.
Exposure	Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including in vitro, in vivo (by various routes of exposure), and ex vivo. In silico studies will also be included if the model system can be linked to a PECO-relevant species. PFOA (CAS number 335-67-1). Other names: perfluorooctanoate, perfluorooctanoic acid, perfluoroctanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroheptanecarboxylic acid, octanoic acid,

PECO Element	Evidence
	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluoroctanoate PFOS (CAS number 1763-23-1). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctane sulfonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid, heptadecafluorooctanesulfonic acid, lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate, ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate
Comparator	**Human:** Comparison to group with no exposure or lower exposure. **Animal, ex vivo, in vitro, in silico:** Comparison to an appropriate vehicle or no treatment control.
Outcome	Any mechanistic data related to the MOA of PFOA/PFOS toxicity. This may include molecular initiating events with PFOA/PFOS or downstream key events that inform the MOA or adverse outcome pathway linking PFOA/PFOS exposure to disease.

Notes: MOA = mode of action; CAS = Chemical Abstracts Service.

A.1.5 Literature Search

EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this updated toxicity assessment based on three data streams: 1) literature published from 2013 through 2019 and then updated in the course of this review (i.e., through February 6, 2023) identified via literature searches of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g., searches of the gray literature, studies shared with EPA by the SAB, and studies submitted through public comment), and 3) literature identified in EPA's 2016 PFOA and PFOS HESDs, which captured literature through 2013 (U.S. EPA, 2016c, d).

A.1.5.1 Literature Search Strategies

The following sections describe literature search strategies used for databases and for additional sources. They also describe methods used to incorporate studies from the 2016 PFOS HESD and other sources into the literature database. The literature search strategy included searches within core literature databases (e.g., PubMed®, Web of Science™) as well as relevant domestic and international non-periodical "gray" literature, such as technical reports, monographs, and conference and symposium proceedings prepared by select committees or bodies (e.g., those convened by the National Academy of Sciences or the World Health Organization (WHO)).

A.1.5.2 Database Searches

The database literature searches for this updated assessment focused only on the chemical name (PFOS and related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, in vitro, *in silico*) or health outcomes. These searches comprised all literature related to health effects resulting from acute, subchronic, and chronic exposure durations, and from inhalation, oral, dermal, and injection exposure studies. Epidemiological, animal toxicological, and in vitro studies that provide MOA information were included, and data specifically useful for addressing risks to children and other susceptible populations (e.g., the elderly, pregnant or lactating women, genetically susceptible populations) were identified. The searches likewise included ADME studies and models useful for dose-response assessment, such as dosimetry and

PBPK models. The initial database search covered from January 2013 through April 11, 2019 (the 2019 literature search). That was subsequently updated by a search covering April 2019 through September 3, 2020 (2020 literature search), another covering September 2020 through February 3, 2022 (2022 literature search), and a final supplemental search covering February 2022 through February 6, 2023 (described in Section A.3 below). The date field tag used for these searches may reflect either the date the article was published in print or e-published which may result in small amounts of literature being captured in a literature search despite being published prior to the start date. At the recommendation of SAB peer reviewers, the 2022 literature search and supplemental 2023 literature search focused on the five priority health outcomes that have been concluded to have the strongest evidence (developmental, hepatic, immune, and cardiovascular effects, and cancer). EPA considered mechanistic and toxicokinetic data identified through the September 2020 literature search, as well as any more recent studies recommended by the SAB.

The databases listed below were searched for literature containing the search strings identified in Table A-4 and Table A-5:

- Web of Science™ (Thomson Reuters),
- PubMed® (National Library of Medicine),
- ToxLine (incorporated into PubMed post 2019), and
- TSCATS (Toxic Substances Control Act Test Submissions).

Table A-4. Search String for April 2019 Database Searches

Database	Search String	Date Run
WoS	((TS="perfluorooctanoic acid" OR TS="perfluorooctane sulfonic acid") AND PY=(2013–2019) OR (TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride" OR TS="Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-" OR TS="Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-" OR TS="Pentadecafluoro-1-octanoic acid" OR TS="Pentadecafluoro-n-octanoic acid" OR TS="Pentadecafluorooctanoic acid" OR TS="Perfluorocaprylic acid" OR TS="Perfluoroctanoic acid" OR TS="Perfluoroheptanecarboxylic acid" OR TS="perfluorooctanyl sulfonate" OR TS="Perfluorooctanoic acid" OR TS="Octanoic acid, pentadecafluoro-" OR TS="Perfluorooctanoate" OR TS="perfluorooctane sulfonate" OR TS="A 5717" OR TS="EF 201" OR TS="Eftop EF 201" OR TS="Perfluoro-1-heptanecarboxylic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid" OR TS="1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-" OR TS="1-Perfluorooctanesulfonic acid" OR TS="EF 101" OR TS="Eftop EF 101" OR TS="Heptadecafluoro-1-octanesulfonic acid" OR TS="Heptadecafluorooctane-1-sulphonic acid" OR TS="Perfluorooctane sulfonate" OR TS="perfluorooctane sulfonate" OR TS="Perfluorooctane sulfonic acid" OR TS="Perfluorooctanesulfonic acid" OR TS="Perfluorooctylsulfonic acid" OR TS="perfluorooctane sulphonate" OR TS="perfluorooctane sulfonate" OR TS="1-Octanesulfonic acid, heptadecafluoro-"OR TS="Heptadecafluorooctanesulfonic acid" OR TS="Perfluoro-n-octanesulfonic acid" OR TS="Perfluorooctane Sulphonic Acid" OR TS="Perfluorooctanesulfonate" OR TS="Perfluorooctylsulfonate" OR ((TS="PFOA" OR TS="PFOS") AND (TS="fluorocarbon*" OR	4/10/2019

Database	Search String	Date Run
	TS="fluorotelomer*" OR TS="polyfluoro*" OR TS="perfluoro-*" OR TS="perfluoroa*" OR TS="perfluorob*" OR TS="perfluoroc*" OR TS="perfluorod*" OR TS="perfluoroe*" OR TS="perfluoroh*" OR TS="perfluoron*" OR TS="perfluoroo*" OR TS="perfluorop*" OR TS="perfluoros*" OR TS= "perfluorou*" OR TS="perfluorinated" OR TS="fluorinated" OR TS="PFAS"))) AND PY=(2013-2019))	
PubMed	(335-67-1[rn] OR 1763-23-1[rn] OR 45298-90-6[rn] OR "perfluorooctanoic acid"[nm] OR "perfluorooctane sulfonic acid"[nm]) AND (2013/01/01:3000[pdat] OR 2013/01/01:3000[mhda] OR 2013/01/01:3000[edat] OR 2013/01/01:3000[crdt]) OR (("2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride"[tw] OR "Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR “perfluorooctanyl sulfonate”[tw] OR "Perfluorooctanoic acid"[tw] OR "Octanoic acid, pentadecafluoro-"[tw] OR "Perfluorooctanoate"[tw] OR “perfluorooctane sulfonate”[tw] OR "A 5717"[tw] OR "EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR “perfluorooctane sulfonate”[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR (("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw]))) AND (2013/01/01:3000[pdat] OR 2013/01/01:3000[mhda] OR 2013/01/01:3000[edat] OR 2013/01/01:3000[crdt]))	4/10/2019
Toxline	@AND+@OR+("perfluorooctane sulfonate"+"pfos"+"perfluorooctanesulfonic acid"+"perfluorooctane sulfonic acid"+"perfluorooctane sulphonate"+"perfluorooctane sulfonate"+"perfluorooctanyl sulfonate"+"Heptadecafluorooctane-1-sulphonic"+"Heptadecafluoro-1-octanesulfonic acid"+"1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid"+"perfluorooctanoate"+"perfluorooctanoic acid"+"perfluoroctanoic acid"+"pfoa"+"2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"+"Pentadecafluoro-1-octanoic acid"+"Pentadecafluoro-n-octanoic acid"+"Octanoic acid, pentadecafluoro-"+"Perfluorocaprylic acid"+"Pentadecafluorooctanoic acid"+"perfluoroheptanecarboxylic acid"+@TERM+@rn+335-67-	4/11/2019

Database	Search String	Date Run
	1+@TERM+@rn+1763-23-1+@TERM+@rn+45298-90-6)+@NOT+@org+pubmed+@AND+@RANGE+yr+2013+2019	
TSCATS	@AND+@OR+@rn+"335-67-1"+@AND+@org+TSCATS+@NOT+@org+pubmed @AND+@OR+@rn+"1763-23-1"+@AND+@org+TSCATS+@NOT+@org+pubmed	4/11/2019

Table A-5. Search String for September 2020, February 2022, and February 2023 Database Searches

Database	Search String	Date Run
PubMed Batch IDs: 39678, 46137	(335-67-1[rn] OR 1763-23-1[rn] OR 45298-90-6[rn] OR "perfluorooctanoic acid"[nm] OR "perfluorooctane sulfonic acid"[nm] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride"[tw] OR "Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "perfluorooctanyl sulfonate"[tw] OR "Perfluorooctanoic acid"[tw] OR "Octanoic acid, pentadecafluoro-"[tw] OR "Perfluorooctanoate"[tw] OR "perfluorooctane sulfonate"[tw] OR "A 5717"[tw] OR "EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR "perfluorooctane sulfonate"[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR (("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw]))) AND (2020/09/03:3000[dp])	9/3/2020, 2/2/2022, 2/6/2023
Web of Science Batch IDs: 39681, 46144	(TS="perfluorooctanoic acid" OR TS="perfluorooctane sulfonic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride" OR TS="Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-" OR TS="Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-" OR TS="Pentadecafluoro-1-	9/3/2020, 2/3/2022, 2/6/2023

Database	Search String	Date Run
	octanoic acid" OR TS="Pentadecafluoro-n-octanoic acid" OR TS="Pentadecafluorooctanoic acid" OR TS="Perfluorocaprylic acid" OR TS="Perfluoroctanoic acid" OR TS="Perfluoroheptanecarboxylic acid" OR TS="perfluorooctanyl sulfonate" OR TS="Perfluorooctanoic acid" OR TS="Octanoic acid, pentadecafluoro-" OR TS="Perfluorooctanoate" OR TS="perfluorooctane sulfonate" OR TS="A 5717" OR TS="EF 201" OR TS="Eftop EF 201" OR TS="Perfluoro-1-heptanecarboxylic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid" OR TS="1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-" OR TS="1-Perfluorooctanesulfonic acid" OR TS="EF 101" OR TS="Eftop EF 101" OR TS="Heptadecafluoro-1-octanesulfonic acid" OR TS="Heptadecafluorooctane-1-sulphonic acid" OR TS="Perfluorooctane sulfonate" OR TS="perfluorooctane sulfonate" OR TS="Perfluorooctane sulfonic acid" OR TS="Perfluorooctanesulfonic acid" OR TS="Perfluorooctylsulfonic acid" OR TS="perfluorooctane sulphonate" OR TS="perfluorooctane sulfonate" OR TS="1-Octanesulfonic acid, heptadecafluoro-"OR TS="Heptadecafluorooctanesulfonic acid" OR TS="Perfluoro-n-octanesulfonic acid" OR TS="Perfluorooctane Sulphonic Acid" OR TS="Perfluorooctanesulfonate" OR TS="Perfluorooctylsulfonate" OR ((TS="PFOA" OR TS="PFOS") AND (TS="fluorocarbon*" OR TS="fluorotelomer*" OR TS="polyfluoro*" OR TS="perfluoro-*" OR TS="perfluoroa*" OR TS="perfluorob*" OR TS="perfluoroc*" OR TS="perfluorod*" OR TS="perfluoroe*" OR TS="perfluoroh*" OR TS="perfluoron*" OR TS="perfluoroo*" OR TS="perfluorop*" OR TS="perfluoros*" OR TS= "perfluorou*" OR TS="perfluorinated" OR TS="fluorinated" OR TS="PFAS"))) AND PY=(2020-2022)	
TOXLINE	TOXLINE taken down, cannot search.	–
TSCATS	Incorporated into PubMed post 2019.	–

The database searches were conducted by EPA and/or contractor information specialists and librarians on April 11, 2019, September 3, 2020, February 2 and 3, 2022, and February 6, 2023 and all search results were stored in the Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608). After deduplication (i.e., removal of duplicate results) in HERO, the database search results were imported into SWIFT Review software for filtering/prioritization. SWIFT Review identifies those references most likely to be applicable to human health risk assessment (https://www.sciome.com/swift-review/; see also (Howard et al., 2016)). In brief, SWIFT Review has preset literature search strategies ("filters") developed and applied by information specialists to identify and prioritize studies that are most likely to be useful for identifying human health content from those that likely are not (e.g., studies on analytical methods). The filters function like a typical search strategy in which studies are tagged as belonging to a certain category if the terms in the filter literature search strategy appear in title, abstract, keyword, and/or medical subject headings (MeSH) fields content. The applied SWIFT Review filters focused on the following evidence types: human (epidemiology), animal models for human health, and in vitro studies. The details of the search strategies that underlie the filters are available online (https://hawcprd.epa.gov/media/attachment/SWIFT-Review_Search_Strategies.pdf). The use of SWIFT Review is consistent with the IRIS Handbook (U.S. EPA, 2022b) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b)

For all literature searches, the evidence stream filters used were human, animal (all), animal (human health model), [no tag], epidemiological quantitative analysis, and in vitro (with one exception – for the 2022 and 2023 literature searches, the in vitro evidence stream filter was not used because the goal of those literature search was to identify studies relevant to dose-response only). Studies not captured using these filters were not considered further. Studies that were captured with these SWIFT Review evidence stream filters were exported as a RIS (Research Information System) file for title and abstract screening using either DistillerSR or SWIFT ActiveScreener software (described in subsequent sections of this appendix).

A.1.5.3 Additional Sources

The literature search strategies used were designed to be broad; however, like any search strategy, studies may be missed (e.g., if the chemical of interest is not mentioned in title, abstract, or keyword content; or if gray literature is not indexed in the databases that were searched). Thus, additional sources were reviewed to identify studies that could have been missed in the database searches. Reviews of additional sources included the following:

1. Review of studies cited in assessments published by other U.S. federal agencies, as well as international, and U.S. state-level agencies (including Agency for Toxic Substances and Disease Registry (ATSDR) and California Environmental Protection Agency (CalEPA) assessments that were ongoing at the time of searching).

    - Manual review of the reference list from ATSDR's *Toxicological Profile for Perfluoroalkyls* (ATSDR, 2021) (not date limited).

    - Manual review of the reference list from CalEPA's *First Public Review Draft of Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water* (CalEPA, 2021) (not date limited).

    - Manual review of National Toxicology Program (NTP) publications (https://ntp.niehs.nih.gov/data/index.html). In 2020, the NTP website was searched for PFOS toxicity study final reports that could provide relevant health effects information.

    - Manual review of PFAS toxicity studies identified by the New Jersey Department of Environmental Protection (NJDEP).

2. Review of studies identified during mechanistic or toxicokinetic evidence synthesis:

    - Manual review of the reference lists of studies screened as PECO-relevant after full-text review were reviewed at the title level for potential relevance (backward citation search).

    - Manual review of other EPA PFAS assessments or literature searches under development by IRIS.

3. Review of studies identified by the SAB PFAS Panel peer reviewers in their final report (published in August 2022).

4. Review of studies submitted through public comment by May 2023 (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

A.1.5.4 Incorporation of Data from the 2016 PFOS Health Effects Support Document

The 2016 PFOS HESD contained a comprehensive summary of relevant literature based on searches conducted through 2013. The 2016 PFOS HESD underwent a public comment period in February 2014, and an independent external public panel peer review in August 2014. EPA incorporated key studies from the 2016 PFOS HESD that addressed one or more of the five priority health outcomes into this updated PFOS assessment, as described below.

Over 140 epidemiological studies were captured in the 2016 PFOS HESD. The 2016 PFOS HESD did not use the epidemiological data quantitatively. For this updated assessment, EPA reviewed the epidemiological studies that were included in the 2016 PFOS HESD summary tables and identified those that were relevant to one or more of the five priority health outcomes (i.e., developmental, immune, hepatic, cardiovascular, and cancer). A total of 47 epidemiological studies were included and are listed in Table A-6 (studies relevant to more than one health outcome are listed under each applicable category in the table).

Table A-6. Key Epidemiological Studies of Priority Health Outcomes Identified From 2016 PFOS Health Effects Support Document

HERO ID	Reference	Title
		Cancer
4727072	Alexander and Olsen (2007)	Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers
1291101	Alexander et al. (2003)	Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility
2150988	Bonefeld-Jørgensen et al. (2011)	Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case-control study
2851186	Bonefeld-Jørgensen et al. (2014)	Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort
2919344	Eriksen et al. (2009)	Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population
4930271	Grice et al. (2007)	Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers
2968084	Hardell et al. (2014)	Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer
		Cardiovascular
1291101	Alexander et al. (2003)	Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility
2919285	Château-Degat et al. (2010)	Effects of perfluorooctanesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec)
2919150	Eriksen et al. (2013)	Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population
2850962	Fitz-Simon et al. (2013)	Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid
1430763	Frisbee et al. (2010)	Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project
3749193	Fu et al. (2014)	Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population

HERO ID	Reference	Title
2850925	Geiger et al. (2014a)	The association between PFOA, PFOS and serum lipid levels in adolescents
2851286	Geiger et al. (2014b)	No association between perfluoroalkyl chemicals and hypertension in children
1290820	Lin et al. (2009)	Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults
1291110	Nelson et al. (2010)	Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general US population
1290020	Olsen et al. (2003a)	Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations
10228462	Olsen et al. (2001)	A longitudinal analysis of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) levels in relation to lipid and hepatic clinical chemistry test results from male employee participants of the 1994/95, 1997 and 2000 fluorochemical medical surveillance program. Final report.
2850928	Starling et al. (2014b)	Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study
1290816	Stein et al. (2009)	Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome
2850370	Timmermann et al. (2014)	Adiposity and glycemic control in children exposed to perfluorinated compounds
		Developmental
1429893	Andersen et al. (2010)	Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy
1290833	Apelberg et al. (2007b)	Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth
1290900	Apelberg et al. (2007a)	Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland
1332466	Chen et al. (2012a)	Perfluorinated compounds in umbilical cord blood and adverse birth outcomes
2850274	Darrow et al. (2014)	PFOA and PFOS serum levels and miscarriage risk
2850966	Darrow et al. (2013)	Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005–2010
1005775	Fei et al. (2007)	Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort
1290822	Fei et al. (2008a)	Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy
2349574	Fei et al. (2008b)	Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort
1290814	Hamm et al. (2010)	Maternal exposure to perfluorinated acids and fetal growth
1332465	Maisonet et al. (2012)	Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls
2349575	Monroy et al. (2008)	Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples
1290816	Stein et al. (2009)	Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome
1291133	Washino et al. (2009)	Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth
		Hepatic
1291101	Alexander et al. (2003)	Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility

HERO ID	Reference	Title
1276142	Gallo et al. (2012)	Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure
4930271	Grice et al. (2007)	Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers
1291111	Lin et al. (2010)	Investigation of the Associations Between Low-Dose Serum Perfluorinated Chemicals and Liver Enzymes in US Adults
1290020	Olsen et al. (2003a)	Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations
10228462	Olsen et al. (2001)	A longitudinal analysis of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) levels in relation to lipid and hepatic clinical chemistry test results from male employee participants of the 1994/95, 1997 and 2000 fluorochemical medical surveillance program. Final report.
		Immune
1937230	Dong et al. (2013)	Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children
1290805	Fei et al. (2010)	Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood
1248827	Grandjean et al. (2012)	Serum vaccine antibody concentrations in children exposed to perfluorinated compounds
1937228	Granum et al. (2013)	Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood
2851240	Humblet et al. (2014)	Perfluoroalkyl chemicals and asthma among children 12–19 yr of age: NHANES (1999–2008)
2850913	Looker et al. (2014)	Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate
1424977	Wang et al. (2011)	The effect of prenatal perfluorinated chemicals exposures on pediatric atopy
		Serum Lipids
2919285	Château-Degat et al. (2010)	Effects of perfluorooctanesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec)
2919150	Eriksen et al. (2013)	Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population
2919156	Fisher et al. (2013)	Do perfluoroalkyl substances affect metabolic function and plasma lipids? – Analysis of the 2007–2009, Canadian Health Measures Survey (CHMS) Cycle 1
2850962	Fitz-Simon et al. (2013)	Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid
1430763	Frisbee et al. (2010)	Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project
3749193	Fu et al. (2014)	Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population
2850925	Geiger et al. (2014a)	The association between PFOA, PFOS and serum lipid levels in adolescents
1290820	Lin et al. (2009)	Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults
3981585	Maisonet et al. (2015b)	Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females
1291110	Nelson et al. (2010)	Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General US Population

HERO ID	Reference	Title
1290020	Olsen et al. (2003a)	Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations
10228462	Olsen et al. (2001)	A longitudinal analysis of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) levels in relation to lipid and hepatic clinical chemistry test results from male employee participants of the 1994/95, 1997 and 2000 fluorochemical medical surveillance program. Final report.
2850928	Starling et al. (2014b)	Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study
1291109	Steenland et al. (2009)	Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant
2850370	Timmermann et al. (2014)	Adiposity and glycemic control in children exposed to perfluorinated compounds

Notes: NHANES = National Health and Examination Survey.

EPA also reviewed the animal toxicological studies in the 2016 PFOS HESD summary tables that were identified as relevant for the five priority health outcomes. A total of nine "key" animal toxicological studies that were either considered quantitatively in the 2016 PFOS HESD or provided data that may quantitatively impact the assessment conclusions were included and listed in Table A-7 (studies relevant to more than one health outcome are listed under each applicable category in the table).

Table A-7. Key Toxicological Animal Toxicological Studies Identified From 2016 PFOS Health Effects Support Document

HERO ID	Reference	Title
		Cardiovascular
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Endocrine
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
757854	Lau et al. (2003)	Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Developmental
757873	Butenhoff et al. (2009)	Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: Developmental neurotoxicity
757854	Lau et al. (2003)	Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation

HERO ID	Reference	Title
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al. (2005a)	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
		Hematologic
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Hepatic
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
2919266	Kawamoto et al. (2011)	Ultrasonic-induced tonic convulsion in rats after subchronic exposure to perfluorooctane sulfonate (PFOS)
757854	Lau et al. (2003)	Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Immune
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Metabolic
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
		Nervous
757873	Butenhoff et al. (2009)	Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: Developmental neurotoxicity
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
1276160	Luebker et al. (2005a)	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
		Renal
1276144	Butenhoff et al. (2012)	Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague-Dawley rats
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)

HERO ID	Reference	Title
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Reproductive
757873	Butenhoff et al. (2009)	Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: Developmental neurotoxicity
757854	Lau et al. (2003)	Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al. (2005a)	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys
		Systemic
757873	Butenhoff et al. (2009)	Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: Developmental neurotoxicity
757871	Curran et al. (2008)	Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS)
757857	Luebker et al. (2005b)	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al. (2005a)	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
1290852	Seacat et al. (2003)	Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats
757853	Seacat et al. (2002)	Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys

A.1.6 Literature Screening Process to Target Dose-Response Studies and PK Models

This section summarizes the methods used to screen the literature search results against the PECO criteria to identify relevant studies potentially suitable for use in dose-response analyses and studies featuring PK models. Literature search results were screened at both title/abstract and full-text levels. These screening steps are described further below.

The PECO criteria used to screen the literature search results are the same as those used to frame the initial literature search (Table A-1) and are outlined again in Table A-8 below.

Table A-8. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects From Exposure to PFOA and PFOS

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages).

PECO Element	Inclusion Criteria
	In vitro/cell studies or in silico/modeling toxicity studies should be tagged as supplemental.
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including but not limited to:** PFOA (*Chemical Abstracts Service (CAS) number 335-67-1*). Other names: perfluorooctanoate; perfluorooctanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic-acid, pentadecafluoro- Relevant Salts of PFOA: ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluorooctanoate PFOS (*CAS number 1763-23-1*). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, Heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid Relevant salts of PFOS: lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate **Human:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, or unknown/multiple routes will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." **Animal:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, injection or unknown/multiple routes, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." Studies involving exposures to mixtures will be included only if they include exposure to PFOA or PFOS alone. Studies with less than 28 d of dosing, with the exception of reproductive, developmental, immune, and neurological health outcome studies, should be tagged as supplemental.
Comparator	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcome	All health outcomes (both cancer and noncancer).
PBPK Models	Studies describing PBPK models will be included.

Notes: PBPK = physiologically based pharmacokinetic.

Following SWIFT Review filtering (see Section A.1.5.2), literature search results were imported into either DistillerSR (Evidence Partners; https://www.evidencepartners.com/products/distillersr-systematic-review-software) or SWIFT ActiveScreener (Sciome; https://www.sciome.com/swift-activescreener/) software and were screened against the PECO criteria at the title and abstract level to identify PECO-relevant studies that could influence the derivation of an oral RfD and/or CSF. Studies that met the PECO criteria were tagged as having relevant human data, relevant animal data (in a mammalian model), or a PBPK model. Studies that did not meet the PECO criteria as determined by title/abstract screening but did appear to include potentially important supplemental information were categorized according to the type of supplemental information they provided (described below). Following completion of title/abstract screening (described further in Sections A.1.6.3 and A.1.6.4), the literature search results were re-screened at the full-text level (described further in Section A.1.6.5).

The title/abstract and full-text level screenings were performed by two independent reviewers using structured forms in DistillerSR, with a process for conflict resolution that included discussion of conflicts with the screening team. During full-text screening, literature inventories identifying evidence types and health effect systems were created for PECO-relevant studies and studies tagged as containing potentially relevant supplemental material to facilitate review of studies by topic-specific experts. These procedures are consistent with those outlined in the IRIS Handbook (U.S. EPA, 2022c).

Studies that did not meet the PECO criteria but contained potentially relevant supplemental information were inventoried during the literature screening process. Potentially relevant supplemental materials included the following (see Table A-11 for full list):

- Mechanistic data (including in vitro/ex vivo/in silico studies),
- Studies in non-mammalian or transgenic mammalian model systems,
- Non-oral routes of administration (for animal toxicological studies),
- ADME and toxicokinetic studies (including the application of existing PBPK models),
- Exposure assessment or characterization studies (no health outcome assessment),
- Mixture studies (animal toxicological studies on mixtures of PFOS and other substances or epidemiological studies that only report associations based on sum or total PFAS),
- Human case reports (n = 1–3 cases per report),
- Records or other assessments with no original data (e.g., reviews, editorials, commentaries),
- Conference abstracts, and
- Non-English language studies.

Following title/abstract and full-text level screening, studies tagged as containing potentially relevant mechanistic, ADME, or toxicokinetic data underwent additional screening and data extraction steps that were separate from steps followed for PECO-relevant studies. Additionally, studies that were tagged as containing relevant PBPK models were sent to the modeling technical experts for scientific and technical review. Details on the screening and data extraction methods for ADME and mechanistic studies are described below.

A.1.6.1 Screening ADME Studies

Studies identified as containing potentially relevant supplemental ADME data during title/abstract and/or full-text screening underwent further screening against the ADME-specific PECO criteria outlined in Table A-2. For studies that met the ADME-specific PECO criteria (see Table A-2), key study information was extracted using litstream™ software. Methods for this ADME screening and extraction of some key study information into litstream is described further in Section A.1.6.7.

A.1.6.2 Screening Mechanistic Studies

Studies identified as containing potentially relevant supplemental mechanistic data during full-text screening underwent further screening against the mechanistic-specific PECO criteria outlined in Table A-3. Studies that met the mechanistic-specific PECO criteria were extracted into litstream™. Methods for this mechanistic information screening and extraction of some key study information into litstream is described further in Section A.1.6.8.

A.1.6.3 Title/Abstract Screening Questions – DistillerSR

Studies identified from the 2016 PFOS HESD and recent systematic literature search and review efforts (searches through 2020) were imported into DistillerSR software for title/abstract screening. For each study, the screeners reviewed the title and abstract and responded to a series of prompts within structured DistillerSR forms to assess PECO relevance and identify evidence stream(s). Table A-9 below lists the prompts within the DistillerSR forms used for title/abstract screening and the response options for each prompt.

Table A-9. DistillerSR Form for Title/Abstract Screening

	Question/Prompt	Response Options
1	**Does the article meet PECO criteria?** *[Select one]*	• Yes[a] • No • Tag as potentially relevant supplemental material • Unclear
If "Yes" to Question #1:		
2a	**What type of evidence?** *[Select all that apply]*	• Human • Animal (mammalian models) • PBPK model
If "Tag as potentially relevant supplemental material" to Question #1:		
2b	**What kind of supplemental material?[b]** *[Select all that apply]*	• Mechanistic[c] • Non-mammalian model • ADME/toxicokinetic • Acute/short-term duration exposures • Non-oral route of administration • Exposure characteristics (no health outcome) • Susceptible population (no health outcome) • Environmental fate or occurrence (including food) • Mixture study • Case study or case series • Other assessments or records with no original data (e.g., reviews, editorials, commentaries) • Conference abstract • Bioaccumulation data in fish

Notes: PBPK = physiologically based pharmacokinetic.
[a] Errata, corrections, and corrigenda were tagged to the original study and not considered a separate relevant record.
[b] Refer to list of supplemental tags in Appendix A.1.6.4.1.
[c] Refer to list of mechanistic information in Appendix A.1.6.4.2.

A.1.6.4 Title/Abstract Screening Questions – SWIFT-Active

Studies identified from the most recent literature search (2020–2022) were imported into SWIFT-Active Screener software for title/abstract screening. For each study, screeners reviewed the title and abstract and responded to a set of prompts designed to ascertain PECO relevance and identify evidence stream(s). Table A-10 below lists the prompts within SWIFT-Active that were used for title/abstract screening and the response options for each prompt.

Table A-10. SWIFT-Active Form for Title/Abstract Screening

	Question/Prompt	Response Options
1	**Include this reference?** Select "Yes, include the reference" if unsure. *[Select one]*	• Yes, include the reference[a] • No, exclude the reference
If "Yes" to Question #1:		
2a	**Identify the Type of Evidence** *[Select all that apply]*	• Human/Epidemiological • Animal • Unsure
If "No, exclude the reference" to Question #1:		
2b	**Not Relevant or Supplemental?[b]** Select whether the reference is not relevant to PECO and should be excluded or if the reference contains supplemental information. *[Select all that apply]*	• Exclude/Not Relevant • Supplemental

Notes:
[a] Errata, corrections, and corrigenda were tagged to the original study and not considered a separate relevant record.
[b] Refer to the list of supplemental tags in Appendix A.1.6.4.1.

A.1.6.4.1 Supplemental Tags

The categories shown in Table A-11 were considered supplemental throughout the title/abstract and full-text screening processes. With the exception of studies tagged as containing mechanistic or ADME/TK information, which were further considered as described in Section A.1.6.7 and Section A.1.6.8 of this appendix, studies identified as not PECO-relevant but containing potentially useful supplemental material were not considered for the subsequent steps of the systematic review process.

Table A-11. Supplemental Tags for Title/Abstract and Full-Text Screening

Category	Evidence
Mechanistic Studies	Studies reporting measurements related to a health outcome that inform the biological or chemical events associated with phenotypic effects, in both mammalian and non-mammalian model systems, including in vitro, in vivo (by various routes of exposure), ex vivo, and in silico studies. When possible, mechanistic studies will be sub-tagged as pertinent to cancer, noncancer, or unclear/unknown.
Non-Mammalian Model Systems	Studies in non-mammalian model systems, e.g., fish, birds, *C. elegans.*
ADME and Toxicokinetic	Studies designed to capture information regarding absorption, distribution, metabolism, and excretion, including toxicokinetic studies. Such information may be helpful in updating or revising the parameters used in existing PBPK models.
Acute/Short-Term Duration Exposures	Animal studies of less than 28 d (unless the study is a developmental/reproductive, neurological, or immune study).
Only One Exposure Group	Animal studies with only one exposure group, e.g., control and 1 mg/kg/day PFOA/S.
Non-Oral Routes of Exposure	Studies not addressing routes of exposure that fall outside the PECO scope, include inhalation and dermal exposure routes.

Category	Evidence
Exposure Characteristics (No Health Outcome)	Exposure characteristic studies include data that are unrelated to toxicological endpoints, but which provide information on exposure sources or measurement properties of the environmental agent (e.g., demonstrate a biomarker of exposure).
Susceptible Populations (No Health Outcome)	Studies that identify potentially susceptible subgroups; for example, studies that focus on a specific demographic, lifestage, or genotype.
Environmental Fate or Occurrence (Including Food)	Studies that focus on describing where the chemical will end up after it is used and released into the environment.
Mixture Studies	Mixture studies that are not considered PECO-relevant because they do not contain an exposure or treatment group assessing only the chemical of interest.
Case Studies or Case Series	Case reports and case series will be tracked as potentially relevant supplemental information.
Records With No Original Data	Records that do not contain original data, such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Other Assessments or Records with No Original Data (e.g., Reviews, Editorials, Commentaries)	Secondary studies (e.g., reviews, editorials, commentaries, assessments) that do not provide any primary research/results.
Conference Abstracts	Records that do not contain sufficient documentation to support study evaluation and data extraction.
Bioaccumulation in Fish	Retained records relevant to other EPA projects mentioned in the PFAS Action Plan.
Non-English Reports	Studies not reported in English.

Notes: PK = pharmacokinetic; PBPK = physiologically based pharmacokinetic; ADME = absorption, distribution, metabolism, and/or excretion; *C. elegans* = *Caenorhabditis elegans.*

A.1.6.4.2 Mechanistic Study Categories and Keywords

The following categories were considered mechanistic throughout the title/abstract and full-text screening (Table A-12). Studies tagged as containing potentially relevant supplemental mechanistic information were further considered as described in Section A.1.6.8 of this appendix.

Table A-12. Mechanistic Study Categories Considered as Supplemental

Category	Examples of Keywords
Chromosome or DNA structure, function, repair, or integrity	Genotoxicity, micronuclei, DNA strand break, sister chromatid exchange, aneuploidy, genomic instability, gene amplification, epigenomics, DNA methylation, DNA methyltransferase, histone, DNA repair, base excision repair, nucleotide excision repair, DNA mismatch repair
Gene expression and transcription	Individual genes, pathway-related genes, transcriptomics, epigenetics, transcription factors, microRNAs, noncoding RNAs
Protein synthesis, folding, function, transport, localization, or degradation	Proteomics, translation, ribosomes, chaperones, heat shock proteins, ubiquitin, proteasome, ER stress, UPR, PERK
Metabolism	Anabolic or catabolic pathways for lipids, carbohydrates, amino acids, nucleotides; energy metabolism; biochemical pathways; metabolomics; lipidomics; enzyme or coenzyme activity or function

Category	Examples of Keywords
Cell signaling or signal transduction pathway	Ligand interactions with membrane, cytoplasmic and nuclear receptors (e.g., AHR, ER, AR, CAR, RAR, neurotransmitter receptors, insulin receptor, G-protein coupled receptors), tyrosine kinases, phosphatase, phospholipases, GTPase, second messengers (calcium, diacylglycerol, ceramide, NO), signaling pathways (NF-ƙB, MAPK/ERK, AKT, mTOR, IP3/DAG, cAMP-dependent, Wnt, β-catenin, TGFβ, etc.)
Cell or organelle structure, motility, integrity	Membrane integrity, cell scaffolding, cytoskeleton, actin, microtubules, ER, Golgi, mitochondria, lysosome, endosome, phagosome, nucleus, chemotaxis, atrophy, hypertrophy
Extracellular matrix or molecules	ECM proteins (collagens, elastins, fibronectins and laminins), proteoglycans, matrix metalloproteinases (MMPs)
Cell growth, differentiation, proliferation, or viability	Cell cycle (G1, S, G2, M), cyclins, CDKs, p53, p27, Rb, E2F stem cell, progenitor, apoptosis, Annexin V, TUNEL, necrosis, blebbing, pyknosis, Bax, Bcl-2, hyperplasia, dysplasia
Activation of intrinsic cell defense molecules or systems	Cytokines, chemokines, caspases, MHC/HLA molecules, pattern recognition receptors (PRRs), NLR, proteasomes, autophagy
Oxidative stress	Reactive oxygen species (ROS), oxidative stress, hydroxyl radical, hydrogen peroxide, reactive nitrogen species, superoxide anion, peroxyl radicals, antioxidant response, catalase, superoxide dismutase, EROD, glutathione (GSH), GSH peroxidase, glutathione-S-transferase, 8-OHdG
Hormone function	GnRH, CRF, ADH/vasopressin, FSH, LH, ACTH, GH, TH, TSH, PTH, cortisol, epinephrine/norepinephrine, melatonin, oxytocin, estrogen, testosterone, adiponectin, leptin, insulin, glucagon
Biomarkers of cerebral function	Apoptotic neurodegeneration protein markers, cerebral glucose metabolism, brain glucose levels
Other (provide details)	Please provide specific details regarding reason for supplemental tag in the notes section.

Notes: DNA = deoxyribonucleic acid; microRNA = micro ribonucleic acid; RNA = ribonucleic acid; ER = estrogen receptor; UPR = unfolded protein response; PERK = protein kinase R-like endoplasmic reticulum kinase; AHR = aryl hydrocarbon receptor; CAR = constitutive androstane receptor; RAR = retinoic acid receptor; GTPase = guanosine triphosphate; NO = nitric oxide; NF-ƙB = nuclear factor kappa B; mTOR = rapamycin; DAG = diacylglycerol; TGFβ = transforming growth factor beta; ECM = extracellular matrix; ; CDK = cyclin-dependent kinase; Bcl-2 = B-cell lymphoma 2; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; MHC/NHLA = major histocompatibility complex/human leukocyte antigen; NLR = nucleotide-binding oligomerization domain-like receptors; EROD = ethoxyresorufin-O-dealkylase; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; GnRH = gonadotropin-releasing hormone; CRF = corticotropin-releasing factor; ADH = Antidiuretic hormone; FSH = follicle stimulating hormone; LH = luteinizing hormone; ACTH = adrenocorticotropic hormone; GH = growth hormone; TH = thyroid hormone; PTH = parathyroid hormone.

A.1.6.5 Full-Text Screening Questions

All studies identified as PECO-relevant from title/abstract screening advanced to full-text screening – which was performed in DistillerSR. Screeners reviewed each full study report and any supplemental study materials to respond to prompts pertaining to PECO relevance, evidence stream, and health outcome(s), and whether PFOS and/or PFOA was evaluated (some screening efforts for PFOA and PFOS were performed concurrently). Table A-13 below lists the prompts and response options that were used for full-text screening.

Table A-13. DistillerSR Form for Full-Text Screening

	Question/Prompt	Response Options
1	**Source of study if not identified from database search.** *[Select one]*	• Source other than HERO database search
2	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No • Tag as potentially relevant supplemental material • Unclear
If "Yes" to Question #1:		
3a	**If meets PECO, what type of evidence?** *[Select all that apply]*	• Human • Animal (mammalian models) • PBPK model
4a	**If meets PECO, which health outcome(s) apply?**[a] *[Select all that apply]*	• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and developmental • Hematological, including non-immune/hepatic/renal clinical chemistry measures • Hepatic, including liver measures and serum markers (e.g., ALT, AST) • Immune/inflammation • Musculoskeletal • Nervous system, including behavior and sensory function • Nutrition and metabolic • Ocular

	Question/Prompt	Response Options
		• PBPK or PK model • Renal, including urinary measures (e.g., protein) • Reproductive • Respiratory • Skin and connective tissue effects • Dermal • Unsure • Other
		If meets PECO and endocrine outcome, which endocrine tags apply? *[Select all that apply]* • Adrenal • Sex hormones (e.g., androgen, estrogen, progesterone) • Neuroendocrine • Pituitary • Steroidogenesis • Thyroid
		If "Unsure" or "Other" is selected for health outcome, write reasoning in the respective free text-box. *[Free-text]*
If "Tag as potentially relevant supplemental material" to Question #1:		
3b	**If supplemental, what type of information?[b,c]** *[Select all that apply]*	• Mechanistic • Non-mammalian model • ADME/toxicokinetic • Acute/short-term duration exposures[d] • Non-oral route of administration • Exposure characteristics (no health outcome) • Susceptible population (no health outcome) • Environmental fate or occurrence (including food) • Mixture study • Case study or case series • Other assessments or records with no original data (e.g., reviews, editorials, commentaries) • Conference abstract • Bioaccumulation data in fish
4b		**If "Acute," which health outcome(s) apply?** *[Select all that apply]*

	Question/Prompt	Response Options
		• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and developmental • Hematological, including non-immune/hepatic/renal clinical chemistry measures • Hepatic, including liver measures and 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 • Dermal • Unsure
If "Yes," "Tag as potentially relevant supplemental material," or "Unclear" to Question #1:		
5	**Which PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS • Other PFAS

Notes: PBPK = physiologically based pharmacokinetic; ALT = alanine transaminase; AST = aspartate aminotransferase; PK = pharmacokinetic; ADME = absorption, distribution, metabolism, and/or excretion.

[a] Refer to list of health outcomes and examples in Appendix A.1.6.5.1.

[b] Refer to list of supplemental tags in Appendix A.1.6.4.1.

[c] Refer to list of mechanistic information in Appendix A.1.6.4.2.

[d] Refer to definition of acute/short-term duration exposures in Appendix A.1.6.6.

A.1.6.5.1 Health Effect Categories and Example Outcomes for Epidemiological Studies

The following health effects categories were considered throughout the full-text screening and subsequent steps of the systematic review process for epidemiological studies (Table A-14).

Table A-14. Health Effect Categories Considered for Epidemiological Studies

Health Effect Category	Example Health Outcomes	Notes
Cancer	• Tumors • Precancerous lesions (e.g., dysplasia)	–
Cardiovascular	• Serum lipids (e.g., cholesterol, LDL, HDL, triglycerides) • Blood pressure • Hypertension • Atherosclerosis • Coronary heart disease • Other cardiovascular disease	–
Dermal	• Skin sensitivity	–
Developmental	• Birth size (birth weight; birth length; small for gestational age) • Preterm birth • Sex ratio • Postnatal growth	• Markers of development specific to other systems are organ/system-specific (e.g., tests of sensory maturation are under **Nervous System**) • Pubertal development is under **Reproductive**.
Endocrine	• Thyroid hormones (e.g., T3, T4, TSH) • Thyroid weight and histopathology • Hormonal measures in any tissue or blood (non-reproductive)	• Reproductive hormones (e.g., estrogen, progesterone, testosterone) are under **Reproductive**.
Gastrointestinal	• Symptoms of the stomach and intestines (e.g., diarrhea, nausea, vomiting, abdominal pain, and cramps)	–
Hematologic	• Blood count • Red blood cells • Blood Hematocrit or hemoglobin • Corpuscular volume • Blood Platelets or reticulocytes • Blood biochemical measures (e.g., sodium, calcium, phosphorus)	• White blood cell counts and globulin are under **Immune**. • Serum lipids are under **Cardiovascular**. • Serum liver markers are under **Hepatic**.
Hepatic	• Liver enzymes (e.g., ALT; AST; ALP) • Liver disease • Liver-specific serum biochemistry (e.g., albumin)	• Serum lipids are under **Cardiovascular**. • Biochemical markers, such as albumin, are under **Hepatic**. Liver tissue cytokines are under **Immune**. • Globulin is under **Immune**. • Serum glucose is under **Metabolic.**
Immune	• Asthma • Allergy • Atopic dermatitis/eczema • Vaccine response • IgE • Autoimmune or infectious disease • Hypersensitivity	• Red blood cells are under **Hematological**. • Non-immune measures of pulmonary function are under **Respiratory**. • Interleukin 6 (IL-6) is considered a **Mechanistic** outcome.

Health Effect Category	Example Health Outcomes	Notes
	• General immune assays (e.g., white blood cell counts) • Immune responses in the respiratory system • Stress-related factors in blood (e.g., glucocorticoids or other adrenal markers)	
Metabolic/Systemic	• Obesity • BMI • Adiposity • Diabetes (including gestational diabetes) • Insulin resistance • Blood glucose • Allostatic load • Metabolic syndrome	• Waist circumference, ponderal index, BMI SDS, BMI z-scores, are all included here. • Gestational weight gain, adult weight change also included here.
Musculoskeletal/Connective Tissue	• Bone health • Osteoporosis • Bone density	–
Nervous	• Cognition • Behavior • Autism • Attention (ADHD) • Depression • Communication • Motor	–
Ocular	• Vison changes • Eye irritation	–
Reproductive, female	• Reproductive hormones • Breastfeeding • Fecundity • PCOS • Spontaneous abortion • Menopause • Endometriosis • Pubertal development • Menstrual cycle characteristics • Anogenital distance (females)	• If data indicate altered birth parameters are likely attributable to female fertility, these data may be discussed under **Female Reproductive**.
Reproductive, male	• Reproductive hormones • Semen parameters • Sperm DNA damage • Pubertal development • Anogenital distance (males)	–
Respiratory	• Non-immune measures of pulmonary (lung) function (e.g., FEV1, FVC, lung capacity)	• Asthma, wheeze, lower/upper respiratory trat infections are **Immune**.
Renal	• GFR • Uric acid • Creatinine • Renal function	–

Health Effect Category	Example Health Outcomes	Notes
	• Urinary measures (e.g., protein; volume; pH; specific gravity)	
Other	• Select this category if the outcome does not fit in any of the above categories.	–

Notes: LDL = low-density lipoprotein; HDL = high-density lipoprotein; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; ALT = alanine transaminase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; IgE = immunoglobulin E; BMI = body mass index; ADHD = attention deficit hyperactivity disorder; PCOS = polycystic ovary syndrome; DNA = deoxyribonucleic acid; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; GFR = glomerular filtration rate.

A.1.6.6 Animal Toxicological Study Design Definitions

The following definitions were used throughout full-text screening and data extraction for animal toxicological studies:

- Acute/short-term: Exposure duration between 1–28 days.
- Subchronic: Exposure duration between 28–90 days.
- Chronic: Exposure duration greater than 90 days.
- Developmental: Exposure occurs during gestation and dams are sacrificed prior to birth. These studies are typically focused on the pups and evaluate viability, developmental milestones, and other growth and developmental effects in pups.
- Reproductive: Exposure begins prior to mating and may continue through birth and, in some cases, through a second generation. These studies will typically evaluate reproductive outcomes in the dams (e.g., copulation and fertility indices, numbers of corpora lutea and implantation sites, pre- and post-implantation loss).

A.1.6.7 ADME Screening and Light Data Extraction

All studies identified as containing ADME data during title/abstract or full-text screening were imported into litstream and underwent additional screening. Studies that met certain criteria (e.g., PECO relevant and evaluated multiple timepoints, tissues, and/or dose levels) underwent light data extraction. For each study, at least two reviewers (one primary screener/extractor and one quality assurance (QA) reviewer) reviewed the full study and any supplemental study materials to respond to prompts pertaining to key study elements (e.g., tested species or population, tissues evaluated, dose levels tested, ADME endpoints measured). Table A-15 below describes the prompts and response options that were used for ADME screening of epidemiological or animal toxicological studies.

Table A-15. Litstream Form for ADME Screening and Light Data Extraction

	Question/Prompt	Response Options	Suggested Considerations
1	**General Questions**		
1.1	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No	• Use ADME-specific PECO statement (See Toxicity Assessment, (U.S. EPA, 2024)) and "Draft EPA IRIS Handbook: Principles and Procedures for Integrated Risk Information System (IRIS) Toxicological Reviews" to inform the answer. • Examples of exclusions may include abstract-only, foreign language, secondary data sources, exposure studies, physical-chemical properties, and species that aren't relevant. • If "No" is selected, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly explaining why the study does not meet PECO.
1.2	**What PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS	–
1.3	**Does this study contain multiple time points, multiple tissues, and/or multiple doses?** *[Select one]*	• Yes • No	• If "No" is selected, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly explaining why the study meets PECO but does not contain multiple time points, multiple tissues, and/or multiple doses.

	Question/Prompt	Response Options	Suggested Considerations
1.4	**Does this study contain supporting epidemiological information?** *[Select one]*	• Yes • No	• Supporting epidemiological information includes studies that compare PFAS levels in women of different parity status or weeks of breastfeeding as well as studies that compare PFAS levels across multiple age groups or multiple time points even if it is not the same individuals who are being followed over time (e.g., a cross-sectional study that enrolls people of various ages and compares PFOS/PFOA levels in a specific tissue in children vs. older adults).
1.5	**Indicate if there is supplemental data for this study.** *[Select all that apply; Free-text]*	• MOA/Mechanistic • Exposure Study	• Use the free text field below to provide a brief description of the type of MOA/mechanistic (refer to Appendix A.1.6.4.2 for examples) and/or exposure information that is available. • Examples of exposure information include studies of PFAS levels in environmental media not directly linked to human exposure (e.g., soil, sediment, microbes, water [except drinking water], birds, or fish [except those typically consumed by humans]).
1.6	**Identify the species, system, or model.** *[Select all that apply]*	• Human • Nonhuman primate • Rat • Mouse • Mammalian cells (in vitro studies) • PBPK/TK models (or in silico studies)	• If a study only contains PBPK/TK models, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly describing the model.
2	**Human Studies Sub-Form** If the study does **not** contain a human study, **skip** this section and move on to Section 3 – Animal Studies Sub-Form.		
2.1	**Population Name** *[Free-Text]*	–	• Name a population (e.g., Females – pregnant, PFOS) • Separate populations should be made for each chemical, population sex, lifestage where ADME data was collected, exposure route, etc. combination.
2.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• Note: PFOA and PFOS are not metabolized so "metabolism" is an unlikely selection.
2.3	**List the specific ADME endpoints addressed.**	–	• List all the ADME endpoints analyzed for this population.

	Question/Prompt	Response Options	Suggested Considerations
	[Free-text]		
2.4	**Exposure Category** Use the free text field if additional information is needed (e.g., it is a unique exposure, occupational setting, etc.). *[Select one; Free-text]*	• General environmental • Poisoning • Occupational • Developmental	–
2.5	**Identify the Exposure Route** *[Select one; Free-text]*	• Inhalation • Oral • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intranasal)	• If "other" option is selected, use the free text field to describe exposure route. • If the study population is exposed through more than one route (e.g., oral and dermal), select one route from the list and use the free text field to describe the other exposure routes listed in the paper. • If the study population is offspring that were exposed "in utero/placental" AND by "lactational transfer," select "in utero/placental" and use the free text field to note that lactational transfer also occurred. • If exposure route is unknown, select "other" option and write in "Unknown" in the free text field. • If the route is unspecified or multiple routes were suspected based on the exposure vehicle, select "other" and write in suspected exposure route in the free text field.
2.6	**What is the exposure vehicle?** *[Select one]*	• Drinking water • Diet • Breast milk • In utero/placental transfer • Occupational • Unknown • Other	• If "other" option is selected, use the free text field to describe exposure vehicle. • If the study population is offspring that were exposed "in utero/placental" AND by "breast milk," select "in utero/placental" and use the free text field to note that lactational transfer also occurred via breast milk. • If "occupational" option is selected, use the free text field to describe exposure vehicle.
2.7	**What is the sex of the population?** *[Select one]*	• Male • Female • Unspecified	• If results are given separately for each sex, separate sub-forms should be used for each population.
2.8	**Number of Subjects**	–	• Example: Total number of subjects = 428

	Question/Prompt	Response Options	Suggested Considerations
	Use the free text field to add additional details on number of subjects if they are broken up by groups or quartiles. *[Free-text]*		
2.9	**What is the lifestage when the ADME data was collected?** Use the free text field to provide additional lifestage notes. *[Select one; Free-text]*	• Prenatal: conception to birth • Infancy: 0–12 mo • Childhood: 13 mo to 11 yr • Adolescence: 12 to 20 yr • Adult: 21 to 65 yr • Elderly: >65 yr	• If there is more than one lifestage when ADME data was collected, add an additional population in another form.
2.10	**Exposure Levels** Use the free text field to enter the numeric exposure levels (if known/estimated in an environmental medium such as air, water, dust, food, breast milk, etc.). *[Free-text]*	–	• Do not report levels in serum or urine for this question.
2.11	**Exposure Units** Use the free text field to report the exposure units as presented in the paper. *[Free-text]*	–	• Examples: mg/kg-d; mg/m3; ppm • Use "Not Reported" if appropriate
2.12	**Exposure Duration** Use the free text field to enter the details of the exposure duration if known. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y). o Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.
2.13	**Time Points Analyzed** Use the free text field to enter the time points data were analyzed. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y). o Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.
2.14	**Measured Tissues** Use the free text field to enter the tissues measured in the study (e.g., plasma, breast milk, cord blood). *[Free-text]*	–	–

	Question/Prompt	Response Options	Suggested Considerations
3	**Animal Studies** If the study does not contain an animal study, skip this section and move on to Section 4 – Mammalian Cells/In vitro.		
3.1	**Population Name** *[Free-text]*	–	• Name a population (e.g., Females dams, PFOS). • Separate populations should be made for each chemical, species, population sex, lifestage where ADME data was collected, exposure route, etc. combination.
3.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• PFOA and PFOS are not metabolized, so "metabolism" is an unlikely selection.
3.3	**List the specific ADME Endpoints addressed.** Use the free text field below to list all the ADME endpoints analyzed for this population. *[Free-text]*	–	–
3.4	**Identify the Exposure Route** *[Select one]*	• Inhalation (nose only) • Inhalation (whole head exposure) • Inhalation (whole body exposure) • Oral (diet) • Oral (drinking water) • Oral (gavage) • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intravenous, intranasal)	• If "other" option is selected, use the free text field below to describe exposure route • If the study population is offspring that were exposed "in utero/placental" AND by "lactational transfer," select "in utero/placental" and use the free text field to note that lactational transfer also occurred • If there is more than one exposure route identified, add an additional population in another form.
3.5	**What is the exposure vehicle?** *[Select one]*	• Diet • Water • Breast milk • In utero/placental transfer • Corn oil • Filtered air • Olive oil • Ethanol	• If "other" option is selected, use the free text field below to describe exposure vehicle • If the study population is offspring that were exposed "in utero/placental" AND by "breast milk," select "in utero/placental" and use the free text field to note that lactational transfer also occurred via breast milk.

	Question/Prompt	Response Options	Suggested Considerations
		• DMSO • Mineral oil • Corn oil:acetone • Other	
3.6	**What is the strain?** Use the free text field to list the strain (e.g., Sprague-Dawley). *[Free-text]*	–	• If there is more than one species studied, add an additional population in another form.
3.7	**What is the sex?** *[Select one]*	• Male • Female • Male and Female	• If results are given separately for each sex, add an additional population in another form.
3.8	**What is the lifestage when the animal was dosed?** *[Select all that apply]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Prenatal o Nonhuman primates: conception to birth o Rodents: GD0 to birth • Weaning o Nonhuman primates: 1–130 d (0.35 yr) o Rodents: PND 1–21 • Adolescent o Nonhuman primates: 130–1,825 d (0.35–5 yr) o Rodents: 21–50 d (3–7 wk) • Adult o Nonhuman primates: 5–35 yr o Rodents: >50 d (>7 wk) • Elderly o Nonhuman primates: >35 yr
3.9	**What is the reported average age of the animals when dosing began?** *[Free-text]*	–	• Use “Not Reported” if appropriate.
3.10	**What is the average initial body weight of the animals when dosing began?** *[Free-text]*	–	• Use “Not Reported” if appropriate.
3.11	**What is the lifestage when the ADME data was collected?** *[Select all that apply; Free-text]*	• Prenatal • Weaning • Adolescent • Adult	• Prenatal o Nonhuman primates: conception to birth o Rodents: GD 0 to birth • Weaning

Question/Prompt	Response Options	Suggested Considerations
	• Elderly	○ Nonhuman primates: 1–130 d (0.35 yr) ○ Rodents: PND 1–21 • Adolescent ○ Nonhuman primates: 130–1,825 d (0.35–5 yr) ○ Rodents: 21–50 d (3–7 wk) • Adult ○ Nonhuman primates: 5–35 yr ○ Rodents: >50 d (>7 wk) • Elderly ○ Nonhuman primates: >35 yr; use the free text field to provide additional lifestage notes. • If there is more than one lifestage when ADME data were collected, add an additional population in another form.
3.12 **What is the number of animals per dosing group?** Use the free text field to report the number of animals per dosing group. *[Free-text]*	–	• Example: Control = 10, low dose = 20, high dose = 20; All groups = 20 • Use "Not Reported" if appropriate.
3.13 **Dose Levels** Use the free text field to enter the numeric dose levels. *[Free-text]*	–	• Example: 0, 450, 900
3.14 **Dose Units** Use the free text field to report the dosage units as presented in the paper. *[Free-text]*	–	• Examples: mg/kg-d; mg/m3; ppm • Use "Not Reported" if appropriate.
3.15 **Dose Duration** Use the free text field to enter the details of the dose duration if known. *[Free-text]*	–	• Use abbreviations (h, d, wk, mo, y). • For reproductive and developmental studies, where possible instead include abbreviated age descriptions such as "GD 1–10" or "GD 2–PND 10" ○ Examples: 14 d, 13 w (6 h/d × 5 d/wk); GD 2–PND 10 • Use "Not Reported" if appropriate.
3.16 **Time Points Analyzed**	–	• Use abbreviations (h, d, wk, mo, y) ○ Examples: 14 or 28 d; 13 wk; 2 y

	Question/Prompt	Response Options	Suggested Considerations
	Use the free text field to enter the time points data were analyzed. *[Free-text]*		• Use "Not Reported" if appropriate.
3.17	**Measured Tissues** Use the free text field to enter the tissues measured in the study (e.g., plasma, liver, adipose). *[Free-text]*	–	–
4	**Mammalian Cells/In vitro** If the study does not contain an in vitro component, skip this section and move on to Section 5 – Notes.		
4.1	**Population Name** *[Free-text]*	–	• Name a population (e.g., Primary Human Hepatic, PFOA; A549, PFOS) • Separate populations should be made for each chemical, population sex, lifestage where ADME data was collected, exposure route, etc. combination. Use the "Clone" button to copy forms/information for easier extraction if the study populations are similar.
4.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• PFOA and PFOS are not metabolized so "metabolism" is an unlikely selection.
4.3	**List the specific ADME Endpoints addressed.** Use the free text field below to list all the ADME endpoints analyzed for this population. *[Free-text]*	–	–
4.4	**Does the study present data on protein binding?** *[Select one; Free-text]*	• Yes • No	• If "Yes" option is selected, use the free text field to list the binding proteins.
4.5	**Does the study present data on active transport?** *[Select one; Free-text]*	• Yes • No	• If "Yes" option is selected, use the free text field to list the transporters.
4.6	**Cell Line Name or Tissue Source** Use the free text field to list the cell line name or tissue source the cells were derived from.	–	• Examples: A549; liver tissue from adult Sprague-Dawley female rats

	Question/Prompt	Response Options	Suggested Considerations
	[Free-text]		• If there is more than one cell line name or tissue source studied, add an additional population in another form.
4.7	**In vitro System** *[Select one; Free-text]*	• Mammalian cells • Cell-free system • In silico system • Other	• If "other" option is selected, use the free text field below to describe the in vitro system. • If there is more than one in vitro source studied, add an additional population in another form.
4.8	**Select all study design elements that apply.** *[Select all that apply]*	• Multiple time points • Multiple cell/tissue types • Multiple dose levels	–
4.9	**Exposure Design** Use the free text field to describe the exposure design, be as succinct as possible. *[Free-text]*	–	–
4.10	**What is the exposure vehicle?** Use the free text field to describe the exposure vehicle, be as succinct as possible *[Free-text]*	–	–
4.11	**Dose Levels** Use the free text field to enter the numeric dose levels. *[Free-text]*	–	• Example: 0, 450, 900
4.12	**Dose Units** Use the free text field to report the dosage units as presented in the paper. *[Free-text]*	–	• Examples: ppm; mg/mL • Use "Not Reported" if appropriate.
4.13	**Dose Duration** Use the free text field to enter the details of the exposure duration. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y) o Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.
4.14	**Time Points Analyzed** Use the free text field to enter the time points data were analyzed. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y) o Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.

	Question/Prompt	Response Options	Suggested Considerations
5	• Notes		
5.1	**General Study Notes** *[Free-text]* Use the free text field to add any general study notes not captured above that may be of interest to the QC reviewer or PBPK modelers	–	• Please indicate whether the study contains information on PFOA/PFOS that is broken up by linear/branched isomers. Use the following phrase: "Contains linear/branched isomer information"
5.2	**Notes from Initial Extractor to QA/QC Team** Use the free text field to add any general study notes not captured above that may be of interest to the QC reviewer. *[Free-text]*	–	–
5.3	**Notes from QA/QC Team** Use the free text field to add any general study notes not captured above that may be of interest to the PBPK modelers. *[Free-text]*	–	–

Notes: ADME = absorption, distribution, metabolism, and/or excretion; QA/QC = quality assurance/quality control; MOA = mode of action; PBPK = physiologically based pharmacokinetic; TK = toxicokinetic; GD = gestational day; PND = postnatal day; ppm = parts per million.

A.1.6.8 Mechanistic Screening and Light Data Extraction

All studies identified as mechanistic in title/abstract or full-text screening were imported into litstream and underwent additional screening. Studies that were confirmed to be PECO relevant underwent light data extraction. For each study, at least two reviewers (one primary screener/extractor and one QA reviewer) reviewed the full study and any study materials to respond to prompts pertaining to key study elements (e.g., tested species or population, mechanistic endpoint(s) evaluated, lifestage(s) at which evaluations were performed). Table A-16 below describes the prompts and response options that were used for screening studies with mechanistic evidence.

Table A-16. Litstream Form for Mechanistic Screening and Light Data Extraction

	Question	Options	Suggested Considerations
1	**General Questions**		
1.1	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No	–
1.2	**What PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS	–
1.3	**Publication Type** *[Select one]*	• Primary research • Review article	–
1.4	**Indicate if there is hazard ID or supplemental data for this study.** *[Select all that apply; Free-text]*	• Animal tox • Epi • ADME	• Use free text field to provide an explanation.
2	**Human Studies Sub-Form** If the study does not contain a human study, skip this section and move on to Section 3 – Animal Studies Sub-Form.		
2.1	**Population/Study Group Name** *[Free-text]*	–	–
2.2	**Exposure Category** *[Select one; Free-text]*	• General environmental • Poisoning • Occupational • Developmental • Controlled experimental	• Free text field if additional information is needed.
2.3	**Identify the Exposure Route** *[Select all that apply]*	• Inhalation • Oral • Dermal • Lactational transfer	• Free text field to elaborate on “other” and “unknown” options.

	Question	Options	Suggested Considerations
		• In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intranasal) • Unknown	
2.4	**What is the exposure vehicle?** *[Select one]*	• Drinking water • Diet • Breast milk • In utero/placental transfer • Occupational • Unknown • Other	• Free text field to elaborate on "other" and "unknown" options.
2.5	**What is the lifestage when the mechanistic data was collected?** *[Select one; Free-text]*	• Prenatal • Infancy • Childhood • Adolescence • Adult • Elderly	• Free text for lifestage notes.
2.6	**What is the corresponding health outcome system?** *[Select one]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.

	Question	Options	Suggested Considerations
2.7	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics • Chromosome/DNA structure, function, repair or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, Morphology, or Morphometry • Other	• Free text field for "other" option.
2.8	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, non-targeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction • Extracellular matrix or molecules; Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and Immune Response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	• Free text field for "other" option.
2.9	**Mechanistic Endpoints** *[Free-text]*	–	• Free text field to list mechanistic endpoints.
3	• Animal Studies Sub-Form • If the study does not contain an animal study, skip this section and move on to Section 4 – In vitro Sub-Form.		
3.1	**Population/Study Group Name** *[Free-text]*	–	–
3.2	**What is the species?** *[Select one; Free-text]*	• Nonhuman primate • Zebrafish • Rat • Mouse • Rabbit • Guinea pig	• Free text field to list species for "other rodent model" option.

	Question	Options	Suggested Considerations
		• Other rodent model	
3.3	**What is the strain?** *[Free-text]*	–	–
3.4	**Identify the Exposure Route** *[Select one]*	• Inhalation (nose only) • Inhalation (whole head exposure) • Inhalation (whole body exposure) • Oral (diet) • Oral (drinking water) • Oral (gavage) • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intravenous, intranasal)	• Free text field for "other" option.
3.5	**What is the exposure vehicle?** *[Select one]*	• Diet • Water • Breast milk • In utero/placental transfer • Corn oil • Filtered air • Olive oil • Ethanol • DMSO • Mineral oil • Corn oil: acetone • Other	• Free text field for other "other" option.
3.6	**What is the lifestage when the animal was dosed?** *[Select one; Free-text]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Free text field for lifestage notes.
3.7	**What is the lifestage when the mechanistic data was collected?** *[Select one; Free-text]*	• Prenatal • Weaning • Adolescent • Adult	• Free text field for lifestage notes.

	Question	Options	Suggested Considerations
		• Elderly	
3.8	**What is the corresponding health outcome system?** *[Select all that apply; Free-text]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free text field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.
3.9	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics chromosome/DNA structure, function, repair, or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, Morphology, or Morphometry • Other	• Free text field for "other" option.
3.10	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, non-targeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction	• Free text field for "other" option.

	Question	Options	Suggested Considerations
		• Extracellular matrix or molecules • Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and Immune Response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	
3.11	**Mechanistic Endpoints** *[Free-text]*	–	• Free text field to list mechanistic endpoints
4	**In vitro Sub-Form** If the study does not contain an in vitro component, skip this section and move on to Section 5 – Notes.		
4.1	**Population/Study Group Name** *[Free-text]*	–	–
4.2	**Does the study present data on protein binding?** *[Select one; Free-text]*	• Yes • No	• Free text field if "Yes" to list binding proteins.
4.3	**Does the study present data on active transport?** *[Select one; Free-text]*	• Yes • No	• Free text field if "Yes" to list transporters.
4.4	**In vitro System** *[Select one; Free-text]*	• Mammalian cells • Cell-free system • In silico system • Other	• Free text field for "other" option.
4.5	**If a cellular model is used, is it a cell line or primary cells?** *[Select one]*	• Cell line • Primary cell	–
4.6	**Cell Or Tissue Source for In vitro/Ex Vivo Studies** *[Select one; Free-text]*	• Human • Zebrafish • Nonhuman primate • Rat • Mouse • Rabbit • Guinea pig	• Free text field to list "other rodent model" option.

	Question	Options	Suggested Considerations
		• Other rodent model	
4.7	**What is the corresponding health outcome system?** *[Select all that apply; Free-text]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free text field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.
4.8	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics chromosome/DNA structure, function, repair, or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, morphology, or morphometry • Other	• Free text field for "other" option.
4.9	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, non-targeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction	• Free text field for "other" option.

	Question	Options	Suggested Considerations
		• Extracellular matrix or molecules • Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and immune response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	
4.10	**Mechanistic Endpoints** *[Free-text]*	–	–
5	• Notes		
5.1	**General Study Notes** Use the free text field to add any general study notes not captured above that may be of interest to the QC reviewer or PBPK modelers. *[Free-text]*	–	• Please indicate whether the study contains information on PFOA/PFOS that is broken up by linear/branched isomers. Use the following phrase: "Contains linear/branched isomer information"
5.2	**Notes from Initial Extractor to QA/QC Team** Use the free text field to add any general study notes not captured above that may be of interest to the QC reviewer. *[Free-text]*	–	–
5.3	**Notes from QA/QC Team** Use the free text field to add any general study notes not captured above that may be of interest to the PBPK modelers. *[Free-text]*	–	–

Notes: ADME = absorption, distribution, metabolism, and/or excretion; DNA = deoxyribonucleic acid; DMSO = dimethyl sulfoxide, PBPK = physiologically based pharmacokinetic; QA/QC = quality assurance/quality control.

A.1.7 Study Quality Evaluation Overview

After literature search results were screened and inventoried, epidemiological and animal toxicological studies that met PECO criteria underwent study quality evaluation to assess each study's validity and utility. As outlined in the IRIS Handbook (U.S. EPA, 2022c), the key concerns during the review of epidemiological and animal toxicological studies are potential bias (factors that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect; low sensitivity is a bias toward the null when an effect exists). Study quality evaluations produce overall judgments about confidence in the reliability of study results. The general approach for study quality evaluation is outlined in Figure A-1, which has been adapted from Figure 4-1 in the IRIS Handbook (U.S. EPA, 2022c) (previously Figure 6-1 in the draft IRIS Handbook (U.S. EPA, 2020a)). Study quality evaluations were performed using the structured platform for study evaluation housed within EPA's Health Assessment Workplace Collaborative (HAWC).

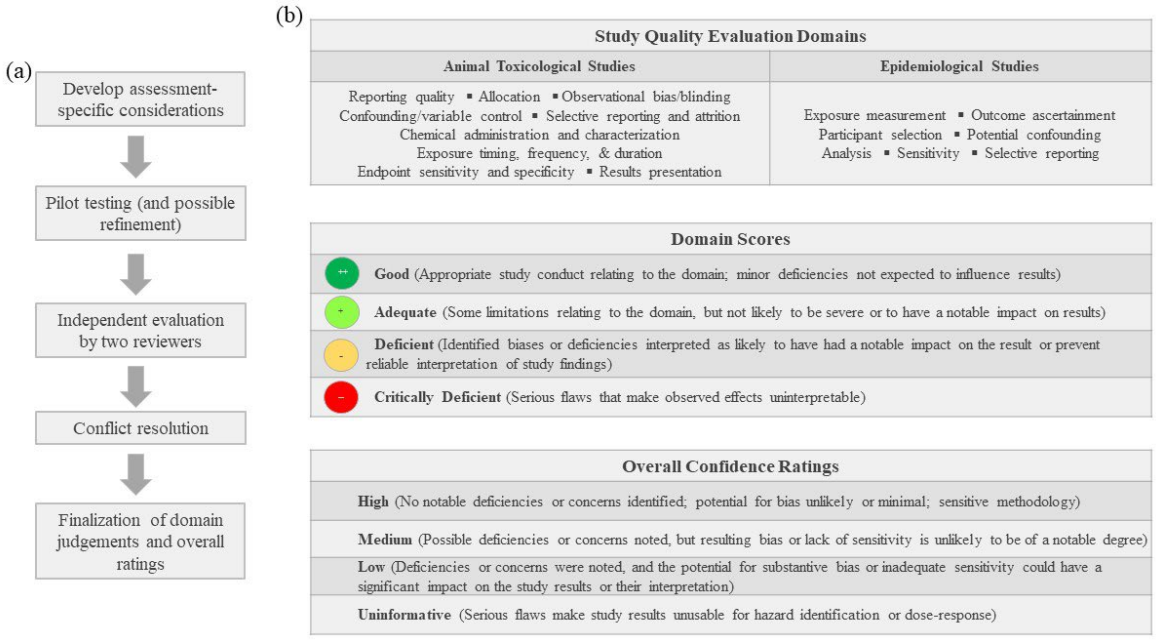


Figure A-1. Overview of Study Quality Evaluation Approach

(a) An overview of the study quality evaluation process; (b) Evaluation domains and ratings definitions (i.e., domain scores and overall confidence ratings, performed on an outcome-specific basis as applicable).

The overall aims of study quality evaluation are the same for both epidemiological and animal toxicological studies, but some aspects of the approaches are different. Therefore, study quality evaluation procedures for epidemiological and animal toxicological studies are described separately in the following sections. In brief, at least two primary reviewers independently judged the reliability of the study results according to multiple study quality evaluation domains presented in the IRIS Handbook. Domain-specific core and prompting questions are provided to guide the reviewer in assessing different aspects of study design and conduct related to reporting, risk of bias, and study sensitivity. For each domain, each reviewer assigned a rating of good, adequate, deficient (or "not reported," which carried the same functional interpretation as

deficient), or critically deficient (see Figure A-1 and Table A-17). A QA reviewer (in accordance with protocols outlined in the IRIS Handbook) engaged in conflict resolution with the two independent reviewers as needed and made a final determination (reflected as study confidence ratings; see Figure A-1 and Table A-18) regarding each health outcome or outcome grouping of interest; thus, different "judgments" were possible for different health outcomes within the same study. The overall confidence rating should, to the extent possible, reflect interpretations of the potential influence on the results across all domains. The rationale supporting the overall confidence rating is documented clearly and consistently and includes a brief description of any important study strengths and/or limitations and their potential impact on the overall confidence.

The specific study limitations identified during study quality evaluation were carried forward to inform the synthesis of findings within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform "judgments" in isolation).

Studies containing PBPK, mechanistic or ADME data did not undergo study quality evaluation, as study quality domains for these types of studies are not currently available (U.S. EPA, 2022b).

Table A-17. Possible Domain Scores for Study Quality Evaluation

Good	Intended to represent a judgment that there was appropriate study conduct relating to the domain (as defined by consideration of the criteria listed below), and any minor deficiencies that were noted would not be expected to influence interpretation of the study findings.
Adequate	Indicates a judgment that there were study design limitations relating to the domain (as defined by consideration of the criteria listed below), but that those limitations are not likely to be severe and are expected to have minimal impact on interpretation of the study findings.
Deficient	Denotes identified biases or limitations that are interpreted as likely to have had a substantial impact on the results or that prevent reliable interpretation of the study findings. **Note: Not reported** indicates that the information necessary to evaluate the domain was not available in the study. Generally, this term carries the same functional interpretation as **Deficient** for the purposes of the study confidence classification.
Critically Deficient	Reflects a judgment that the study design limitations relating to the domain introduced a flaw so serious that the study should not be used without exceptional justification (e.g., it is the only study of its kind and may highlight possible research gaps). This judgment should only be used if there is an interpretation that the limitation(s) would be the primary driver of any observed effect(s), or if it makes the study findings uninterpretable.

Table A-18. Overall Study Confidence Classifications

***High* Confidence**	No notable concerns were identified (e.g., most or all domains rated **Good**).
***Medium* Confidence**	Some concerns are identified but expected to have minimal impact on the interpretation of the results (e.g., most domains rated **Adequate** or **Good**; may include studies with **Deficient** ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.
***Low* Confidence**	Identified concerns are expected to significantly impact the study results or their interpretation (e.g., generally, **Deficient** ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis.

Uninformative	Serious flaw(s) make the study results unusable for informing hazard identification (e.g., generally, **Critically Deficient** rating in any domain; many **Deficient** ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.

A.1.7.1 Study Quality Evaluation for Epidemiological Studies

Study quality evaluation domains for assessing risk of bias and sensitivity in epidemiology studies of health effects are: participant selection, exposure measurement, outcome ascertainment, potential confounding, analysis, study sensitivity, and selective reporting. As noted in the IRIS Handbook, this framework is adapted from the Risk Of Bias in Nonrandomized Studies of Interventions (ROBINS-I) tool (https://methods.cochrane.org/methods-cochrane/robins-i-tool), modified by IRIS for use with the types of studies more typically encountered in EPA's work. As outlined in Section A.1.7 of this appendix, study quality evaluations are performed for a set of established domains, and core and prompting questions are provided for each domain to guide the reviewer. Each domain is assigned a score of **Good**, **Adequate**, **Deficient**, **Not Reported** or **Critically Deficient,** and rationales to support the scores are developed. Once all domains are evaluated, a confidence rating of ***High***, ***Medium***, or ***Low*** confidence or ***Uninformative*** is assigned.

The tables presented in the following sections describe the epidemiological study quality evaluation domains and the prompting questions and considerations for assessing study quality in relation to each domain.

A.1.7.1.1 Participant Selection

The aim of study quality evaluation for this domain is to ascertain whether the reported information indicates that selection in or out of the study (or analysis sample) and participation was not likely to be biased (i.e., the exposure-outcome distribution of the participants is likely representative of the exposure-outcome distribution in the overall population of eligible persons) (Table A-19).

Table A-19. Study Quality Evaluation Considerations for Participant Selection

Core Question: Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
For longitudinal cohort: Did participants volunteer for the cohort based on knowledge of exposure and/or preclinical disease symptoms? Was entry into the cohort or continuation in the cohort related to exposure and outcome? ***For occupational cohort:*** Did entry into the cohort begin with the start of the exposure? Was follow-up or outcome assessment incomplete, and if so, was follow-up related to both exposure and outcome status? Could exposure produce symptoms that would result in a change in work assignment/work status ("healthy worker survivor effect")? ***For case-control study:*** Were controls representative of population and time periods from which cases were drawn?	Were differences in participant enrollment and follow-up evaluated to assess the potential for bias? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)? Were appropriate analyses performed to address changing exposures over time in relation to symptoms? Is there a comparison of participants and	**Good**	• Minimal concern for selection bias based on description of recruitment process (e.g., selection of comparison population, population-based random sample selection, recruitment from sampling frame including current and previous employees) such that study participants were unlikely to differ from a larger cohort based on recruitment or enrollment methods (or data provided to confirm a lack of difference) • Exclusion and inclusion criteria specified and would not be likely to induce bias. • Participation rate is reported at all steps of study (e.g., initial enrollment, follow-up, selection into analysis sample). If rate is not high, there is appropriate rationale for why it is unlikely to be related to exposure (e.g., comparison between participants and nonparticipants or other available information indicates differential selection is not likely). • Comparison groups are similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers).

Core Question: Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?			
Are hospital controls selected from a group whose reason for admission is independent of exposure? Could recruitment strategies, eligibility criteria, or participation rates result in differential participation relating to both disease and exposure? ***For population based-survey:*** Was recruitment based on advertisement to people with knowledge of exposure, outcome, and hypothesis?	nonparticipants to address whether differential selection is likely?	**Adequate**	• Enough of a description of the recruitment process (i.e., recruitment strategy, participant selection or case ascertainment) to be comfortable that there is no serious risk of bias. • Inclusion and exclusion criteria specified and would not induce bias. • Participation rate is incompletely reported for some steps of the study, but available information indicates participation is unlikely to be related to exposure. • Comparison groups are largely similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers) or these are mostly accounted for in the study analysis.
		Deficient	• Little information on recruitment process, selection strategy, sampling framework and/or participation OR aspects of these processes raises the likelihood of bias (e.g., healthy worker effect, survivor bias). *Example: Enrollment of "cases" from a specific clinic setting (e.g., diagnosed autism), which could be biased by referral practices and services availability, without consideration of similar selection forces affecting recruitment of controls.*
		Critically Deficient	• Aspects of the processes for recruitment, selection strategy, sampling framework, or participation result in concern that the likelihood of selection bias is high (e.g., convenience sample with no information about recruitment and selection, cases and controls are recruited from different sources with different likelihood of exposure, recruitment materials stated outcome of interest and potential participants are aware of or are concerned about specific exposures). • Convenience sample, and recruitment and selection not described. • Case report, case series, or other study designs lacking a comparison group (these should be excluded if they do not meet assessment PECO criteria).

A.1.7.1.2 Exposure Measurement

This domain may need to be evaluated multiple times for a single study if more than one measurement of exposure is assessed. Therefore, different sets of criteria may be applied for different exposure assessments in the same study. Table A-20 outlines criteria that apply across exposure assessments (first row), and specific *additional* criteria for specific types of exposure assessments (e.g., biomarkers, occupational) in subsequent rows.

Table A-20. Study Quality Evaluation Considerations for Exposure Measurement

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?

Prompting Questions	Follow-Up Questions	Suggested Considerations	
Does the exposure measure capture the variability in exposure among the participants, considering intensity, frequency, and duration of exposure? Does the exposure measure reflect a relevant time window? If not, can the relationship between measures in this time and the relevant time window be estimated reliably? Was the exposure measurement likely to be affected by a knowledge of the outcome? Was the exposure measurement likely to be affected by the presence of the outcome (i.e., reverse causality)?	Is the degree of exposure misclassification likely to vary by exposure level? If the correlation between exposure measurements is of concern, is there an adequate statistical approach to ameliorate variability in measurements? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Valid exposure assessment methods used, which represent the etiologically relevant time period for reported effects (e.g., exposure during a critical developmental window or exposure preceding the evaluation of the outcome). • Exposure misclassification is expected to be minimal.
		Adequate	• Valid exposure assessment methods used, which represent the etiologically relevant time period of interest. • Exposure misclassification may exist but is not expected to greatly impact the effect estimate.
		Deficient	• Specific knowledge about the exposure and outcome raise concerns about reverse causality, but there is uncertainty whether it is influencing the effect estimate. • Exposed groups are expected to contain a notable proportion of unexposed or minimally exposed individuals, the method did not capture important temporal or spatial variation, or there is other evidence of exposure misclassification that would be expected to notably change the effect estimate.
		Critically Deficient	• Exposure measurement does not characterize the etiologically relevant time period of exposure or is not valid. • There is evidence that reverse causality is very likely to account for the observed association. • Exposure measurement was not independent of outcome status.

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?

Additional prompting questions for biomarkers of exposure: Is a standard assay used? What are the intra- and inter-assay coefficients of variation? Is the assay likely to be affected by contamination? Are values less than the limit of detection dealt with adequately? What exposure time period is reflected by the biomarker? If the half-life is short, what is the correlation between serial measurements of exposure?	***Additional suggested considerations for biomarkers of exposure (should be evaluated in addition to the general considerations above):***	
	Good	• Use of appropriate analytic method such as [specific gold standard exposure assessment method for the exposure of interest].
	Adequate	• Use of appropriate (but not gold standard) analytic method.
	Deficient	• Did not identify analytical methods used to measure exposure. • Failure to report LOD, percentage less than LOD, and methods used to account for values below the LOD. • Failure to report QA/QC measures and results.
	Critically Deficient	• Use of inappropriate analytical method or use of an appropriate method with measurement issues that are likely to impact the interpretation of results.
Additional prompting questions for case-control studies of occupational exposures: Is exposure based on a comprehensive job history describing tasks, setting, time period, and use of specific materials?	***Additional suggested considerations for occupational exposures (should be evaluated in addition to the general considerations above):***	
	Good	• Describes the use of personal protective equipment. • Confirmed contrast in exposure between groups using biomarker measurements. • Expert assessment method based on a detailed lifetime occupational history and using a high-quality, validated job exposure matrix (JEM) or a JEM that incorporates industry, time period, population/country, tasks, and material used.
	Adequate	• Describes the use of personal protective equipment. • Confirmed contrast in exposure between groups using biomarker measurements.
	Deficient	• Expert assessment method based on incomplete occupational history information (lacking job titles, employers, industries, start and finish years, number of hours worked per day, number of days worked per week, tasks performed, or materials used) – may be Critically Deficient, depending on severity of this limitation.

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?		
	Critically Deficient	• JEM with data indicating it cannot differentiate between exposure levels over time, area, or between individuals.

PFAS-Specific Exposure Measurement Study Quality Evaluation Criteria

Standard analytical methods of individual PFAS in serum or whole blood using quantitative techniques, such as liquid chromatography triple quadrupole mass spectrometry, are considered well-established methods (Table A-21).

Table A-21. Criteria for Evaluating Exposure Measurement in Epidemiology Studies of PFAS and Health Effects

Rating	Criteria
Good	• Evidence that exposure was consistently assessed using well-established analytical methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma). **OR** • Exposure was assessed using less established methods (e.g., measurement of PFAS in breast milk) or methods that indirectly measure exposure (e.g., drinking water concentrations and residential location/history, questionnaire or occupational exposure assessment by a certified industrial hygienist) that are supported by well-established methods (i.e., inter-methods validation: one method vs. another) in the target population of interest. **And all the following:** • Exposure was assessed in a relevant time-window (i.e., temporality is established, and sufficient latency occurred prior to disease onset) for development of the outcome based on current biological understanding. • There is evidence that sufficient exposure data measurements are above the limit of quantification for the assay. • The laboratory analysis included data on standard quality control measures with demonstrated precision and accuracy.
Adequate	• Exposure was assessed using less established methods or indirect measures that are validated but not in the target population of interest. **OR** • Evidence that exposure was consistently assessed using methods described in Good, but there were some concerns about quality control measures or other potential for nondifferential misclassification. **And all the following:** • Exposure was assessed in a relevant time-window for development of the outcome. • There is evidence that sufficient exposure data measurements are above the limit of quantification for the assay. • The laboratory analysis included some data on standard quality control measures with demonstrated precision and accuracy.
Deficient	**Any of the following:** • Some concern, but no direct evidence, that the exposure was assessed using methods that have not been validated or empirically shown to be consistent with methods that directly measure exposure. • Exposure was assessed in a relevant time window(s) for development of the outcome, but there could be some concern about the potential for bias due to reverse causality[a] between exposure and outcome, yet no direct evidence that it is present; or has somehow been mitigated by the design, etc.
Critically Deficient	**Any of the following:** • Exposure was assessed in a time window that is unknown or not relevant for development of the outcome. This could be due to clear evidence of bias from reverse causality between exposure and outcome, or other concerns such as the lack of temporal ordering of exposure and disease onset, insufficient latency, or having exposure measurements that are not reliable measures of exposure during the etiologic window(s). • Direct evidence that bias was likely because the exposure was assessed using methods with poor validity.

Rating	Criteria
	• Evidence of differential exposure misclassification (e.g., differential recall of self-reported exposure). • There is evidence that an insufficient number of the exposure data measurements were above the limit of quantification for the assay.

Notes:

[a] Reverse causality refers to a situation where an observed association between exposure and outcome is not due to causality from exposure to outcome, but rather due to the outcome of interest causing a change in the measured exposure.

A.1.7.1.3 Outcome Ascertainment

This domain may need to be evaluated multiple times for a single study if more than one PECO-relevant outcome is reported. Therefore, outcome-specific criteria (Radke et al., 2019) may be applied for each outcome measured in a study. Table A-22 presents criteria that apply across outcomes.

Table A-22. Study Quality Evaluation Considerations for Outcome Ascertainment

Core Question: Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?

Prompting Questions	Follow-Up Questions		Suggested Considerations
Is outcome ascertainment likely to be affected by knowledge of, or presence of, exposure (e.g., consider access to healthcare, if based on self-reported history of diagnosis)? ***For case-control studies:*** Is the comparison group without the outcome (e.g., controls in a case-control study) based on objective criteria with little or no likelihood of inclusion of people with the disease? ***For mortality measures:*** How well does cause of death data reflect occurrence of the disease in an individual? How well do mortality data reflect incidence of the disease? ***For diagnosis of disease measures:*** Is the diagnosis based on standard clinical criteria? If it is based on self-report of the diagnosis, what is the validity of this measure? ***For laboratory-based measures (e.g., hormone levels):*** Is a standard assay used? Does the assay have an acceptable level of inter-assay variability? Is the sensitivity of the assay appropriate for the	Is there a concern that any outcome misclassification is nondifferential, differential, or both? What is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• High certainty in the outcome definition (i.e., specificity and sensitivity), minimal concerns with respect to misclassification. • Assessment instrument was validated in a population comparable to the one from which the study group was selected.

Core Question: Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?

outcome measure in this study population? Were QA/QC measures and results reported?		
	Adequate	• Moderate confidence that outcome definition was specific and sensitive, some uncertainty with respect to misclassification but not expected to greatly change the effect estimate. • Assessment instrument was validated but not necessarily in a population comparable to the study group.
	Deficient	• Outcome definition was not specific or sensitive. • Uncertainty regarding validity of assessment instrument.
	Critically Deficient	• Invalid/insensitive marker of outcome. • Outcome ascertainment is very likely to be affected by knowledge of, or presence of, exposure. **Note:** Lack of blinding should not be automatically construed to be ***Critically Deficient***.

Notes: QA/QC = quality assurance/quality control.

A.1.7.1.4 Potential Confounding

The aim of evaluating this domain is to ascertain whether confounding of the relationship between the exposure and health outcome of interest is likely to exist, and if so, whether it was considered in the design and/or analysis of the study (Table A-23). Co-exposures to other PFAS were considered in this domain.

Table A-23. Study Quality Evaluation Considerations for Confounding

Core Question: Is confounding of the effect of the exposure likely?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
Is confounding adequately addressed by considerations in: • Participant selection (matching or restriction)? • Accurate information on potential confounders and statistical adjustment procedures? • Lack of association between confounder and outcome, or confounder and exposure in the study? • Information from other sources? Is the assessment of confounders based on a thoughtful review of published literature, potential relationships (e.g., as can be gained through directed acyclic graphing), and minimizing potential overcontrol (e.g., inclusion of a variable on the pathway between exposure and outcome)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Conveys strategy for identifying key confounders. This may include: a priori biological considerations, published literature, causal diagrams, or statistical analyses; with recognition that not all "risk factors" are confounders. • Inclusion of potential confounders in statistical models not based solely on statistical significance criteria (e.g., $p < 0.05$ from stepwise regression). • Does not include variables in the models that are likely to be influential colliders or intermediates on the causal pathway. • Key confounders are evaluated appropriately and considered to be unlikely sources of substantial confounding. This often will include: o Presenting the distribution of potential confounders by levels of the exposure of interest and/or the outcomes of interest (with amount of missing data noted); o Consideration that potential confounders were rare among the study population, or were expected to be poorly correlated with exposure of interest; o Consideration of the most relevant functional forms of potential confounders; o Examination of the potential impact of measurement error or missing data on confounder adjustment; o Presenting a progression of model results with adjustments for different potential confounders, if warranted.

Core Question: Is confounding of the effect of the exposure likely?		
	Adequate	• Similar to **Good** but may not have considered all potential confounders (though all key confounders were considered), or less detail may be available on the evaluation of confounders (e.g., sub-bullets in **Good**). It is possible that residual confounding could explain part of the observed effect, but concern is minimal.
	Deficient	• All key confounders were not considered by design or in the statistical analysis. • Assessed an outcome based on report of medical diagnosis that would have required access to a health professional (e.g., autism, ADHD, depression) and failed to consider some marker of socioeconomic status (e.g., maternal education, household income, marital status, crowding, poverty, job status) as a potential confounder. • Does not include variables in the models that are likely to be influential colliders or intermediates on the causal pathway. **And any of the following:** • The potential for bias to explain some of the results is high based on an inability to rule out residual confounding, such as a lack of demonstration that key confounders of the exposure-outcome relationships were considered; • Descriptive information on key confounders (e.g., their relationship relative to the outcomes and exposure levels) is not presented; or • Strategy of evaluating confounding is unclear or is not recommended (e.g., only based on statistical significance criteria or stepwise regression [forward or backward elimination]).
	Critically	• Includes variables in the models that are colliders and/or intermediates in the causal pathway, indicating that substantial bias is likely from this adjustment; or • Substantial confounding is likely present and not accounted for, such that all of the results were most likely due to bias. • If confounders not considered by design or in the analysis (e.g., only simple correlations presented).

Notes: ADHD = attention deficit hyperactivity disorder.

A.1.7.1.5 Analysis

Information relevant to evaluation of analysis includes, but is not limited to: the extent (and if applicable, treatment) of missing data for exposure, outcome, and confounders; approach to modeling; classification of exposure and outcome variables (continuous vs. categorical); testing of assumptions; sample size for specific analyses; and relevant sensitivity analyses (Table A-24).

Table A-24. Study Quality Evaluation Considerations for Analysis

Core Question: Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?			
Prompting Questions	**Follow-Up Questions**	**Suggested Considerations**	
Are missing outcome, exposure, and covariate data recognized, and if necessary, accounted for in the analysis? Does the analysis appropriately consider variable distributions and modeling assumptions? Does the analysis appropriately consider subgroups of interest (e.g., based on variability in exposure level or duration or susceptibility)? Is an appropriate analysis used for the study design? Is effect modification considered, based on considerations developed a priori? Does the study include additional analyses addressing potential biases or limitations (i.e., sensitivity analyses)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Use of an optimal characterization of the outcome variable. • Quantitative results presented (effect estimates and confidence limits or variability in estimates (e.g., standard error, standard deviation); i.e., not presented only as a p-value or "significant"/"not significant"). • Descriptive information about outcome and exposure provided (where applicable). • Amount of missing data noted and addressed appropriately (discussion of selection issues – missing at random vs. differential). • Where applicable, for exposure, includes LOD (and percentage below the LOD), and decision to use log transformation. • Includes analyses that address robustness of findings, e.g., examination of exposure-response (explicit consideration of nonlinear possibilities, quadratic, spline, or threshold/ceiling effects included, when feasible); relevant sensitivity analyses; effect modification examined based only on a priori rationale with sufficient numbers. • No deficiencies in analysis evident. Discussion of some details may be absent (e.g., examination of outliers).
		Adequate	• Same as Good, except: • Descriptive information about exposure provided (where applicable) but may be incomplete; might not have discussed missing data, cut points, or shape of distribution. • Includes analyses that address robustness of findings (examples in Good), but some important analyses are not performed.

Core Question: Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?		
	Deficient	• Descriptive information about exposure levels not provided (where applicable). • Effect estimate and p-value presented, without standard error or confidence interval (where applicable). • Results presented as statistically "significant"/"not significant."
	Critically Deficient	• Results of analyses of effect modification examined without clear a priori rationale and without providing main/principal effects (e.g., presentation only of statistically significant interactions that were not hypothesis driven). • Analysis methods are not appropriate for design or data of the study.

Notes: LOD = limit of detection.

A.1.7.1.6 Selective Reporting

This domain concerns the potential for misleading results that can arise from selective reporting (e.g., of only a subset of the measures or analyses that were conducted). The concept of selective reporting involves the selection of results from among multiple outcome measures, multiple analyses, or different subgroups, based on the direction or magnitude of these results (e.g., presenting "positive" results) (Table A-25).

Table A-25. Study Quality Evaluation Considerations for Selective Reporting

Core Question: Is there reason to be concerned about selective reporting?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
Were results provided for all the primary analyses described in the methods section? Is there appropriate justification for restricting the amount and type of results that are shown? Are only statistically significant results presented?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Adequate**	• The results reported by study authors are consistent with the primary and secondary analyses described in a registered protocol or methods paper **OR** • The authors described their primary (and secondary) analyses in the methods section and results were reported for all primary analyses.
		Deficient	• Concerns were raised based on previous publications, a methods paper, or a registered protocol indicating that analyses were planned or conducted that were not reported, or that hypotheses originally considered to be secondary were represented as primary in the reviewed paper. • Only subgroup analyses were reported; results for the entire group were omitted without any justification (e.g., to address effect measure modification). • Of the PECO-relevant outcomes examined, only statistically significant results were reported.

A.1.7.1.7 Study Sensitivity

The aim of evaluation of this domain is to determine if there are features of the study that affect its ability to detect a true association (Table A-26). Some of the study features that can affect study sensitivity may have already been included in the outcome, exposure, or other categories, such as the validity of a method used to ascertain an outcome, the ability to characterize exposure in a relevant time period for the outcome under consideration, selection of affected individuals out of the study population, or inappropriate inclusion of intermediaries in a model.

Other features may not have been addressed, and so should be included here. Examples include the exposure range (e.g., the contrast between the "low" and "high" exposure groups within a study), the level or duration of exposure, and the length of follow-up. In some cases (for very rare outcomes), sample size or number of observed cases may also be considered within this "sensitivity" category.

Table A-26. Study Quality Evaluation Considerations for Study Sensitivity

Core Question: Is there a concern that sensitivity of the study is not adequate to detect an effect?			
Prompting Questions	**Follow-Up Questions**	**Suggested Considerations**	
Is the exposure range/contrast adequate to detect associations that are present? Was the appropriate (at risk) population included? Was the length of follow-up adequate? Is the time/age of outcome ascertainment optimal given the interval of exposure and the health outcome? Are there other aspects related to risk of bias or otherwise that raise concerns about sensitivity?	–	**Adequate**	• The range of exposure levels provides adequate variability to evaluate primary hypotheses in study. • The population was exposed to levels expected to have an impact on response. • The study population was sensitive to the development of the outcomes of interest (e.g., ages, lifestage, sex). • The timing of outcome ascertainment was appropriate given expected latency for outcome development (i.e., adequate follow-up interval). • The main effects and stratified analyses were fairly precise (relatively small confidence bounds) • The study was adequately powered to observe an effect. Consider sample size, precision (e.g., width of confidence intervals), anticipated power, exposure ranges and contrasts. • No other concerns raised regarding study sensitivity.
		Deficient	• Concerns were raised about the issues described for ***Adequate*** that are expected to notably decrease the sensitivity of the study to detect associations for the outcome.

A.1.7.1.8 Overall Confidence

Table A-27. Evaluation Considerations for Overall Study Confidence – Overall Confidence, Epidemiological Studies

Provide judgement and rationale for each endpoint or groups of endpoints. The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results. Evaluation Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?

Prompting Questions		Suggested Considerations
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects? *NOTE: Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias towards the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	***High* confidence**	• No notable concerns are identified (e.g., most or all domains rated Good).
	***Medium* confidence**	• Some concerns are identified but expected to have minimal impact on the interpretation of the results. (e.g., most domains rated Adequate or Good; may include studies with Deficient ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.
	***Low* confidence**	• Identified concerns are expected to significantly impact on the study results or their interpretation (e.g., generally, Deficient ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note).
	Uninformative	• Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, Critically Deficient rating in any domain; many Deficient ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.

A.1.7.2 Study Quality Evaluation for Animal Toxicological Studies

As noted in the IRIS Handbook, the approach to evaluating study quality for animal toxicological studies considers study design and experimental conduct in the context of reporting quality, risk of bias, and study sensitivity. As outlined in Section A.1.7 of this appendix, study quality evaluations are performed for a set of established domains, and core and prompting questions are provided for each domain to guide the reviewer. Each domain is assigned a score of **Good**, **Adequate**, **Deficient**, **Not Reported** or **Critically Deficient,** and rationales to support the scores are developed. Once all domains are evaluated, a confidence rating of ***High***, ***Medium***, or ***Low*** confidence or ***Uninformative*** is assigned for each endpoint/outcome from the study.

The tables in the following sections describe the core and prompting questions and considerations for assessing each domain during animal toxicological study quality evaluation. Tables within each section also provide example evaluations for each domain.

A.1.7.2.1 Reporting Quality

Evaluation of this domain is focused on ascertaining whether the study reports enough information to enable evaluation of the study (Table A-28).

Table A-28. Study Quality Evaluation Considerations for Reporting Quality

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
Does the study report the following? **<u>Critical information</u> necessary to perform study evaluation:** • Species; test article name; levels and duration of exposure; route (e.g., oral; inhalation); qualitative or quantitative results for at least one endpoint of interest **<u>Important information</u> <u>for evaluating the study methods:</u>** • Test animal: strain, sex, source, and general husbandry procedures • Exposure methods: source, purity, method of administration • Experimental design: frequency of exposure, animal age and lifestage during exposure and at endpoint/outcome evaluation • Endpoint evaluation methods: assays or procedures used to measure the endpoints/outcomes of interest	**Good**	• Minimal concern for selection bias based on description of recruitment process (e.g., selection of comparison population, population-based random sample selection, recruitment from sampling frame including current and previous employees) such that study participants were unlikely to differ from a larger cohort based on recruitment or enrollment methods (or data provided to confirm a lack of difference) • Exclusion and inclusion criteria specified and would not be likely to induce bias. • Participation rate is reported at all steps of study (e.g., initial enrollment, follow-up, selection into analysis sample). If rate is not high, there is appropriate rationale for why it is unlikely to be related to exposure (e.g., comparison between participants and nonparticipants or other available information indicates differential selection is not likely). • Comparison groups are similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers).	• Good. Important information is provided for test species, strain, sex, age, exposure methods, experimental design, endpoint evaluations and the presentation of results. • The authors report that "the study was conducted in compliance with the OECD guidelines for Good Laboratory Practice [c(81) 30 (Final)]."

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?			
Note: • Reviewers should reach out to authors to obtain missing information when studies are considered key for hazard evaluation and/or dose-response. • This domain is limited to reporting. Other aspects of the exposure methods, experimental design, and endpoint evaluation methods are evaluated using the domains related to risk of bias and study sensitivity.	**Adequate**	• Enough of a description of the recruitment process (i.e., recruitment strategy, participant selection or case ascertainment) to be comfortable that there is no serious risk of bias. • Inclusion and exclusion criteria specified and would not induce bias. • Participation rate is incompletely reported for some steps of the study, but available information indicates participation is unlikely to be related to exposure. • Comparison groups are largely similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers) or these are mostly accounted for in the study analysis.	• Adequate. All critical information is reported but some important information is missing. Specifically, it is unclear what strain of rats was used.
	Deficient	• Little information on recruitment process, selection strategy, sampling framework and/or participation OR aspects of these processes raises the likelihood of bias (e.g., healthy worker effect, survivor bias). *Example: Enrollment of "cases" from a specific clinic setting (e.g., diagnosed autism), which could be biased by referral practices and services availability, without consideration of similar selection forces affecting recruitment of controls.*	• Deficient. All critical information is reported, but some important information is missing that makes additional study evaluation and interpretation of the results difficult. Specifically, it is not reported (and cannot be inferred) what age/lifestage the animals were at outcome evaluation.
	Critically Deficient	• Aspects of the processes for recruitment, selection strategy, sampling framework, or participation result in concern that the likelihood of selection bias is high (e.g., convenience sample with no information about recruitment and selection, cases and controls are recruited from different sources with different likelihood of exposure, recruitment materials stated outcome of interest and	• **Example 1**: Critically Deficient. Critical information is missing. Authors did not report the duration of the exposure or the results (qualitative or quantitative). • **Example 2:** Critically Deficient. Critical information is missing. The study reports animals were exposed to per-and polyfluoroalkyl substances (PFAS), but the specific chemicals tested were not provided.

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?		
		potential participants are aware of or are concerned about specific exposures). • Convenience sample, and recruitment and selection not described. • Case report, case series, or other study designs lacking a comparison group (these should be excluded if they do not meet assessment PECO criteria).

Notes: OECD = Organisation for Economic Co-operation and Development.

For the Reporting domain, the **Deficient** rating was used as a flag to potentially reach out to study authors to obtain missing critical information (e.g., blinding, randomization) that may impact the overall confidence rating of the study (e.g., from b confidence to ***Medium*** confidence). A **Deficient** rating does not necessarily relegate the study to ***Low*** confidence, but it is an indicator that obtaining information from the study authors may change the overall confidence rating. EPA could then judge if it was necessary to contact the study authors. If the study received a **Deficient** rating for this domain and correspondence with the study authors could potentially increase the confidence, a statement was added to indicate that obtaining information from the study authors could impact the confidence.

If EPA followed up with authors to obtain missing information, the study details page was updated to note that the authors were contacted and provided the corresponding details.

A.1.7.2.2 Selection and Performance – Allocation

Table A-29. Study Quality Evaluation Considerations for Selection and Performance – Allocation

Core Question: Were animals assigned to experimental groups using a method that minimizes selection bias?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
For each study: Did each animal or litter have an equal chance of being assigned to any experimental group (i.e., random allocation)? Is the allocation method described? Aside from randomization, were any steps taken to balance variables across experimental groups during allocation?	**Good**	• Experimental groups were randomized and any specific randomization procedure was described or inferable (e.g., computer-generated scheme). [Note that normalization is not the same as randomization (see response for 'Adequate').]	• Good. The study authors report that "Fifty males and fifty females were randomly assigned to groups by a computer-generated weight-ordered distribution such that individual body weights did not exceed + 20% of the mean weight for each sex."
	Adequate	• Authors report that groups were randomized but do not describe the specific procedure used (e.g., 'animals were randomized'). Alternatively, authors used a non-random method to control for important modifying factors across experimental groups (e.g., body weight normalization).	• **Example 1**: Adequate. Randomization was not performed. However, normalization procedures that balance important variables across groups were performed. Specifically, the authors state that animals were "allocated into groups with similar distributions in body weight." • **Example 2**: Adequate. The study authors state that "animals were randomly distributed to exposure groups." However, the specific randomization method used was not described. • **Example 3**: Adequate. Randomization was not explicitly reported. However, the study was performed according to OECD 416 and EPA OPPT 870.3800 guidelines which both specify randomization, although the specific methods of randomization used in the current study could not be inferred. OECD 416 guidelines state "animals should be randomly assigned to the control and

Core Question: Were animals assigned to experimental groups using a method that minimizes selection bias?			
			treated groups (stratification by body weight is recommended).” The EPA OPPT 870.3800 guidelines state “animals should be randomly assigned to the control and treatment groups, in a manner which results in comparable mean body weight values among all groups.” • • **Example 4:** Adequate. The study authors state that "Animals were randomized by weight into treatment groups," and do not present the specific randomization procedural details.
	Not Reported (Interpreted as Deficient)	• No indication of randomization of groups or other methods (e.g., normalization) to control for important modifying factors across experimental groups.	• Not reported (interpreted as Deficient). The authors did not indicate randomization or other normalization procedures for balancing important variables across groups.
	Critically Deficient	• Bias in the animal allocations was reported or inferable.	• Critically Deficient. There is direct evidence that animals were allocated to treatment groups in a subjective way, involving the judgment of the investigator. Specifically, the study authors report “the heavier dams were assigned to the higher dose groups to reduce toxicity from [chemical]”; dam weight is an important variable for these developmental outcomes.

Notes: OECD = Organisation for Economic Co-operation and Development; OPPT = Office of Pollution Prevention and Toxics.

A.1.7.2.3 Selection and Performance – Observational Bias/Blinding

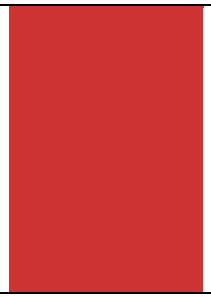
Table A-30. Study Quality Evaluation Considerations for Selection and Performance – Observational Bias/Blinding

Core Question: Did the study implement measures to reduce observational bias?

Prompting Questions		Suggested Considerations	Example Answers
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the study report blinding or other methods/procedures for reducing observational bias? If not, did the study use a design or approach for which such procedures can be inferred? What is the expected impact of failure to implement (or report implementation) of these methods/procedures on results?	**Good**	• Measures to reduce observational bias were described (e.g., blinding to conceal treatment groups during endpoint evaluation; consensus-based evaluations of histopathology lesions[a]).	• **Example 1:** Good. Histopathology: Although the study did not indicate blinding, blinding during the initial evaluation of tissues for initial or non-targeted evaluations is generally not recommended as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004). The study did include a secondary evaluation by a pathology working group (PWG) review on coded pathology slides which minimized the potential for observational bias. • **Example 2:** Good. Organ weights, FOB, motor activity, swim maze and histopathology: Authors reported that the investigators were blinded to the animal treatment group during evaluation for all outcome measures. Although blinding is not recommended for initial or non-targeted evaluations (Crissman et al., 2004), this study evaluated prespecified outcomes in targeted evaluations for which blinding is appropriate (cell counts in the CA3 region of the hippocampus).
	Adequate	• Methods for reducing observational bias (e.g., blinding) can be inferred or were reported but described incompletely.	• Adequate. Histopathology measures: Authors report "lesions were counted by 2 observers in a blinded fashion" although it should be noted that blinding during the initial evaluation of tissues is generally not recommended for initial or non-targeted

Core Question: Did the study implement measures to reduce observational bias?			
			evaluations as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004).
	Not Reported (Interpreted as Adequate)	• Measures to reduce observational bias were not described. • The potential concern for bias was mitigated based on use of automated/computer driven systems, standard laboratory kits, relatively simple, objective measures (e.g., body or tissue weight), or screening-level evaluations of histopathology.	• **Example 1**: Not reported (interpreted as Adequate). Body and organ weights, developmental landmarks, and hormone measures: Authors did not indicate whether investigators were blinded during outcome assessment. Potential concern for bias was mitigated for these endpoints which were measured using automated/computer driven systems, standard laboratory kits, relatively simple, objective measures (e.g., body or tissue weight). • **Example 2**: Not reported (interpreted as Adequate). Histopathology: Blinding during the initial evaluation of tissues is generally not recommended as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004). Histopathology was evaluated by an independent laboratory (Toxicology Pathology Associates Little Rock, Arkansas, John Pletcher, D.V.M., DACPV). No subsequent steps to minimize the potential for observational bias were reported (i.e., conducting a secondary targeted blinded review, independent prospective or retrospective peer-review, formation of a pathology working group).

Core Question: Did the study implement measures to reduce observational bias?			
			• **Example 3**: Not reported (interpreted as Adequate). Fetal evaluation for malformations: Blinding during initial evaluation of fetuses is typically not conducted as masked evaluation can make the task of separating treatment-related changes from normal developmental variation more difficult and may result in subtle developmental anomalies being overlooked. Fetal evaluations were conducted in accordance with regulatory test guideline recommendations, using standardized nomenclature. No subsequent steps to minimize the potential for observational bias were reported (e.g., conducting a secondary targeted blinded review, or an independent prospective or retrospective peer-review).
	Not Reported (Interpreted as Deficient)	• Measures to reduce observational bias were not described. • The potential impact on the results is major (e.g., outcome measures are highly subjective).	• Not reported (interpreted as Deficient). Neurobehavior (auditory and visual sensory reactivity): Procedural methods addressing observational bias were not described for these endpoints, which were measured using highly subjective methods (i.e., it appears that investigators measured reactivity using manually operated timers).
	Critically Deficient	• Strong evidence for observational bias that could have impacted results.	• Critically Deficient. Neurobehavior after restraint stress: There is direct evidence of observational bias in testing methods. Specifically, the study reported that, to minimize stress from changing investigators across trials, one investigator consistently stressed control mice each day for 30 minutes and subsequently tested behaviors, while a separate investigator conducted stress and behavioral testing in treated mice. There

Core Question: Did the study implement measures to reduce observational bias?			
			was no mention of blinding of investigators.

Notes: FOB = functional observed battery.

[a] For non-targeted or screening-level histopathology outcomes often used in guideline studies, blinding during the initial evaluation of tissues is generally not recommended as masked evaluation can make 'the task of separating treatment-related changes from normal variation more difficult' and 'there is concern that masked review during the initial evaluation may result in missing subtle lesions.' Generally, blinded evaluations are recommended for targeted secondary review of specific tissues or in instances when there is a pre-defined set of outcomes that is known or predicted to occur (Crissman et al., 2004).

A.1.7.2.4 Confounding/Variable Control

Table A-31. Study Quality Evaluation Considerations for Confounding/Variable Control

Core Question: Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each study: Are there differences across the treatment groups (e.g., co-exposures, vehicle, diet, palatability, husbandry, health status, etc.) that could bias the results? If differences are identified, to what extent are they expected to impact the results?	**Good**	• Outside of the exposure of interest, variables that are likely to confound or modify results appear to be controlled for and consistent across experimental groups.	• Good. On the basis of the study report, vehicle (deionized water with 2% Tween 80) and husbandry practices were inferred to be the same in controls and treatment groups. The experimental conditions described provided no indication of concern for uncontrolled variables or different practices across groups.
	Adequate	• Some concern that variables that were likely to confound or modify results were uncontrolled or inconsistent across groups but are expected to have a minimal impact on the results.	• **Example 1 (oral):** Adequate. Hormone measurements: Authors did not use a soy-free diet. Soy-based rodent feeds contain phytoestrogens that may act as a confounder for endocrine-related measures. Since this study includes relatively high doses (100 and 1500 mg/kg-d) the concern is minimal. • **Example 2 (inhalation):** Adequate. Behavior, immunological responses, and hormonal changes: control rats did not appear to receive chamber air exposures (they were left in their home cages). As this might introduce a difference in stressors across groups, this difference is interpreted as a possible confounder for measures shown to be sensitive to stress, although the impact of this limitation on the results is expected to be minimal.

Core Question: Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?			
	Deficient	• Notable concern that potentially confounding variables were uncontrolled or inconsistent across groups and are expected to substantially impact the results.	• Deficient. Dams in the medium and high exposure groups (1500 and 15,000 ppm, respectively) showed significantly lower consumption of the treated food throughout the exposure period (gestation) that increased to control levels after the exposure ended. Addition of the test chemical may have affected the palatability of the food and reduced food intake during gestation may have significantly impacted the developmental outcomes in the pups.
	Critically Deficient	• Confounding variables were presumed to be uncontrolled or inconsistent across groups, and are expected to be a primary driver of the results.	• Critically Deficient. The study did not include a vehicle-only control group, and, given the high concentration of DMSO required to solubilize the test article in other experiments using a similar exposure design, this is interpreted as likely to be a significant driver of any observed effects.

Notes: ppm = parts per million; DMSO = dimethyl sulfoxide.

A.1.7.2.5 Reporting and Attrition Bias

Table A-32. Study Quality Evaluation Considerations for Selective Reporting and Attrition – Reporting and Attrition Bias

Core Question: Did the study report results for all prespecified outcomes and tested animals?

Prompting Questions		Suggested Considerations	Example Answers
For each study: ***Selective reporting bias:*** Are all results presented for endpoints/outcomes described in the methods (see note)? ***Attrition bias:*** Are all animals accounted for in the results? If there are discrepancies, do authors provide an explanation (e.g., death or unscheduled sacrifice during the study)? If unexplained results omissions and/or attrition are identified, what is the expected impact on the interpretation of the results? *NOTE: This domain does **not** consider the appropriateness of the analysis/results presentation. This aspect of study quality is evaluated in another domain.*	**Good**	• Quantitative or qualitative results were reported for all prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Data not reported in the primary article is available from supplemental material. If results omissions or animal attrition are identified, the authors provide an explanation and these are not expected to impact the interpretation of the results.	• Good. Animal loss was reported (the authors treated 10 rats/sex/dose group and noted one death in a high-dose male rat at day 85 of study). All endpoints described in methods were reported qualitatively or quantitatively.
	Adequate	• Quantitative or qualitative results are reported for most prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Omissions and/or attrition are not explained but are not expected to significantly impact the interpretation of the results.	• Adequate. Animal loss occurred and was reported (see below), but these are not expected to significantly impact the interpretation of the results. All endpoints described in methods were reported qualitatively or quantitatively. • "In the high dose (1000 mg/kg-day) group no male animals were able to complete the entire study; whereas all male rats exposed at other doses completed the 4-week experiment. In the female group, 1 rat was removed in the 250 mg/kg-day group at day 25, 1 rat in the 500 mg/kg-day was removed at day 21 and 8 rats in the 1000 mg/kg/day group were removed between days 16 and 27 of the experiment." Justification for removals was provided by the study authors.

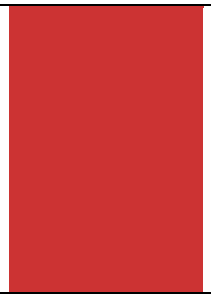
Core Question: Did the study report results for all prespecified outcomes and tested animals?			
	Deficient	• Quantitative or qualitative results are missing for many prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints and/or high animal attrition; omissions and/or attrition are not explained and may significantly impact the interpretation of the results.	• **Example 1:** Deficient. Unaccounted for loss of animals was difficult to assess because the study authors do not provide a clear description of the number of animals per exposure group or the selection of animals for outcome analysis. Table 1 states there were 8 animals used in experiment 1 and 6 animals used in experiments 2 and 3. The figures and tables report data for varying numbers of animals (from 4 to 8), but the authors do not provide a description of the approach used to sample animals for each outcome. • **Example 2:** Deficient. Although the authors indicated that "the liver, kidneys, and spleen were weighed and processed for routine histopathology at study termination," qualitative or quantitative findings were not reported for liver or kidney weights, nor for liver, kidney, or spleen histopathology ("spleen weights" were described as unchanged during the description of changes in cultured splenic immune cells).
	Critically Deficient	• Extensive results omission and/or animal attrition are identified and prevents comparisons of results across treatment groups.	• Critically Deficient. None of the animals in the high and medium dose groups survived and there was high mortality (>75%) in the low-dose group.

A.1.7.2.6 Exposure Methods Sensitivity – Chemical Administration and Characterization

Table A-33. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Chemical Administration and Characterization

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
For each study: Does the study report the source and purity and/or composition (e.g., identity and percent distribution of different isomers) of the chemical? If not, can the purity and/or composition be obtained from the supplier (e.g., as reported on the website) Was independent analytical verification of the test article purity and composition performed? Did the authors take steps to ensure the reported exposure levels were accurate? For inhalation studies: were target concentrations confirmed using reliable analytical measurements in chamber air? For oral studies: if necessary, based on consideration of chemical-specific knowledge	**Good**	• Chemical administration and characterization are complete (i.e., source, purity, and analytical verification of the test article are provided). There are no concerns about the composition, stability, or purity of the administered chemical, or the specific methods of administration. For inhalation studies, chemical concentrations in the exposure chambers are verified using reliable analytical methods.	• **Example 1 (oral):** Good. Source (3M) and purity (98%) are described, and the authors provided verification using analytical methods (GC/MS). Addressing concerns about known instability in solution for this chemical, the authors verified the dosing solutions twice weekly over the course of the experiment. Animals were exposed via gavage with all dose groups receiving the same volume. • **Example 2 (inhalation):** Good. Source (3M) and purity (98%) of the test article are described. All animals were transferred to dynamic inhalation exposure chambers for the exposures. The concentration of the test chemical in the air was continuously monitored from the animals' breathing zone throughout the 6-hour exposure periods and mean daily average concentrations and variability were reported.
(e.g., instability in solution; volatility) and/or exposure design (e.g., the frequency and duration of exposure), were chemical concentrations in the dosing solutions or diet analytically confirmed? Are there concerns about the methods used to administer the chemical (e.g., inhalation chamber type, gavage volume, etc.)? *NOTE: Consideration of the appropriateness of the route of exposure is not evaluated at the*	**Adequate**	• Some uncertainties in the chemical administration and characterization are identified but these are expected to have minimal impact on interpretation of the results (e.g., source and vendor- reported purity are presented, but not independently verified; purity of the test article is sub-optimal but not concerning; For inhalation studies, actual exposure concentrations are	• **Example 1 (oral):** Adequate. Purity (98%) is described, but source is missing. Purity is assumed to be vendor reported because independent analytical verification of the purity is not described. Authors were contacted to try to obtain the vendor information however they did not respond. Stability assessments were not necessary because fresh dosing solutions were prepared daily.

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
individual study level. Relevance and utility of the routes of exposure are considered in the PECO criteria for study inclusion and during evidence synthesis.		missing or verified with less reliable methods).	• **Example 2 (inhalation):** Adequate. Source (3M) and purity (98%) of the test article are described. All animals were transferred to dynamic inhalation exposure chambers for the exposures. The nominal/target concentrations of the test chemical were not verified by analytical measurements of the chamber air.
		• Uncertainties in the exposure characterization are identified and expected to substantially impact the results (e.g., source of the test article is not reported; levels of impurities are substantial or concerning; deficient administration methods, such as use of static inhalation chambers or a gavage volume considered too large for the species and/or lifestage at exposure).	• **Example 1 (oral):** Deficient. Test chemical supplied by the chemical manufacturer. Purity and isomeric composition are not described and could not be obtained from manufacturer's website. Analytical verification of the test article's purity and composition was not provided, and the stability of chemical in the diet across the 1-year exposure period does not appear to have been assessed. • **Example 2 (inhalation):** Deficient. Source (3M) and vendor-reported purity are described, although these were not independently verified. The animals appear to have been exposed in static (i.e., without dynamic airflow) chambers; this is not interpreted as a critical deficiency due to the relatively short (2-hour) durations of daily exposure.
	Critically Deficient	• Uncertainties in the exposure characterization are identified and there is reasonable certainty that the results are largely attributable to factors other than exposure to the chemical of interest (e.g., identified impurities are expected to be a primary driver of the results).	• **Example 1 (oral):** Critically Deficient. The test article contains large amounts of a known impurity [specify] that has previously been shown to cause the outcome(s) of interest. On the basis of the doses tested (and inferences regarding the administered doses of the impurity), this is likely to be a significant driver of any observed effects.

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?		
		• **Example 2 (inhalation):** Critically Deficient. Dams were exposed in static chambers during gestation, and there was evidence of overt toxicity (i.e., gasping) throughout the 12-hr daily exposures at all tested concentrations. This is likely to be a substantial driver of any observed developmental effects.

Notes: GC/MS = gas chromatography mass spectrometry.

A.1.7.2.7 Exposure Methods Sensitivity – Exposure Timing, Frequency, and Duration

Table A-34. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Exposure Timing, Frequency, and Duration

Core Question: Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the exposure period include the critical window of sensitivity? Was the duration and frequency of exposure sensitive for detecting the endpoint of interest?	**Good**	• The duration and frequency of the exposure was sensitive and the exposure included the critical window of sensitivity (if known).	• **Example 1**: Good. Study uses a standard OECD short-term (28-day) study design to examine toxicological effects that are routinely evaluated in this testing guideline. • **Example 2:** Good. experimental potential male effects. The experiment was designed to evaluate recommendations guidelines.
	Adequate	• The duration and frequency of the exposure was sensitive and the exposure covered most of the critical window of sensitivity (if known).	• Adequate. The study does not include the full developmental window of exposure most informative to evaluating potential effects on androgen-dependent development of male reproductive organs. Specifically, the study exposed rats from GD 18–GD 21, whereas the critical window for the development of these endpoints (i.e., cryptorchidism; testes and seminal vesicle weights; and male reproductive organ histopathology) begins on GD 15, and peaks around GD 17 (NRC 2008 [635834]; Scott et al 2009 [673313]) in rats. The incomplete coverage of this critical window in this study is expected to result in a minor bias towards the null.

Core Question: Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?			
	Deficient	• The duration and/or frequency of the exposure is not sensitive and did not include the majority of the critical window of sensitivity (if known). These limitations are expected to bias the results towards the null.	• Deficient. The experimental design is not considered appropriate for evaluation of male fertility. Male rats were exposed for *chemical X* for 1 wk and fertility was assessed on wk 2 of the study. This design is considered deficient because in most rodent species "damage to spermatogonial stem cells will not appear in samples from the cauda epididymis or in ejaculates for 8 to 14 wk" (U.S. EPA, 1996).
	Critically Deficient	• The exposure design was not sensitive and is expected to strongly bias the results towards the null. The rationale should indicate the specific concern(s).	• Critically Deficient. The experimental design is not appropriate for evaluation of cancer endpoints. Animals were necropsied and tissues evaluated for the presence of tumors and/or neoplasms 4 wk after only a 28-day exposure period. Notably, because this critical deficiency is due to insensitivity, depending on other identified limitations, the utility of this study will depend on whether effects were observed in the study (i.e., if tumors were observed, this study could be adjusted to a higher rating).

Notes: OECD = Organisation for Economic Co-operation and Development; OPPT = Office of Pollution Prevention and Toxics; GD = gestation day.

A.1.7.2.8 Outcome Measures and Results Display – Endpoint Sensitivity and Specificity

Table A-35. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Endpoint Sensitivity and Specificity

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Are there concerns regarding the specificity and validity of the protocols? Are there serious concerns regarding the sample size (see note)? Are there concerns regarding the timing of the endpoint assessment? *NOTE: Sample size alone is not a reason to conclude an individual study is critically deficient.*	**Good**	–	• **Example 1:** Good. Lipid/Lipoproteins: There are no notable concerns about aspects of the procedures, or for the timing of these evaluations. Study authors used standard methodology (i.e., commercial kits) appropriate for use in adult liver tissue samples. • **Example 2:** Good. Organ weight, body weights, and hormone measures: no concerns regarding the specificity and validity of the protocols and measures were identified. Study authors used standard methodology for evaluating organ and body weights. Thyroid hormones were measured using commercial electrochemiluminescence-immunoassay methods, and the known diurnal variation in these measures was accounted for during blood collection.
	Adequate	–	• **Example 1**: Adequate. Histopathology: Tissues were fixed in 10% neutral buffered formalin, trimmed, sectioned (5 microns) and embedded and stained with H&E. Evaluations included 12 tissues from all animals in the control and highest dose groups. Although not explicitly stated, it is inferred that tissues from animals in the low- and mid-dose groups would have been evaluated if significant increases in lesion incidence were observed at the highest dose. This practice

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			is consistent with NTP pathology guidelines (ref) and is expected to be of minimal concern unless effects are observed at the high dose. Additionally, the report did not provide information on sampling (e.g., # sections evaluated/tissue, sections evaluated at *x* micron or section intervals). Together, the missing study details introduce some concern for potential insensitivity. • **Example 2:** Adequate. Clinical chemistry: Some concern was raised regarding the procedural methods, as no information was provided on the diagnostic kits and, for some of the specific measures (i.e., those without specific data reported), it is unclear whether serum or plasma was analyzed.
	Deficient	–	• **Example 1**: Deficient. Histopathology (testis): Concerns regarding the method used to preserve testis for histological analysis: 10% formalin. For evaluation of histopathological effects in the testis, conventional immersion fixation in buffered formalin is not recommended as it gives very poor penetration of fixative and may result in artifacts (Haschek (ed) et al 2009 [3987435]; Foley et al 2001 [PMID: 11215684]). • **Example 2:** Deficient. Nipple retention: Concerns for insensitivity were raised due to the timing of endpoint evaluation. Specifically, the authors examined nipple retention in rats at PND 9, whereas this endpoint is more appropriately evaluated around PNDs 12–14.

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			• **Example 3:** Deficient. <u>Motor activity:</u> Concerns were raised regarding the small sample sizes used to evaluate these outcomes. Specifically, the authors tested 4 animals (sex not specified, but assumed males) per group. Ideally, it is preferable to have more than 10 animals/sex/group for this type of evaluation, according to OECD guidelines.
	Critically Deficient	–	• Critically Deficient. [<u>Endpoint name</u>]: [Assay X] has been shown to be unreliable for evaluating [endpoint of interest]. Currently best practice is to use [Assay Y] for this endpoint.

Notes: NTP = National Toxicology Program; PND = postnatal day; OECD = Organisation for Economic Co-operation and Development.

A.1.7.2.9 Outcome Measures and Results Display – Results Presentation

Table A-36. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Results Presentation

Core Question: Are the results presented in a way that makes the data usable and transparent?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the level of detail allow for an informed interpretation of the results? Are the data analyzed, compared, or presented in a way that is inappropriate or misleading?	**Good**	–	• Good. There are no notable concerns about the way the results are analyzed or presented.
	Adequate	–	• **Example 1:** Adequate. Reproductive organ weights, hormone measures: results are presented graphically; however, the authors do not clarify whether error bars correspond to SD or SE. • **Example 2:** Adequate. Developmental effects: the study failed to report information on potential maternal toxicity; however, all tested doses other than the highest dose are not expected to cause overt toxicity in adults, reducing the level of concern. • **Example 3:** Adequate. Anogenital distance (AGD): The authors reported AGD without adjusting for body weight, which is preferred (Daston 1998 [3393032]). However, because the study also provided body weight data, approximation was possible, limiting concern.
	Deficient	–	• **Example 1:** Deficient. Histopathology: Incidence and severity of individual effects was unclear, as only scores across multiple, disparate pathological endpoints were reported. • **Example 2:** Deficient. Behavior (neuromuscular function and dexterity):

Core Question: Are the results presented in a way that makes the data usable and transparent?			
			Performance on the rotarod was presented as incidence of falling off the rod within an arbitrary time, rather than as time spent on the rod (the preferred metric). This dichotomization of continuous data without sound justification is expected to strongly bias the results towards observing an effect. • **Example 3:** Deficient. Brain weight: Authors presented only relative brain weights, and absolute weights could not be calculated. The adult CNS is highly protected, and absolute brain weight data are preferred [include reference]. • **Example 4:** Deficient. Birth outcomes: Data on pup viability, weights, and malformations were reported as pup averages, without addressing potential litter effects.
	Critically Deficient	–	• Critically Deficient. Endpoint name: The study presents the results for this endpoint in both a table and figure; however, the data do not match (e.g., mean ± SE reported for the control group is 2.3 ± 0.5 in the table and 1.9 ± 0.2 in the figure). This reporting discrepancy could not be resolved from the information provided in the study and study authors did not respond to queries for clarification.

A.1.7.2.10 Overall Confidence

The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results (Table A-37).

Table A-37. Evaluation Considerations for Overall Study Confidence – Overall Confidence, Animal Toxicological Studies

Provide judgement and rationale for each endpoint or groups of endpoints. The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results. Evaluation Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects? *NOTE: Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias towards the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	**High confidence**	• No notable concerns are identified (e.g., most or all domains rated Good).	• *High* confidence. Reproductive and developmental effects other than behavior: The study was well-designed for the evaluation of reproductive and developmental toxicity induced by chemical exposure. The study applied established approaches, recommendations, and best practices, and employed an appropriate exposure design for these endpoints. Evidence was presented clearly and transparently.
	Medium confidence	• Some concerns are identified but expected to have minimal impact on the interpretation of the results. (e.g., most domains rated Adequate or Good; may include studies with Deficient ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.	• **Example 1:** *Medium* confidence. Developmental effects: The study was adequately designed for the evaluation of developmental toxicity. Although the authors failed to describe randomized allocation of animals to exposure groups and some concerns were raised regarding the sensitivity (i.e., timing) and sample sizes (i.e., n = 6 litters/group) used for the evaluation of potential effects on male reproductive system development with gestational exposure, these limitations are expected to have a minimal impact on the results.

Provide judgement and rationale for each endpoint or groups of endpoints. The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results. Evaluation Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?			
			• **Example 2:** *Medium* confidence. Histopathology: The study authors did not report information on the severity of histological effects for which this is routinely provided. The authors also failed to describe use of methods to reduce potential observational bias.
	Low confidence	• Identified concerns are expected to significantly impact on the study results or their interpretation (e.g., generally, Deficient ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note).	• **Example 1:** *Low* confidence. Developmental effects: Substantial concerns were raised regarding quantitative analyses without addressing potential litter effects. Other significant limitations included incomplete data presentation (sample sizes for outcome assessment were unclear; no information on maternal toxicity was provided), and methods for selection of animals for outcome assessment. • **Example 2:** *Low* confidence. Behavioral measures: The cursory cage-side observations of activity are considered insensitive and nonspecific methods for detecting motor effects, with a strong bias towards the null.
	Uninformative	• Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, Critically Deficient rating in any domain; many Deficient ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.	• **Example 1:** *Uninformative*. Critical information was not reported. Specifically, the study authors did not report the duration of the exposure or the results (qualitative or quantitative). Given this critical deficiency, the other domains were not evaluated. • **Example 2**: *Uninformative*. Concerns were raised over the lack of information on test animal strain and allocation, and

Provide judgement and rationale for each endpoint or groups of endpoints. The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results. Evaluation Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?			
			chemical source/purity. The lack of information on blinding or other methods to reduce observational blinding is also of significant concern for the endpoints of interest (i.e., follicle counts, ova counts, and evaluation of estrous cyclicity). Finally, concerns were also raised over the apparent self-plagiarism in similar chromium studies published in 1996 by this group of authors. Taken together, this combination of limitations resulted in an interpretation that the results were unreliable. • **Example 3:** *Uninformative*. Sperm Measures: Issues were identified with the methods used to prepare samples for analysis, which are likely to introduce artifacts. Concerns were also raised regarding results presentation (i.e., lack of group variability), missing information on sample sizes and loss of animals, and a lack of information on the timing of these evaluations. Taken together, the evaluation of this endpoint was considered *uninformative*.

A.1.8 Data Extraction for Epidemiological Studies

All epidemiological studies identified as PECO-relevant after full-text screening were considered eligible for data extraction. As noted in the IRIS Handbook (U.S. EPA, 2022c), during data extraction, relevant results from each study are extracted to facilitate organization, visualization, comparison, and analysis of findings and results. Data from PECO-relevant epidemiological studies were extracted if they received a *medium* or *high* confidence study quality evaluation rating. In cases where data were limited (e.g., thyroid cancer) or when there was a notable effect, results from *low* confidence studies were extracted. Data extracted from *low* confidence studies was considered qualitatively only (e.g., in the evidence synthesis and integration). Studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction. Extraction was targeted towards the five priority health outcomes recommended by the SAB (i.e., cancer, cardiovascular, developmental, hepatic, and immune). Results from main analyses were extracted, and age- and sex-stratified analyses were extracted if available. Results from other stratified and sensitivity analyses were extracted when deemed appropriate for a given outcome (e.g., medication use status for cardiovascular outcomes).

Data extraction of epidemiological studies was carried out using a set of structured forms in DistillerSR. Studies slated for extraction were pre-screened by an expert epidemiologist who identified the relevant results to be extracted. Data extraction was performed by one reviewer and then independently verified by at least one other reviewer for quality control. Any conflicts or discrepancies related to data extraction were resolved by discussion and confirmation within the extraction team.

Table A-38 outlines the content of the DistillerSR forms that were populated during data extraction of epidemiological studies, including the extraction questions or prompts and response options.

Table A-38. DistillerSR Form for Data Extraction of Epidemiological Studies

	Question/Prompt	Response Options	Suggested Considerations
1	**Has this study been QC'd?** *[Select one]*	• No (select if doing data extraction) • Yes, no corrections needed • Yes, corrections were needed and completed during QC (please list any major revisions, e.g., incomplete responses, NOEL/LOEL incorrect, etc.) • Study is not PECO-relevant (please specify why)	–
2	**Reference (short form)** e.g., Smith et al. (1978) *[Free-text]*	–	• Enter author information; use the format specified in the Distiller form.
3	**Population** *[Select one]*	• General population, adults and children • General population, adults	• Do not select "pregnant women" if pregnant women are only included as part of a general population sample.

	Question/Prompt	Response Options	Suggested Considerations
		• General population, children and adolescents <18 yr • Occupational • Pregnant women • Occupational/general population, adults • Other	• When exposure is measured in cord blood and outcome in children, the study population would be "children."
4	**Population Summary** *[Free-text]*	–	• Briefly describe the study population (e.g., women undergoing fertility treatment, NHANES adults 18+). Try to capture anything outside a typical general population sample. Keep it brief – does not need to be in full sentences. • For studies of mother-child cohorts, when exposure is in maternal blood and outcome is evaluated in children, use "pregnant women and their children." • For example, if any of these (non-exhaustive) scenarios apply, capture them in this field: • Known potential for PFAS exposure (e.g., contamination event/lawsuit) • Follow-up timing • Participants are drawn from a specific population, such as people with a specific health condition, narrow age range within "adults" and "children" (e.g., infants, seniors), specific environments (e.g., assisted living facility, daycare, farmers), etc.
5	**Study Design** *[Select one]*	• Cohort • Case-control • Cross-sectional • Ecological • Controlled trial • Other • Nested caste-control • Cross-sectional and prospective analyses • Cohort and cross-sectional • Case-control and cross-sectional	• See Appendix A.1.8.1 for different types of study design. • Note: Third trimester samples with outcome measured at birth should be classified as cohort studies. • Cohort studies reporting prospective and cross-sectional analyses should be classified as Cohort and cross-sectional. • Case-control studies reporting cross-sectional analyses among the whole study population or within cases or controls should be classified as Case-control and cross-sectional.
6	**Study Name (if applicable)** *[Free-text]*	–	• Only use the name of an official study or cohort. Leave blank if there is no name.
7	**Country (or Countries)** *[Free-text]*	–	• Use full names such as "United States" (not US).
8	**Year of Data** List which year(s) the data came from. *[Free-text]*	–	• For prospective cohort studies that only state the period the population was recruited (e.g., 2012–2015) and mention the outcomes were assessed at follow-up (e.g., state "5 yr later" but do not provide dates), extract "recruitment

	Question/Prompt	Response Options	Suggested Considerations
			2012–2015, outcome assessed at 5-year follow-up."
9	**Exposure Measurement** *[Select all that apply]*	• Biomonitoring • Air • Food • Drinking water • Occupational (use in cases where exposure is based on factors such as job function, place in building where people worked, job exposure matrices) • Modeled • Questionnaire • Direct administration – oral • Direct administration – inhalation • Other	–
10	**If "biomonitoring" was selected, indicate the matrix.** *[Select all that apply]*	• Blood • Serum • Plasma • Maternal blood • Cord blood • Urine • Feces • Breast milk • Hair • Saliva • Nails • Teeth • Semen • Cerebrospinal fluid • Exhaled breath • Other • Glucose • Maternal serum • Amniotic fluid • Maternal Plasma	• For biomonitoring matrix, if PFAS is measured in serum, select serum (and not also blood). Only select blood if something more specific is not specified (e.g., cord blood, maternal blood, plasma, serum).
11	**Quantitative Data Extraction (Sub-Forms)**		
11.1	**Health Effect Category** *[Select one]*	• Cancer • Cardiovascular • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Metabolic	• See Appendix A.1.6.5.1 for what kind of health outcomes are grouped under which health effect category. Please create a separate form for each outcome.

	Question/Prompt	Response Options	Suggested Considerations
		• Musculoskeletal/Connective Tissue • Nervous • Ocular • Reproductive, female • Reproductive, male • Respiratory • Renal • Other	
11.2	**Measured Outcome/Endpoint** *[Free-text]*	–	• Describe the measured outcome/endpoint and start with most relevant word (e.g., "glucose concentration in serum" preferred to "serum glucose"). • Provide units in parentheses if relevant and readily available. • If the outcome is log transformed, please note it here: o Weight (ln-grams) o Triglyceride (log_{10}-mg/dL) • Some outcomes are dichotomous (e.g., high blood pressure, high cholesterol, etc.), indicate the outcome definition in parentheses. For example: o High cholesterol (>5.0 mg/dL)
11.3	**If developmental, when was the outcome measured?** *[Select all that apply]*	• ≤2 yr of age • 2–5 yr of age • >5 yr of age	–
11.4	**PFAS** *[Select one]*	• PFOA • PFOS	–
11.5	**For neurodevelopmental outcomes, when was PFAS exposure measured?** *[Select all that apply]*	Participants were ≤6 mo of age • Participants were >6 mo of age	–
11.6	**Sub-population** *[Free-text]*	–	• If relevant, specify subgroup within the study (e.g., sex, age group, age at outcome and/or exposure measurement). • Leave blank if not applicable.
11.7	**N** *[Free-text]*	–	• N should be for everyone in the analysis, not just one exposure/comparison group. However, if extracting results for specific population subgroups (age category, gender-specific) and if reported, the N should reflect the number of participants in that specific subgroup (e.g., number of boys in the male-specific result extracted).
11.8	**Exposure Levels** *[Free-text]*	–	• Exposure level should be for everyone in the analysis, not just one comparison group. • Ideally extract median and the 25th–75th percentile range for PFAS being extracted. The following format is preferred:

	Question/Prompt	Response Options	Suggested Considerations
			median = xx (units) (25th–75th percentile: xx-xx). • Provide labels and units (e.g., median = xx (units) (range: min–max: xx–xx)). ○ If median is not available, please extract other measures of distribution, such as mean or geometric mean, range, other percentiles. • Extract levels for the **overall study population**. If only available by subgroups, specify which subgroup. • <u>Example:</u> Males: median = 6.4 ng/mL (25th–75th percentile: 3.6–9.2 ng/mL); Females: median = 5.8 ng/mL (25th–75th percentile: 3.1–8.3 ng/mL) • Note: sometimes manuscripts will incorrectly use IQR rather than 25th–75th percentile. The IQR is the difference between the 75th and the 25th percentile, so it should be a single number, not a range. If a range is labeled IQR, please use "25th–75th percentile."
11.9	**% with Negligible Exposure (e.g., below the LOD)** *[Free-text]*	–	• Number of samples below LOD/LOQ; do not include the percent sign. • Leave blank if not reported.
11.10	**Description of the Effect Estimate, including Comparison Group if applicable** *[Free-text]*	–	• Describe the effect estimate, including comparison group if applicable. • Brief description of the effect estimate: describe the comparison being made (e.g., beta regression coefficient for IQR increase; OR for Q2 vs. Q1). Make sure to specify unit change for continuous measures (e.g., 1 ln-unit, IQR change, SD increase). • Use ln() over log() for natural log transformations. If not ln, specify log(*base*) (e.g., $\log_{10}$ or log(10)). **<u>Good Examples/Formatting:</u>** • regression coefficient (per 1-log2 ng/mL increase in PFOA) • OR (per 1-ln ng/mL increase in estimated plasma PFOS) • OR (for Q2 vs. Q1) • OR [for Q2 (0.83–1.4 ng/mL) vs. Q1 (<0.83 ng/mL)] • OR [for T2 (0.83–1.4 ng/mL) vs. T1 (<0.83 ng/mL)] **<u>Bad Examples/Formatting:</u>** • beta coefficient

	Question/Prompt	Response Options	Suggested Considerations
			• linear regression coefficient (standard error) with one unit increase in log-PFC in adults
11.11	**Rank this Comparison Group by Exposure** *[Free-text]*	–	• For standalone result of unit change, leave blank. • If results are presented for quantiles of exposure, the comparison group for Q2 to Q1 would be ranked as 1, while Q3 to Q1 would be ranked as 2.
11.12	**Effect Estimate Type** *[Select one]*	• Odds Ratio (OR) • Relative Risk Ratio (RR) • Absolute Risk % • Beta Coefficient (b) • Beta Coefficient (standardized) • Standardized Mortality Ratio (SMR) • Standardized Incidence Ratio (SIR) • Incidence Risk Ratio (IRR) • Absolute Risk Reduction/Risk Difference (ARR or RD) • Hazard Ratio (HR) • Comparison of Means • Incidence Rate Ratio • Comparison of Means • Spearman's Correlation Coefficient • Correlation Coefficient • Percent Incidence • Regression Coefficient • Proportionate Mortality Ratio (PMR) • Mean Difference • Percent Difference • Percent Change • Benchmark Dose (BMD) • Mean • Geometric Mean • Least Square Means (LSM) • Geometric Mean Ratio • Fecundability Ratio • Adjusted r^2 • Mean Ratio • Prevalence Ratio (PR)	• If the effect estimate is a regression coefficient (a beta or β), select from the menu "Regression Coefficient" rather than "Beta Coefficient." • If PFOS/PFOA was the outcome of interest (e.g., study looked at the impact of a disease on PFOS/PFOA level), please still extract the data but make a note under the Results Comments (11.19).
11.13	**Effect Estimate** *[Free-text]*	–	• Only report the effect estimate from the adjusted model. If there are multiple adjustment sets, use the final model. • Do not extract the reference group (1) for results comparing exposure levels (i.e.,

	Question/Prompt	Response Options	Suggested Considerations
			extract OR (for Q2 vs. Q1), but don't extract the OR of 1 for the reference group Q1).
11.14	**CI LCL:** Confidence Interval – Lower Confidence Limit *[Free-text]*	–	–
11.15	**CI UCL:** Confidence Internal – Upper Confidence Limit *[Free-text]*	–	–
11.16	**SD or SE** *[Free-text]*	–	• Enter the SD or SE if reported for the effect estimate. • Leave blank if not reported.
11.17	**p-value** *[Free-text]*	–	• Enter the quantitative p-value if available (e.g., "0.0001" or "<0.001") o If the study/table only indicates that p-value is not significant, enter "ns" for not significant. o If the p-value is not reported or does not apply to the estimate being reported, leave blank. o If table footnote mentioned "*p < 0.05" for the results with *, then enter <0.05. If results do not have a * and no p-value was reported, then leave blank. o If the p-value is not reported and text/methods mention significance level is 0.05, and: ▪ the text mentioned the specific result is statistically significant, then enter <0.05 (and make a note in the Results Comments (11.19) which page is this from). ▪ the text mentioned a result as not statistically significant, then enter "ns" (and make a note in the Results Comments (11.19) which page is this from). • Make sure the p-value reported corresponds to the regression coefficient being extracted. Authors will occasionally report p-values for other things such as the model fit. • Other types of p-values such as interaction p-values or trend p-values are reported, these can be placed in Results Comments (11.19).
11.18	**Covariates in Model** *[Free-text]*	–	• If there are multiple adjustment sets, list covariates in the final model, but make a note in the comment field on the main form (14). that additional adjustment sets were available for sensitivity analyses. • List just the covariates, no need to add "adjusted for…" • Example: age, gender, race, SES

	Question/Prompt	Response Options	Suggested Considerations
11.19	**Results Comments** *[Free-text]*	–	• Enter the location of the extracted data (e.g., "Table 3" or "in-text p. 650"). • Enter any relevant p-values, such as interaction p-values or trend p-values. • Enter any additional details on the outcome measurement or definition.
12	**Select PFOS or PFOA if it was measured in the study but not analyzed with health effects.**	• PFOS • PFOA	–
13	**Correlations across the included PFAS presented in paper or supplement.** *[Select one]*	• Yes • No	• Note whether the main manuscript or the supplemental material present a table or text describing the (Spearman) correlation coefficients between concentrations of PFAS included in the paper.
14	**Comments** Include brief description of results provided in supplemental materials but not extracted (e.g., stratified analyses, sensitivity analyses). *[Free-text]*	–	• Briefly mention if **effect modification** is analyzed but not extracted (e.g., stratified analyses by race, by BMI categories, etc.). Note: **Stratification by sex and age should always be extracted.** • Do not need to specify how values below the LOD were handled. • If data is presented by **subgroup/strata** (e.g., race) in the supplemental material, just note that here. Note: **Stratification by sex and age should always be extracted.** • Briefly, describe any other **supplemental results (e.g., sensitivity analyses, etc.)** here; no need to list all confounders other models adjusted for. • Any outcome definitions if study-specific (e.g., how was *elevated ALT* defined in a study reporting ORs of elevated ALT).

Notes: QC = quality control; NOEL = no-observed-effect level; LOEL = lowest-observed-effect level; PECO = populations, exposures, comparators, and outcomes; NHANES = National Health and Nutrition Examination Survey; PFAS = perfluoroalkyl substances; PFOA = perfluorooctanoate acid; PFOS = perfluorooctane sulfonic acid; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; Q2 = quarter 2; Q1 = quarter 1; ln = natural log; SD = standard deviation; T2 = tertile 2S; T1 = tertile 1; PFC = ; Q3 = quarter 3; CI = confidence interval; SE = standard error; ns = not significant; SES = socioeconomic status; BMI = body mass index; ALT = alanine transaminase.

A.1.8.1 Epidemiological Study Design Definitions

Epidemiological studies with cross-sectional, cohort, case-control, ecological, or controlled trial study designs were included. The study design definitions shown in Table A-39 were used throughout full-text screening and data extraction for epidemiological studies.

Table A-39. Epidemiological Study Design Definitions

Study Design	Description
Cross-sectional	Exposure and outcome are examined at the same point in time in a defined study population. Cannot determine if exposure came before or after outcome.
Cohort	A group of people is examined over time to observe a health outcome. Everyone belongs to the same population (e.g., general U.S. population; an occupational group;

Study Design	Description
	cancer survivors). All cohort studies (prospective or retrospective) consider exposure data from before the occurrence of the health outcome.
Case-control	Cases (people with the health outcome) and controls (people without the health outcome) are selected at the start of a study. Exposure is determined and compared between the two groups. A case-control study can be nested within a cohort.
Ecological	The unit of observation is at the group level (e.g., zip code; census tract), rather than the individual level. Ecological studies are often used to measure prevalence and incidence of disease. Cannot make inferences about an individual's risk based on an ecological study.
Controlled Trial	Exposure is assigned to subject and then outcome is measured.

A.1.9 Data Extraction for Animal Toxicological Studies

All animal toxicological studies identified as PECO-relevant after full-text screening in DistillerSR were eligible for data extraction. As noted in the IRIS Handbook (U.S. EPA, 2022c), during data extraction, relevant results from each study are extracted to facilitate organization, visualization, comparison, and analysis of findings and results. PECO-relevant animal toxicological studies that received a *medium* or *high* confidence study quality evaluation rating were extracted.

Data extraction was carried out using a set of structured forms in HAWC (Table A-40). Studies slated for extraction were pre-screened by an expert toxicologist who identified the relevant results. Extraction was performed by one reviewer and then independently verified by at least one other reviewer for quality control. Any conflicts or discrepancies were resolved by discussion and confirmation with a third reviewer.

Table A-40. HAWC Form Fields for Data Extraction of Animal Toxicological Studies

	Questions/Prompts and Options	Suggested Considerations
1	**Experiment**	
1.1	**Name Field** *[Free-text]*	• Name should be short and simple. For example, '28-Day Oral' '2-Year Drinking Water', '1-Week Inhalation'. • Reproductive/developmental if appropriate, then route of exposure (oral/inhalation), not number of generations or acute/short-term/sub-chronic/chronic. • If a study includes multiple experiments (e.g., multiple species, varied exposure durations), create separate experiments for each.
1.2	**Type Field** *[Select one]*	• For reproductive and/or developmental studies, select 'reproductive' or 'developmental' as appropriate (recognizing that a study may contain both reproductive and developmental endpoints, but is typically defined as one or the other based on design). • In general, use reproductive when the study begins treatment prior to mating and continues through birth and in some cases through a second generation. These studies will typically evaluate reproductive outcomes in the dams (e.g., copulation and fertility indices, numbers of corpora lutea and implantation sites, pre- and post-implantation loss). Use developmental when the exposure occurs during gestation and dams are sacrificed prior to birth. These studies are typically focused on the pups and evaluate viability, developmental milestones, and other growth and developmental effects in pups and primarily they are looking for abnormalities in the pups. • If reproductive or developmental are selected, indicate if there are data for more than one generation.
1.3	**Chemical Name Field** *[Free-text]*	• Enter the preferred name of the chemical (i.e., PFOA or PFOS). • Refer to the PECO statement in for a list of synonyms for each chemical.
1.4	**Chemical Identifier (CAS) Field** *[Free-text]*	• Be sure to include the dashes in the CAS number. • The CAS number for the chemical can be found in the PECO statement if they are not listed in the paper.

	Questions/Prompts and Options	Suggested Considerations
1.5	**Chemical Source Field** *[Free-text]*	• If the chemical source is not provided by the authors, add in "Not Reported" to this field.
1.6	**Chemical Purity Fields** *[Checkbox]*	• As a default, the 'Chemical purity available?' box will be checked. If the box is checked, entries for 'Purity qualifier' and 'Chemical purity (%)' are required. • Uncheck this box if chemical purity information is not available.
2	**Animal Group**	
2.1	**Name Field** *[Free-text]*	• Name should include sex, common strain name, and species (e.g., Male Sprague-Dawley Rats). • For reproductive or developmental studies, include the generation before sex in title (e.g., F1 Male Sprague-Dawley Rats or P0 Female C57 Mice). • If a study combines male and female subjects into one group, use "Male and Female" (e.g., Male and Female Sprague-Dawley Rats). • If gender is unclear, do not mention (e.g., Sprague-Dawley Rats). • Use the plural form for species (e.g., Rats, Mice).
2.2	**Animal Source and Husbandry Field** *[Free-text]*	• Copy and paste details directly from the paper using quotation marks. • If the authors do not provide the animal source, add in "Not Reported" to this field. • For multigenerational reproductive or developmental studies, the animal group dosed might be the parental (or P0) group. For example, a P0 female rat may be dosed during pregnancy and/or lactation, and developmental effects are then measured in offspring – or F_1 animals. • For a multigenerational study, specify the 'Generation'.
3	**Add Dosing Regime**	
3.1	**Exposure Duration (Days) Field** *[Free-text]*	• Decimals are allowed, so a 4 h single day study can be represented as 0.17 d. However, decimals are likely not needed for the PFOA/PFOS project since acute studies are not PECO relevant.
3.2	**Exposure Duration (Text) Field** *[Free-text]*	• For all time units, use the following abbreviations: year = yr; month = mo; week = wk; day = d; hour = hr; minute = min; second = sec. • Eliminate unnecessary space between length of time and unit (i.e., "2wk" instead of "2 wk").
3.3	**Description Field** *[Free-text]*	• Include dosing description from materials and methods. Be sure to use quotation marks around all text directly copied/pasted from the paper. • Include any information on how dosing solutions were prepared. • Summarize any results the authors present on analytical work conducted to confirm dose, stability, and purity.
3.4	**Dose-Groups Field** *[Free-text]*	• Dose groups should be listed lowest to highest (dose group 1 = 0 mg/kg-d). • For visualization purposes dose units need to be in mg/kg-d. For studies that provide the units, please use those for extraction purposes. • For dietary or drinking water studies, if they provide BOTH concentration of the dose formulation (e.g., ppm) AND doses as mg/kg-d, please extract both.

	Questions/Prompts and Options	Suggested Considerations
		• For dietary or drinking water studies that ONLY provide the dose concentration, enter the dose concentrations as reported in the study and then utilize the conversions spreadsheet to convert the dosage into mg/kg-day (note that mg/kg body weight/day is the same as mg/kg-d so you just need to use the mg/kg-d). • If PFOA/PFOS are administered as salts and the doses are presented as salts of PFOA/PFOS, please contact senior-level extractors before using the conversion spreadsheet. • If converting doses, add in "Data extractor calculated [PFOS/PFOA] equivalent doses for mg/kg-day" into the "Description" box. • When defining the dosing regime for a multigenerational experiment, creating a new dosing regime may not be needed; instead specify the existing dosing regime of the P_0 (dosed during gestation and/or lactation). • A new dosing regime may be needed if offspring were exposed after weaning and, if applicable, acknowledge parental exposure in the 'Description' field on the 'Dosing regime' page. • If the authors provide internal measurements of PFOS/PFOA in any tissue, add this information in as an additional dose group using the mean tissue levels as the value and the tissue as part of the dose units (e.g., mg/kg bone, ppm brain).
4	**Endpoints (General)**	
4.1	**Endpoint Name Field** *[Free-text]*	• Name should not include descriptive information captured in other fields within HAWC such as sex, strain, species, duration, route, etc. • Include common abbreviation in parenthesis if applicable. • Endpoint detail should be added after main endpoint, ex. "Body Weight, Fetal" NOT "Fetal Body Weight." • In general, specific endpoint names are used except for general categories such as 'Clinical Observations' or histopathology (e.g., 'Kidney Histopathology'), which may comprise a number of observational endpoints. • Examples: Liver Weight, Relative; Triiodothyronine (T3)
4.2	**System Field** *[Free-text]*	• Represents the appropriate system for the endpoint. • Examples: Hepatic; Endocrine
4.3	**Organ (and Tissue) Field** *[Free-text]*	• Represents the appropriate organ or tissue for the endpoint. • Examples: Liver; Thyroid
4.4	**Effect and Effect Subtype Fields** *[Free-text]*	• Represents the appropriate system for the endpoint. • Examples: Hepatic; Endocrine
4.5	**Observation Time Fields** *[Free-text]*	• The 'Observation time' text field is included in visualizations and should be filled in; the 'Observation time' numeric field and 'Observation time units' can be left blank. • For all time units, use the following abbreviations: year = yr; month = mo; week = wk; day = d; hour = hr • Eliminate unnecessary space between length of time and unit (i.e., "2wk" instead of "2 wk"). • Example: 2yr; 6hr; 45d; 90 min • For developmental and reproductive studies, specify observation time in terms of development (e.g., GD 16, PND_0).

	Questions/Prompts and Options	Suggested Considerations
4.6	**Values Estimated Field** *[Free-text]*	• If data was extracted from a figure into HAWC using a measured ruler, check this box. • For data requiring a digital ruler, use the WebPlotDigitizer tool: https://apps.automeris.io/wpd/. • If there are multiple time points, extract only the latest time point (i.e., end of treatment) or if the last time point is not significant and an earlier time point is, extract the earlier time point (this information should be provided in the data to extract instructions, but this is the general rule in case there are no instructions provided). • Provide additional information in the results comment box to make note of what happened at other timepoints that were not extracted.
4.7	**Litter Effects Field** *[Free-text]*	• If the experiment type has been identified as either 'reproductive' or 'developmental', the 'Litter effects' will be required, and a choice other than 'not applicable' must be selected.
4.8	**Dataset Type Field** *[Free-text]*	• Select the appropriate dataset type for the endpoint. In general, 'Dataset type' is continuous except for incidence data, which is dichotomous.
4.9	**NOAEL and LOAEL Fields** *[Free-text]*	• Be sure to enter the significance level (e.g., 0.05) for significant results as well as NOAEL/LOAEL. • The NOAEL is the highest dose at which there was not an observed toxic or adverse effect. If the LOAEL is the lowest (non-control) dose, then NOAEL should be <None>, not 0. • The LOAEL is the lowest dose at which there was an observed toxic or adverse effect. These fields are critical to the visualizations. If there is no LOAEL, leave as <None>. • In cases where the study authors did not conduct statistical tests, use the study authors conclusions to indicate where effects occur. Just make sure to note in the results comments that these were based on author conclusions and no statistical testing was conducted.
4.10	**Statistical Test Field** *[Free-text]*	• If the statistical test is not provided in the study, add "Not Reported" to the text field.
4.11	**Results Notes Field** *[Free-text]*	• If needed, copy and paste details into this field using quotation marks. Although the methods text field can describe all methods used, results comments should be more endpoint specific.
5	**Endpoint (Dummy Variables)** Data to be extracted using dummy variables for the following reasons: • Results that are qualitatively discussed in the text, but actual data are not provided. • For instances where study authors specify that only the significant effects are described – and certain endpoints are then not discussed – assume that no change occurred in these endpoints. Create dummy variables for all endpoints stated to be measured with	• For endpoints for which no quantitative data are provided, create the endpoint as described above with the exceptions below. • 'Dataset type' is dichotomous or continuous based on the data type if there were data available. • For 'Response units,' use whatever units correspond to the effect for which you are creating the dummy variable (e.g., 'incidence' for histopathology observations, 'grams' for body weight) • Under 'Dose-response data', fill in with a dummy variable. Use 0 to indicate no change from control, a 1 to indicate an increase from control and a −1 to indicate a decrease from the control. • 'Significance Level' should be populated if the author indicates significance. Otherwise, 'Significance Level' is left blank. • Multiple clinical observations can be grouped together into a single endpoint. • Example: create an endpoint for clinical observations and add dummy variables to indicate no effect.

Questions/Prompts and Options	Suggested Considerations
the assumption if they are not discussed they were not significant and make sure to document this in the results comments field. • If an endpoint is discussed in the methods, but there is no mention at all in the results (even to indicate that only significant effects were reported), then create an endpoint only and do not extract any data. In this case, uncheck the 'data reported' and 'data extracted' boxes on the endpoint page. • Organs/tissues that were examined for histopathological changes, but no changes were noted. • Clinical observations in which multiple clinical signs or general observations are grouped together.	• If a single endpoint called "Clinical Observation," create the dummy variables above using all 0 with nothing tagged as significant. • Or if there was an effect, still create a single endpoint called "Clinical Observation" and then put a 1 at the dose where the effects were observed and then in the results comment field indicate the effects that were observed. This would be common in reproductive and developmental studies; indicate if there were "Clinical Observations in Dams" and where they occurred but didn't want to have a separate endpoint for each observation. • Example: for any organ listed but not specified any lesions to extract, create a histopathology endpoint and create a dummy variable to indicate no treatment-related effect. • Create an endpoint for each organ (e.g., Liver Histopathology, Kidney Histopathology, Uterus Histopathology), and create the dummy variables described above using all 0 with nothing tagged as significant. • Whenever using dummy variables instead of actual data, make sure to note in the results comment text box that the data are dummy variables using the standard language given in the instructions in HAWC under the 'Results notes' box.

Notes: NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; CAS = Chemical Abstracts Service.

A.1.10 Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes. For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review, considering the conclusions from the individual study quality evaluations. Syntheses of the evidence for human and animal health effects are based primarily on studies of *high* and *medium* confidence; *low* confidence results were given less weight compared with *high* or *medium* confidence results during evidence synthesis and integration. However, in certain instances (i.e., for health outcomes for which few or no studies with higher confidence are available), *low* confidence studies might be used to help evaluate consistency or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect.

The available human and animal evidence pertaining to the potential health effects of PFOS were synthesized separately, and a summary discussion of the available evidence was developed for each evidence stream. For the five priority health outcomes, mechanistic evidence was also considered in the development of each synthesis. Strength-of-evidence judgments were made for each health outcome within each evidence stream (i.e., human or animal) using standard terminology (i.e., *robust, moderate, slight, indeterminate*) and definitions according to the framework described in the IRIS Handbook and outlined in Table A-41 and Table A-42.

Following evidence synthesis, the evidence for humans and animals was integrated for each health outcome. The evidence integration was conducted following the guidance outlined in the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). Integrated judgements were drawn across all lines of evidence for each assessed health outcome as to whether, and to what extent, the evidence supports that exposure to PFOS has the potential to be hazardous to humans. The evidence integration provided a summary of the causal interpretations from the available studies, as well as mechanistic evidence for the five priority health outcomes. Mechanistic evidence was organized by signaling pathway or other categories (e.g., key characteristics of carcinogens) as relevant to each outcome. The integrated judgments are developed through structured review of the evidence against an established set of considerations for causality. These considerations include risk of bias, sensitivity, consistency, strength (effect magnitude) and precision, biological gradient/dose-response, coherence, and mechanistic evidence related to biological plausibility. The evidence integration involved an overall judgment on whether there was sufficient evidence or insufficient evidence for each potential human health effect and an evidence basis rationale. During evidence integration, a structured and documented process was used, as follows:

- Summarize human and animal health effect studies in parallel but separately, using the set of considerations for causality first introduced by Austin Bradford Hill (Hill, 1965) and relevant mechanistic evidence (or mode of action (MOA) understanding).
- Identify strength of the human and animal health evidence in light of inferences across evidence streams.
- Summarize judgment as to whether the available evidence base for each potential health outcome as a whole indicates that PFOS exposure has the potential to cause adverse health effects in humans (see Table A-43) (“evidence demonstrates,” “evidence indicates (likely),” “evidence suggests,” “evidence is inadequate,” or “strong evidence supports no effect”).

The decision points within the structured evidence integration process are summarized in an evidence profile table for each assessed health effect.

Table A-41. Framework for Strength-of-Evidence Judgments for Epidemiological Studies[a]

Strength-of-Evidence Judgment	Description
Robust (⊕⊕⊕)	A set of *high*- or *medium*-confidence studies reporting an association between the exposure and the health outcome, with reasonable confidence that alternative explanations, including chance, bias, and confounding, can be ruled out across studies. The set of studies is primarily consistent, with reasonable explanations when results differ; and an exposure response gradient is demonstrated. Supporting evidence, such as associations with biologically related endpoints in human studies (coherence) or large estimates of risk or severity of the response, may help to rule out alternative explanations. Similarly, mechanistic evidence from exposed humans may serve to address uncertainties relating to exposure-response, temporality, coherence, and biological plausibility (i.e., providing evidence consistent with an explanation for how exposure could cause the health effect based on current biological knowledge) such that the totality of human evidence supports this judgment.
Moderate (⊕⊕⊙)	• Multiple studies showing generally consistent findings, including at least one *high* or *medium* confidence study and supporting evidence, but with some residual uncertainty due to potential chance, bias, or confounding (e.g., effect estimates of low magnitude or small effect sizes given what is known about the endpoint; uninterpretable patterns with respect to exposure levels). Associations with related endpoints, including mechanistic evidence from exposed humans, can address uncertainties relating to exposure response, temporality, coherence, and biological plausibility, and any conflicting evidence is not from a comparable body of higher confidence, sensitive studies • A single *high*- or *medium*-confidence study demonstrating an effect with one or more factors that increase evidence strength, such as: a large magnitude or severity of the effect, a dose-response gradient, unique exposure or outcome scenarios (e.g., a natural experiment), or supporting coherent evidence, including mechanistic evidence from exposed humans. There are no comparable studies of similar confidence and sensitivity providing conflicting evidence, or if there are, the differences can be reasonably explained (e.g., by the population or exposure levels studied)
Slight (⊕⊙⊙)	One or more studies reporting an association between exposure and the health outcome, where considerable uncertainty exists: • A body of evidence, including scenarios with one or more *high*- or *medium*-confidence studies reporting an association between exposure and the health outcome, where either (1) conflicting evidence exists in studies of similar confidence and sensitivity (including mechanistic evidence contradicting the biological plausibility of the reported effects), a (2) a single study without a factor that increases evidence strength (factors described in moderate), OR (3) considerable methodological uncertainties remain across the body of evidence (typically related to exposure or outcome ascertainment, including temporality), AND there is no supporting coherent evidence that increases the overall evidence strength. • A set of only *low* confidence studies that are largely consistent. • Strong mechanistic evidence in well-conducted studies of exposed humans (*medium* or *high* confidence) or human cells, in the absence of other substantive data, where an informed evaluation has determined that the data are reliable for assessing the health effect of interest and the mechanistic events have been reasonably linked to the development of that health effect.
Indeterminate (⊙⊙⊙)	• No studies in humans or well-conducted studies of human cells. • Situations when the evidence is highly inconsistent and primarily of *low* confidence. • May include situations with *medium* or *high* confidence studies, but unexplained heterogeneity exists (in studies of similar confidence and sensitivity), and there are additional outstanding concerns such as effect estimates of low magnitude, uninterpretable patterns with respect to

Strength-of-Evidence Judgment	Description
	exposure levels, or uncertainties or methodological limitations that result in an inability to discern effects from exposure. • A set of largely null studies that does not meet the criteria for compelling evidence of no effect, including evidence bases with inadequate testing of susceptible populations and lifestages.
Compelling evidence of no effect (– – –)	Several *high*-confidence studies showing null results (for example, an odds ratio of 1.0), ruling out alternative explanations including chance, bias, and confounding with reasonable confidence. Each of the studies should have used an optimal outcome and exposure assessment and adequate sample size (specifically for higher exposure groups and for susceptible populations). The set as a whole should include the full range of levels of exposures that human beings are known to encounter, an evaluation of an exposure response gradient, and an examination of at-risk populations and lifestages.

Notes:
[a] Table slightly adapted from Table 11-3 in the IRIS Handbook.

Table A-42. Framework for Strength-of-Evidence Judgments for Animal Toxicological Studies[a]

Strength-of-Evidence Judgment	Description
Robust (⊕⊕⊕)	A set of *high*- or *medium*-confidence studies with consistent findings of adverse or toxicologically significant effects across multiple laboratories, exposure routes, experimental designs (e.g., a subchronic study and a two-generation study), or species; and the experiments reasonably rule out the potential for nonspecific effects to have caused the effects of interest. Any inconsistent evidence (evidence that cannot be reasonably explained based on study design or differences in animal model) is from a set of experiments of lower confidence or sensitivity. To reasonably rule out alternative explanations, multiple additional factors in the set of experiments exist, such as: coherent effects across biologically related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; or consistent observations across animal lifestages, sexes, or strains. Similarly, mechanistic evidence (e.g., precursor events linked to adverse outcomes) in animal models may exist to address uncertainties in the evidence base such that the totality of animal evidence supports this judgment.
Moderate (⊕⊕⊙)	• At least one *high*- or *medium*-confidence study with supporting information increasing the strength of the evidence. Although the results are largely consistent, notable uncertainties remain. However, in scenarios when inconsistent evidence or evidence indicating nonspecific effects exist, it is not judged to reduce or discount the level of concern regarding the positive findings, or it is not from a comparable body of higher confidence, sensitive studies. The additional support provided includes either consistent effects across laboratories or species; coherent effects across multiple related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; or consistent observations across exposure scenarios (e.g., route, timing, duration), sexes, or animal strains. Mechanistic evidence in animals may serve to provide this support or otherwise address residual uncertainties. • A single *high* or *medium* confidence experiment demonstrating an effect in the absence of comparable experiment(s) of similar confidence and sensitivity providing conflicting evidence, namely evidence that cannot be reasonably explained (e.g., by respective study designs or differences in animal model).
Slight (⊕⊙⊙)	• Scenarios in which there is a signal of a possible effect, but the evidence is conflicting or weak: • A body of evidence, including scenarios with one or more *high* or *medium* confidence experiments reporting effects but without supporting or coherent evidence (see description in moderate) that increases the overall evidence strength, where conflicting evidence exists from a

Strength-of-Evidence Judgment	Description
	set of sensitive experiments of similar or higher confidence (including mechanistic evidence contradicting the biological plausibility of the reported effects). • A set of only *low* confidence experiments that are largely consistent. • Strong mechanistic evidence in well-conducted studies of animals or animal cells, in the absence of other substantive data, where an informed evaluation has determined the assays are reliable for assessing the health effect of interest and the mechanistic events have been reasonably linked to the development of that health effect.
Indeterminate (⊙⊙⊙)	• No animal studies or well-conducted studies of animal cells. • The available models (not considering human relevance) or endpoints are not informative to the hazard question under evaluation. • The evidence is inconsistent and primarily of *low* confidence. • May include situations with *medium* or *high* confidence studies, but there is unexplained heterogeneity and additional concerns such as small effect sizes (given what is known about the endpoint) or a lack of dose-dependence. • A set of largely null studies that does not meet the criteria for compelling evidence of no effect.
Compelling evidence of no effect (– – –)	A set of *high* confidence experiments examining a reasonable spectrum of endpoints relevant to a type of toxicity that demonstrate a lack of biologically significant effects across multiple species, both sexes, and a broad range of exposure levels. The data are compelling in that the experiments have examined the range of scenarios across which health effects in animals could be observed, and an alternative explanation (e.g., inadequately controlled features of the studies' experimental designs; inadequate sample sizes) for the observed lack of effects is not available. The experiments were designed to specifically test for effects of interest, including suitable exposure timing and duration, post exposure latency, and endpoint evaluation procedures, and to address potentially susceptible populations and lifestages. Mechanistic data in animals (in vivo or in vitro) that address the above considerations or that provide information supporting the lack of an association between exposure and effect with reasonable confidence may provide additional support such that the totality of evidence supports this judgment.

Notes:
[a] Table slightly adapted from Table 11-4 in the IRIS Handbook.

Table A-43. Evidence Integration Judgments for Characterizing Potential Human Health Effects in the Evidence Integration[a]

Evidence integration judgment level	Explanation and example scenarios
Evidence demonstrates	A strong evidence base demonstrating that [chemical] exposure causes [health effect] in humans • For when there is robust human evidence supporting an effect • Could also be used when there is moderate human evidence and robust animal evidence if there is strong mechanistic evidence that MOA(s) or key precursors identified in animals are expected to occur and progress in humans
Evidence indicates (likely)	An evidence base that indicates that [chemical] exposure likely causes [health effect] in humans, although there may be outstanding questions or limitations. • Used if there is robust animal evidence supporting an effect and slight or indeterminate human evidence, or with moderate human evidence when strong mechanistic evidence is lacking • Could also be used with moderate human evidence supporting an effect and slight or indeterminate animal evidence, or with moderate animal evidence supporting an effect and slight or indeterminate human evidence. In these scenarios, any uncertainties in the moderate evidence are not sufficient to substantially reduce confidence in the reliability of the evidence,

Evidence integration judgment level	Explanation and example scenarios
	or mechanistic evidence in the slight or indeterminate evidence base (e.g., precursors) exists to increase confidence in the reliability of the moderate evidence A decision between "evidence indicates" and "evidence suggests" considers the extent to which findings are coherent or biologically consistent across lines of evidence streams, and may incorporate other supplemental evidence (e.g., structure-activity data; chemical class information)
Evidence suggests	An evidence base that suggests that [chemical] exposure may cause [health effect] in humans, but there are very few studies that contributed to the evaluation, the evidence is weak or conflicting, and/or the methodological conduct of the studies is poor. • Used if there is slight human evidence and indeterminate or slight animal evidence • Used with slight animal evidence and indeterminate or slight human evidence • Could also be used with moderate human evidence and slight or indeterminate animal evidence, or with moderate animal evidence and slight or indeterminate human evidence. In these scenarios, there are outstanding issues regarding the moderate evidence that substantially reduced confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., null results in well-conducted evaluations of precursors) exists to decrease confidence in the reliability of the moderate evidence • When there is general scientific understanding of mechanistic events that result in a health effect, this judgment level could also be used if there is strong mechanistic evidence that is sufficient to highlight potential human toxicity in the absence of informative conventional studies in humans or in animals
Evidence inadequate[b]	This conveys either a lack of information or an inability to interpret the available evidence for [health effect]. On an assessment-specific basis, a single use of this "evidence inadequate" judgment might be used to characterize the evidence for multiple health effect categories. • Used if there is indeterminate human and animal evidence • Used if there is slight animal evidence and compelling evidence of no effect human evidence • Could also be used with slight or robust animal evidence and indeterminate human evidence if strong mechanistic information indicated that the animal evidence is unlikely to be relevant to humans
Strong evidence supports no effect	Extensive evidence across a range of populations and exposure levels has identified no effects/associations. This scenario requires a high degree of confidence in the conduct of individual studies, including consideration of study sensitivity, and comprehensive assessments of the endpoints and lifestages of exposure potentially relevant to the heath effect of interest. • Used if there is compelling evidence of no effect in human studies and compelling evidence of no effect or indeterminate animal evidence • Also used if there is indeterminate human evidence and compelling evidence of no effect animal evidence in models judged as relevant to humans • Could also be used with compelling evidence of no effect in human studies and moderate or robust animal evidence if strong mechanistic information indicated that the animal evidence is unlikely to be relevant to humans

Notes: MOA = mode of action.
[a] Table adapted from Table 11-5 in the IRIS Handbook.
[b] An "evidence inadequate" judgment is not a determination that the chemical does not cause the indicated human health effect(s), but rather an indication that the available evidence is insufficient to reach a judgment.

A.1.10.1 Epidemiological Studies Included From the 2016 PFOS HESD

For the five priority health outcomes (i.e., developmental, immune, hepatic, cardiovascular and cancer), epidemiological studies identified and reviewed in the 2016 PFOS HESD were included in the evidence synthesis, including discussion of study quality considerations, according to the recommendations from the SAB. Inferences drawn from included studies from the 2016 PFOS

HESD were considered in drawing health effects conclusions and were incorporated into the evidence profile tables

For all non-priority health outcomes, epidemiological studies identified and reviewed in the 2016 PFOS HESD were included in the evidence syntheses in summary paragraphs describing previously reached conclusions for each health outcome. Study quality was considered, but domain-based, structured study quality evaluations were not performed for 2016 PFOS HESD studies. Inferences drawn from evidence in the current literature search were compared with the results described from 2016 studies.

A.1.10.2 Epidemiological Studies Excluded From Synthesis

Some epidemiological studies were not included in the evidence synthesis narrative if they included overlapping results (e.g., overlapping NHANES studies). Studies reporting results from the same cohort with the same health outcome were considered overlapping evidence, and these studies were not discussed in the synthesis narrative to avoid duplication or overrepresentation of results from the same group of participants. When participants from the same cohort were included in more than one eligible study, the study with the largest number of participants was included in the evidence synthesis narrative. In general, to best gauge consistency and magnitude of reported associations, EPA focused on the most accurate and most prevalent measures. In some cases, such as developmental outcomes, studies on the same population providing more accurate outcome measures (e.g., birthweight and birth length for fetal growth restriction) were given preference over studies providing less accurate outcome measures (e.g., ponderal index for fetal growth restriction). Overlapping studies were included in study quality figures.

Meta-analyses were considered during evidence integration as support of consistent effects across studies. Details of the identified meta-analyses and assessment implications are summarized in Section A.2.

A.1.11 Dose-Response Assessment: Selecting Studies and Quantitative Analysis

As noted in the IRIS Handbook, selection of studies and endpoints for dose-response assessment involves considerations of the data that build from "judgments" and decisions made during earlier steps of the systematic review and assessment process. EPA guidance and support documents that describe data requirements and other considerations for dose-response modeling include EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

Dose-response assessments are performed for both noncancer and cancer oral health hazards, if supported by existing data. For noncancer hazards, an oral RfD will be derived when possible. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002). Reference values are not predictive risk values; that is, they provide no information about risks at higher or lower exposure levels.

For cancer hazards, a CSF will be derived to estimate human cancer risk when low-dose linear extrapolation for cancer effects is supported. A CSF is a plausible upper bound lifetime cancer risk from chronic ingestion of a chemical per unit of mass consumed per unit body weight per day (mg/kg-day). In contrast to RfDs, CSFs can be used in conjunction with exposure information to predict cancer risk at a given dose.

The derivation of reference values will depend on the conclusions drawn during previous steps of this protocol. Specifically, EPA will attempt dose-response assessments for noncancer outcomes when the evidence integration judgements indicate stronger evidence of hazard (i.e., *evidence demonstrates* and *evidence indicates* integration judgements). Quantitative analyses are generally not attempted for other evidence integration conclusions. Similarly, EPA will attempt dose-response assessments for cancer outcomes for chemicals that are classified as *Carcinogenic* or *Likely to be Carcinogenic to Humans*. When there is *Suggestive Evidence of Carcinogenic Potential to Humans*, EPA generally does not conduct dose-response assessment unless a well-conducted study is available and a quantitative analysis is deemed useful.

A.1.11.1 Study Selection

Evidence synthesis and integration enabled identification of the health outcomes with the strongest weight of evidence supporting causal relationships between PFOS exposure and adverse health effects, as well as the most sensitive cancer and noncancer endpoints within those health outcomes. Dose-response modeling was performed for endpoints within health outcomes with data warranting evidence integration conclusions of *evidence demonstrates* and *evidence indicates (likely)* for noncancer endpoints and carcinogenicity descriptors of *Carcinogenic to Humans* and *Likely to be Carcinogenic to Humans*. Human epidemiological and animal toxicological studies that were consistent with the overall weight of evidence for a specific endpoint were considered for dose-response. Additionally, for human evidence, all *high* or *medium* confidence studies pertaining to a specific endpoint were considered; for animal evidence, only animal toxicological studies with at least two PFOS exposure groups that were of *high* or *medium* confidence were considered. Relevance of the endpoint or species reported by animal toxicological studies to human health effects was also considered. When multiple endpoints for a health outcome are available, endpoints are selected for dose-response analysis based on rationale describing how the endpoint is representative of the broader health outcome (U.S. EPA, 2022c). Studies were evaluated for use in POD derivation following considerations described in Table 7-2 (Table A-44) of the IRIS Handbook (U.S. EPA, 2022c). These attributes support a more complete characterization of the shape of the exposure-response curve and decrease the uncertainty in the associated exposure-response metric (e.g., RfD) by reducing statistical uncertainty in the POD and minimizing the need for low-dose extrapolation. Some important considerations include:

- human data are preferred over animal data to eliminate interspecies extrapolation uncertainties,
- animal species known to respond similarly to humans are preferred over studies of other species,
- *high* or *medium* confidence studies are preferred over *low* confidence studies,
- chronic or subchronic studies, or studies encompassing a sensitive lifestage (i.e., gestational) are preferred for the derivation of chronic toxicity values over acute studies,

- studies with a design or analysis that addresses relevant confounding for a given outcome are preferred,
- human studies providing the most updated data on a population are preferred over prior publications,
- and studies reporting all necessary data (e.g., total population or quartile exposure concentrations) for dose-response analysis are preferred.

The number of studies considered for toxicity value derivation was reduced based on these considerations and others described in EPA (U.S. EPA, 2022c, 2012).

Table A-44. Attributes used to evaluate studies for derivation of toxicity values (adapted from ORD Staff Handbook for Developing IRIS Assessments Table 7-2)

Study Attributes		Considerations	
		Human studies	**Animal studies**
Study confidence		*High* or *medium* confidence studies are highly preferred over *low* confidence studies. The selection of *low* confidence studies should include an additional explanatory justification (e.g., only *low* confidence studies had adequate data for toxicity value derivation). The available high and medium confidence studies are further differentiated on the basis of the study attributes below, as well as a reconsideration of the specific limitations identified and their potential impact on dose-response analyses.	
Rationale for choice of species		Human data are preferred over animal data to eliminate interspecies extrapolation uncertainties (e.g., in pharmacodynamics, dose-response pattern in relevant dose range, relevance of specific health outcomes to humans).	Animal studies provide supporting evidence when adequate human studies are available, and they are considered the studies of primary interest when adequate human studies are not available. For some hazards, studies of particular animal species known to respond similarly to humans would be preferred over studies of other species.
Relevance of exposure paradigm	Exposure route	Studies involving human environmental exposures (oral, inhalation).	Studies by a route of administration relevant to human environmental exposure are preferred. A validated pharmacokinetic model can also be used to extrapolate across exposure routes.
	Exposure durations	When developing a chronic toxicity value, chronic or subchronic studies are preferred over studies of acute exposure durations. Exceptions exist, such as when a susceptible population or lifestage is more sensitive in a particular time window (e.g., developmental exposure).	
	Exposure levels	Exposures near the range of typical environmental human exposures are preferred. Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship (see the EPA *Benchmark Dose Technical Guidance*, §2.1.1) and facilitate extrapolation to more relevant (generally lower) exposures.	
Subject selection		Studies that provide risk estimates in the most susceptible groups are preferred.	
Controls for possible confounding		Studies with a design (e.g., matching procedures, blocking) or analysis (e.g., covariates or other procedures for statistical adjustment) that adequately address the relevant sources of potential critical confounding for a given outcome are preferred.	
Measurement of exposure		Studies that can reliably distinguish between levels of exposure in a time window considered most relevant for development of a causal effect are preferred. Exposure assessment methods that provide measurements at the level of the individual and that reduce measurement error are preferred. Measurements of exposure should not be influenced by knowledge of health outcome status.	Studies providing actual measurements of exposure (e.g., analytical inhalation concentrations vs. target concentrations) are preferred. Relevant internal dose measures might facilitate extrapolation to humans, as would availability of a suitable animal PBPK model in conjunction with an animal study reported in terms of administered exposure.
Health outcome(s)		Studies that can reliably distinguish the presence or absence (or degree of severity) of the outcome are preferred. Outcome ascertainment methods using generally accepted or standardized approaches are preferred.	
		Studies with individual data are preferred in general. For example, individual data allow you to characterize experimental variability more realistically and to characterize overall incidence of individuals affected by related outcomes (e.g., phthalate syndrome).	

Study Attributes	Considerations	
	Human studies	**Animal studies**
	Among several relevant health outcomes, preference is generally given to those outcomes with less concerns for indirectness or with greater biological significance.	
Study size and design	Preference is given to studies using designs reasonably expected to have power to detect responses of suitable magnitude This does not mean that studies with substantial responses, but low power would be ignored, but that they should be interpreted in light of a confidence interval or variance for the response. Studies that address changes in the number at risk (through decreased survival, loss to follow-up) are preferred.	

Notes: PBPK = physiologically based pharmacokinetic.

A.1.11.2 Approach to POD and Candidate RfD Derivation for Noncancer Health Outcomes

The current recommended EPA human health risk assessment approach for noncancer POD derivation described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* includes selection of a benchmark response (BMR), analysis of dose and response within the observed dose range, followed by extrapolation to lower exposure levels (U.S. EPA, 2002). For noncancer health outcomes, EPA performed dose-response assessments to define PODs, including low-dose extrapolation, when feasible, and applied uncertainty factors (UFs) to those PODs to derive candidate RfDs. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002). For PFOS, multiple candidate RfDs were derived within a health outcome as described in Section 4 of the Toxicity Assessment (U.S. EPA, 2024).

Considerations for BMR selection are discussed in detail in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). For the derivation of RfDs, the BMR selected should correspond to a low or minimal level of response in a population for the outcome of interest and is generally the same across assessments, though the BMR could change over time based on new data or developments. The following general recommendations for BMR selection were considered for this assessment:

- For dichotomous data (e.g., presence or absence), a BMR of 10% extra risk is generally used for minimally adverse effects. Lower BMRs (5% or lower) can be selected for severe or frank effects. For example, developmental effects are relatively serious effects, and BMDs derived for these effects could use a 5% extra risk BMR. Developmental malformations considered severe enough to lead to early mortality could use an even lower BMR (U.S. EPA, 2022c, 2012).
- For continuous data, a BMR is ideally based on an established definition of biologic significance in the effect of interest. In the absence of such a definition, a difference of one standard deviation (SD) from the mean response of the control mean is often used and one-half the standard deviation is used for more severe effects. Note that the standard deviation used should reflect underlying variability in the outcome to the extent possible separate from variability attributable to laboratory procedures, etc. (U.S. EPA, 2022c, 2012).

Deviations of these recommendations, if any, are described in Section 4 of the Toxicity Assessment (U.S. EPA, 2024).

For PFOS animal toxicological studies, EPA attempted benchmark dose (BMD) modeling on all studies considered for dose-response to refine the POD. BMD modeling was performed after converting the administered dose reported by the study to an internal dose using a pharmacokinetic model (see Toxicity Assessment, (U.S. EPA, 2024).). This approach resulted in dose levels corresponding to specific response levels near the low end of the observable range of the data and identified the lower limits of the BMDs (BMDLs) which serve as potential PODs (U.S. EPA, 2012). EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). BMDS fits mathematical

models to the data and determines the dose (i.e., BMD) that corresponds to a predetermined level of response (i.e., benchmark response or BMR). For dichotomous data, the BMR is typically set at either 5% or 10% above the background or the response of the control group. For continuous data, a BMR of one-half or one standard deviation from the control mean is typically used when there are no outcome-specific data to indicate what level of response is biologically significant (U.S. EPA, 2012). For dose-response data for which BMD modeling did not produce an adequate model fit, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD. However, a POD derived using a BMD approach typically provides a higher level of confidence in the conclusions for any individual case, as the BMDL takes into account all the data from the dose-response curve, incorporates the evaluation of the uncertainty in the BMD, and is related to a known and predefined potential effect size (i.e., the BMR) (U.S. EPA, 2022b, 2012). For noncancer endpoints, there were several factors considered when selecting the final model and BMD/BMDL, including the type of measured response variable (i.e., dichotomous or continuous), experimental design, and covariates (U.S. EPA, 2012). However, as there is currently no prescriptive hierarchy, selection of model types was often based on the goodness-of-fit and was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. The *Benchmark Dose Technical Guidance* provides a "BMD Decision Tree" to assist in model selection (U.S. EPA, 2012). See Appendix E for additional details on the study-specific modeling.

For the epidemiological studies considered for dose-response assessment, EPA used multiple modeling approaches to determine PODs, depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies, EPA used a hybrid modeling approach that involves estimating the incidence of individuals above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control (U.S. EPA, 2012) because the EPA was able to define a level considered clinically adverse for these outcomes (see Appendix E). As sensitivity analyses for comparison purposes, EPA also performed BMD modeling and provided study LOAELs/NOAELs as PODs for the epidemiological hepatic and serum lipid dose-response studies. For the immune studies, for which a clinically defined adverse level is not established, EPA used multivariate models provided in the studies and determined a BMR according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012). See Appendix E for additional details on the study-specific modeling.

After POD derivation, EPA used a pharmacokinetic model for human dosimetry to estimate human equivalent doses (HEDs) from both animal and epidemiological studies. A pharmacokinetic model for human dosimetry is used to simulate the HED from the animal PODs and is also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal dose POD obtained from dose-response modeling (see Toxicity Assessment, (U.S. EPA, 2024).). Based on the available data, a serum PFOS concentration was identified as a suitable internal dosimetry target for the human and animal endpoints of interest.

Next, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HEDS}) to account for five possible areas of uncertainty and variability For each noncancer dataset analyzed for dose-response, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HEDS}) to

account for five possible areas of uncertainty and variability: extrapolation from animals to humans, human variation, the type of POD being used for reference value derivation, extrapolation to chronic exposure duration, and extrapolation to a minimal level of risk (if not observed in the dataset). The particular value for these adjustments is usually 10, 3, or 1, but different values may be applied based on chemical-specific information if sufficient information exists in the chemical database. The assessment discusses the scientific bases for estimating these data-based adjustments and uncertainty factors (UFs). UFs used in this assessment were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

- Animal-to-human extrapolation: If animal results are used to make inferences about humans, the toxicity value incorporates cross-species differences, which may arise from differences in toxicokinetics or toxicodynamics. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. Otherwise, if the POD is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences.
- Human variation: The assessment accounts for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. If population-based data for the effect or for characterizing the internal dose are available, the potential for data-based adjustments for toxicodynamics or toxicokinetics is considered. Further, "when sufficient data are available, an intraspecies UF either less than or greater than 10× may be justified (U.S. EPA, 2002). However, a reduction from the default (10) is only considered in cases when there are dose-response data for the most susceptible population" (U.S. EPA, 2002). This factor is reduced only if the POD is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, 1991). Otherwise, a factor of 10 is generally used to account for this variation.
- LOAEL to NOAEL: If a POD is based on a LOAEL or a BMDL associated with an adverse effect level, the assessment must infer an exposure level where such effects are not expected. This can be a matter of great uncertainty if there is no evidence available at lower exposures. A factor of up to 10 is generally applied to extrapolate to a lower exposure expected to be without appreciable effects. A factor other than 10 may be used depending on the magnitude and nature of the response and the shape of the dose-response curve.
- Subchronic-to-chronic exposure: If a chronic reference value is being developed and a POD is based on subchronic evidence, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of up to 10 is applied when using subchronic studies to make inferences about lifetime exposure. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response. This factor may also be applied, albeit rarely, for developmental or reproductive effects if exposure covered less than the full critical period.
- In addition to the adjustments above, if database deficiencies raise concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database UF (U.S. EPA, 2002, 1991). The size of the factor depends on the nature of the database deficiency. For example, EPA typically follows the suggestion that

a factor of 10 be applied if a prenatal toxicity study and a two-generation reproduction study are both missing, and a factor of $10^{1/2}$ (rounded to 3) if either one or the other is missing. A database UF would still be applied if this type of study were available but considered to be a *low* confidence study.

The POD for a particular RfD is divided by the product of these factors. The RfD review recommends that any composite factor that exceeds 3,000 represents excessive uncertainty and recommends against relying on the associated RfD.

A.1.11.3 Cancer Assessment

A.1.11.3.1 Approach for Cancer Classification

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, a descriptive weight of evidence expert judgment is made, based on all available animal, human, and mechanistic data, as to the likelihood that a contaminant is a human carcinogen and the conditions under which the carcinogenic effects may be expressed (U.S. EPA, 2005a). A narrative is developed to provide a complete description of the weight of evidence and conditions of carcinogenicity. The potential carcinogenicity descriptors (presented in the 2005 guidelines) are:

- Carcinogenic to Humans
- Likely to Be Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not Likely to Be Carcinogenic to Humans

More than one carcinogenicity descriptor can be applied if a chemical's carcinogenic effects differ by dose, exposure route, or mode of action (MOA)[3]. For example, a chemical may be carcinogenic to humans above but not below a specific dose level if a key event in tumor formation does not occur below that dose. MOA information informs both the qualitative and quantitative aspects of the assessment, including the human relevance of tumors observed in animals. The MOA analysis must be conducted separately for each target organ/tissue type (U.S. EPA, 2005a).

A.1.11.3.2 Derivation of Candidate Cancer Slope Factors

EPA's 2005 *Guidelines for Carcinogen Risk Assessment* recommends a two-step process for the quantitation of cancer risk as a CSF. A CSF is a plausible upper bound lifetime cancer risk from chronic ingestion of a chemical per unit of mass consumed per unit body weight per day (mg/kg-day) (U.S. EPA, 2005a). First, a model is used to fit a dose-response curve to the data, based on the doses and associated tumors observed (U.S. EPA, 2005a). In the second step of quantitation, the POD is extrapolated to the low-dose region of interest for environmental exposures. The approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). When evidence indicates that a chemical causes cancer through a mutagenic MOA (i.e., mutation of deoxyribonucleic acid (DNA)) or the MOA for carcinogenicity is not known, the linear

[3]MOA is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. It is contrasted with "mechanism of action," which implies a more detailed understanding and description of events.

approach is used, and the extrapolation is performed by drawing a line (on a graph of dose vs. response) from the POD to the origin (zero dose, zero tumors). The slope of the line (Δresponse/Δdose) gives rise to the CSF, which can be interpreted as the risk per mg/kg/day.

For animal toxicological studies, EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). First, a PK model converted the administered dose reported by the study to an internal dose (see Toxicity Assessment, (U.S. EPA, 2024)). Then, BMDS fits multistage models, the preferred model type (U.S. EPA, 2012), to the data and the model is used to identify a POD for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD_{10} (the dose corresponding to a 10% increase in tumors) and the $BMDL_{10}$ (the 95% lower confidence limit for that dose) are also reported and are often used as the POD. Similar to noncancer PODs, selection of model types is often based on the goodness-of-fit (U.S. EPA, 2012). For PFOS, after a POD was determined, a PK model was used to calculate the HED for animal oral exposures (POD_{HED}). The CSF is derived by dividing the BMR by the POD_{HED}. See Appendix E for additional details on the study-specific modeling.

In addition, according to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), affirmative determination of a mutagenic MOA (as opposed to defaulting to a mutagenic MOA based on insufficient data or limited data indicating potential mutagenicity) indicates the potential for higher cancer risks from an early-life exposure compared to the same exposure during adulthood, and so requires that the application of age-dependent adjustment factors (ADAFs) be considered in the quantification of risk to account for additional sensitivity of children. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposure to a mutagenic chemical.

In cases for which a chemical is shown to cause cancer via an MOA that is not linear at low doses, and the chemical does not demonstrate mutagenic or other activity consistent with linearity at low doses, a nonlinear extrapolation is conducted. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* state that "where tumors arise through a nonlinear MOA, an oral RfD or inhalation reference concentration, or both, should be developed in accordance with EPA's established practice of developing such values, taking into consideration the factors summarized in the characterization of the POD" (U.S. EPA, 2005a). In these cases, an RfD-like value is calculated based on the key event[4] for carcinogenesis or the tumor response.

A.1.11.4 Selecting Health Outcome-Specific and Overall Toxicity Values

The next step is to select a health outcome-specific toxicity value for each hazard (cancer and noncancer) identified in the assessment. This selection can be based on the study confidence considerations, the most sensitive outcome, a clustering of values, or a combination of such factors; the rationale for the selection is presented in the assessment. Key considerations for candidate value selection are described in the IRIS Handbook (U.S. EPA, 2022c) and include: 1) the weight of evidence for the specific effect or health outcome; 2) study confidence; 3)

[4]The key event is defined as an empirically observed precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element.

sensitivity and basis of the POD; and 4) uncertainties in modeling or extrapolations. The value selected as the organ/system-specific toxicity value is discussed in the assessment.

The selection of overall toxicity values for noncancer and cancer effects involves the study preferences described above, consideration of overall toxicity, study confidence, and confidence in each value, including the strength of various dose-response analyses and the possibility of basing a more robust result on multiple datasets. The values selected as the overall RfD and CSF are discussed in the assessment.

A.2 Meta-Analysis Table

Studies identified in title/abstract and full-text screening as assessments or records with no original data were considered supplemental material. Meta-analysis studies were included among those secondary studies. Consideration of meta-analyses alongside original epidemiology studies could lead to duplication of results and give greater weight to studies included in meta-analyses; therefore, meta-analysis studies were summarized separately. For PFOS, 21 epidemiological meta-analysis studies were identified and summarized below (Table A-45).

Table A-45. Epidemiologic Meta-Analysis Studies Identified from Literature Review

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Meta-Analysis Studies Identified before February 2022				
Verner et al. (2015)	7	Canada, Denmark, Japan, Norway, Taiwan, United Kingdom, United States	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase of PFOS in maternal or cord blood (6 studies) = −5.0 g (−8.9, −1.1) • Physiologically based pharmacokinetic model simulations suggest that the association between PFAS levels and birthweight may be confounded by changes in glomerular filtration rate and due to blood draw timing
Negri et al. (2017)	13	Canada, China, Denmark, Germany, Greenland, Japan, Norway, Poland, South Korea, Taiwan, Ukraine, United Kingdom, United States	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in PFOS in maternal or cord blood (8 studies) = −0.92 g (−3.4, 1.6), I^2 = 74% • Pooled β per 1-ln ng/mL increase in PFOS in maternal or cord blood (8 studies) = −46.1 g (−80.3, −11.9), I^2 = 25%
Dzierlenga et al. (2020)	29	Australia, Belgium, Canada, China, Denmark, Greenland, Japan, Norway, Poland, South Korea, Spain, Sweden, Taiwan, Ukraine, United Kingdom, United States	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in PFOS in maternal or cord blood (29 studies) = −3.22 g (−5.11, −1.33), I^2 = 58.3% • Pooled β per 1 ng/mL in PFOS sampled before or in early pregnancy (8 studies) = −1.35 g (−2.33, −0.37), I^2 = 5% • Pooled β per 1 ng/mL in PFOS sampled in later pregnancy (21 studies) = −7.15 g (−10.93, −3.41), I^2 = 55% • Meta-regression modeling for timing of blood draw (early vs. late) showed that when drawn from before or early pregnancy, there was no significant relationship between birthweight and PFOS: 0.59 g/ng/mL (−1.94, 3.11)

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Cao et al. (2021)	5	South Korea, Spain, Taiwan, United States	Developmental	**LBW:** • Pooled OR for PFOS in maternal blood (5 studies) = 1.32 (1.09, 1.55), I^2 = 0.00% • Stratified by region: positive association in United States (2 studies), pooled OR = 1.44 (1.15, 1.72)
Deji et al. (2021)	21	Brazil, Canada, China, Denmark, Norway, Spain, United States	Developmental, Female Reproductive	**PTB[c]**: • Pooled OR (16 studies) = 1.20 (1.04, 1.38), I^2 = 54.3% • Pooled OR (6 studies in in North America) = 1.09 (1.01, 1.19); I^2 = 0% **Miscarriage:** • Pooled OR (6 studies): 1.01, 95% CI: 0.92, 1.10; I^2 = 35.9%
Gao et al. (2021)	29	Brazil, Canada, China, Denmark, Norway, Spain, Sweden, United States	Developmental, Female Reproductive	**Preeclampsia:** • Pooled OR per 1-log increase in PFOS (4 studies) = 1.27 (1.06, 1.51) **PTB[c]:** • Pooled OR per 1 ng/mL increase in PFOS (8 studies): 1.01 (1.00–1.02) **GDM** (7 studies), **miscarriage** (2 studies), pregnancy-induced hypertension (2 studies), **SGA** (6 studies), **LBW** (2 studies): Associations not statistically significant

Yang et al. (2022b)	22	Belgium, Canada, China, Denmark, Netherlands, Norway, Slovakia, Spain, Sweden, United States	Developmental	**PTB[c]:**
				• Pooled OR (14 studies): 1.54 (1.20, 1.98), I^2 = 63.4%
				  ○ Significant associations between PFOS and PTB in America [5 studies, pooled OR = 1.44 (1.19, 1.76), I^2 = 2.1%]
				  ○ Significant associations for PFOS in maternal blood sampled in 1st–2nd trimester and in 3rd trimester to delivery, and for maternal blood sample type overall
				Miscarriage:
				• Pooled OR (5 studies) = 1.10 (0.93, 1.32), I^2 = 0%
				SGA:
				• Pooled OR (9 studies) = 1.22 (0.92, 1.61), I^2 = 74.3%
				  ○ Significant associations for PFOS in cord blood at delivery [2 studies, pooled OR = 2.51 (1.45, 4.34), I^2 = 0.00%]
				• Pooled OR (7 studies): 1.52 (1.19, 1.94), I^2 = 19.1%
				LBW:
				• Pooled OR (2 studies, U.S. only): 1.71 (1.19, 2.47), I^2 = 0%
				• Pooled OR for PFOS in maternal blood (6 studies): 1.48 (1.16, 1.90), I^2 = 22.9%

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Costello et al. (2022)	25	Asia (NOS), Europe (NOS), United States	Hepatic	**ALT:** • PFOS was associated with higher ALT levels in adults and adolescents o Cross-sectional (6 studies) weighted z-score = 3.55, $p < 0.001$ o Longitudinal (1 study) reported a positive association o No associations for PFOS and ALT in children less than 12 yr of age or other liver enzymes **GGT, AST, and other liver enzymes:** • Associations for PFOS not statistically significant
Abdullah Soheimi et al. (2021)	29	Canada, China, Denmark, Italy, Norway, Spain, Sweden, Taiwan, United States	Cardiovascular (16 studies)	**CVD:** • Strong evidence of association between serum PFOS and CVD risk (14 studies); $z = 3.87$, $p < 0.0001$, $I^2 = 60.13\%$ **CIMT:** • Inconsistent associations between serum PFOS and CIMT (2 studies)
			Serum Lipids (10 studies)	• Consistent associations between serum PFOS and increased serum TC, LDL, and TG levels
			Metabolic (3 studies)	**GDM:** • Inconsistent associations between serum PFOS and increased GDM in pregnant mothers compared with non-pregnant mothers
Kim et al. (2018)	12	Canada, China, South Korea, Japan, Norway, Taiwan, United States	Endocrine	**Free T4:** • Pooled z-value (9 studies): 0.05 (0.03, 0.08), $I^2 = 0\%$ • More pronounced correlation between blood PFOS and free T4 in intermediate exposure group (8–16 ng/mL): 0.07 (0.02, 0.11), $I^2 = 0\%$ • Association not statistically significant among subgroup of pregnant women • **Total T4** (8 studies), **Total T3** (8 studies), **TSH** (12 studies): Associations not statistically significant • Sensitivity analyses removed outlier for total T4 and total T3; total T4 z-value = −0.04 (−0.07, −0.01), $I^2 = 5\%$; total T3 z-value = −0.06 (−0.09, −0.03), $I^2 = 31\%$
Zare Jeddi et al. (2021b)	7	Canada, China, Croatia, Italy, United States	Metabolic	**Metabolic syndrome:** • Pooled OR: 0.94 (0.79, 1.10), $I^2 = 78.7\%$
Stratakis et al. (2022)	21	China, Demark, Faroe Islands, Greenland, Netherlands, Norway, Spain,	Metabolic	**BMI z-score:** • In infancy (3 studies): Pooled β per unit increase in prenatal PFOS: −0.007 (−0.012, −0.003), $I^2 = 0\%$ • In childhood period (2–9 years) (10 studies): Pooled β per unit increase in prenatal PFOS = 0.00 (−0.01, 0.01), $I^2 = 42.9\%$

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
		Sweden, Taiwan, Ukraine, United Kingdom, United States		**Waist circumference:** • In childhood (4 studies): Pooled β per unit increase in prenatal PFOS = −0.06 (−0.19, 0.07), I^2 = 20.5% • Inconsistent associations between PFOA exposure and **fat mass**, **overweight risk**
Qu et al. (2021)	8	Denmark, Greenland, Norway, Poland, Sweden, Ukraine, United States	Neurodevelopmental	**ADHD**: • Pooled OR: 1.01 (0.88, 1.14), I^2 = 54.7% • Subgroup analysis between children's blood and prevalence rate of ADHD (2 studies), pooled OR = 1.05 (1.02, 1.08), I^2 = 48.7% • Subgroup analysis between PFOS exposure and prevalence rate of ADHD in the United States (2 studies), OR = 1.05 (1.02, 1.08), I^2 = 48.7%
Meta-Analyses Studies Identified after February 2022				
Jiang et al. (2022)	8	China, Denmark, France, Japan, The Philippines, United States	Cancer	**Breast cancer:** • PFOS had no association with breast cancer risk (pooled OR = 1.01 [0.87, 1.17], I^2 = 99.8%) • Pooled OR (8 studies) = 1.01 (0.87, 1.17), I^2 = 99.8% • Serious methodological limitations warrant cautious interpretation of results from this publication.
Gui et al. (2022a)	46	Australia, Brazil, Canada, China, Denmark, England, Faroe Islands, Germany, Greenland, Japan, Norway, Poland, South Korea, Spain, Sweden, Taiwan, Ukraine, United States	Developmental	Meta-analysis of 23 studies, pooled change in birthweight per 1-ln ng/mL increase in PFOS (unadjusted for gestational age/unstandardized birth weight). Significant effects observed for birth weight, birth length, ponderal index, and head circumference. No significant associations observed for preterm birth, low birth weight or small for gestational age. Subgroup analyses were included, by fetal sex, time of blood sample collection, blood sample type and whether adjusted for GA/parity, study design, and geographic region. Described assessment of risk of bias for studies included in the meta-analyses. **Birth weight:** • Pooled β per 1 ln(ng/mL) increase in PFOS (23 studies) = −34.88 g (−52.53, −17.24), I^2 = 66.2% **Birth length:** • Pooled β per 1 ng/mL increase in PFOS (3 studies) = − 0.034 cm (−0.062, −0.005), I^2 = 0.0% **Ponderal index:** • Pooled β per 1 ng/mL increase in PFOS (2 studies) = −0.355 g/cm^3 (−0.702, −0.008), I^2 = 0.0% **Head circumference:**

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
				• Pooled β per 1 ng/mL increase in PFOS (2 studies) = −0.021 cm (−0.038, −0.004), I^2 = 0.0% **PTB[c]:** • Pooled OR for the highest *vs*. lowest PFOS exposure (7 studies) = 1.46 (0.97, 2.18) **LBW:** • Pooled OR per 1 ln(ng/mL) increase in PFOS (3 studies) = 1.23 (0.96, 1.57) **SGA:** • Associations for PFOS not statistically significant.
Zhang et al. (2022b)	9	Faroe Islands, Germany, Greenland, Guinea-Bissau, Norway, United States	Immune	**Vaccine antibody production in children:** **Tetanus antibodies:** • Pooled effect estimate (3 studies, 5 results) = −10.04 (−19.12, −0.96), p-value for heterogeneity = 0.546 • Unclear what the effect estimate measures reported are and what units were used for PFOS exposure. **Diphtheria antibodies:** • No association for PFOS exposure.
Gui et al. (2022b)	22	China, Norway, Sweden, South Korea, Taiwan, United States	Metabolic	**Diabetes:** • Case-control studies (number of studies not reported): OR of T2DM incidence for high *vs.* low PFOS exposure = 1.80, (1.09, 2.97), I^2 = 5%; OR per ln-ng/mL increase in PFOS = 0.12 (0.07, 0.20), I^2 = 0% • Cohort studies (6 studies): HR per ln-ng/mL increase in PFOS = 1.40 (1.15, 1.69), I^2 = 47% • No association with PFOS in case-control and cross-sectional studies combined.
Wang et al. (2022a)	7	China, Denmark, Faroe Islands, Greenland, Poland, Ukraine, United States	Male Reproductive	**Semen quality:** • No association with any of the six semen parameters
Pan et al. (2023)	11	China, Italy, Norway, Sweden, United States	Cardiovascular	**Hypertension:** Pooled OR (11 studies, 12 results) = 1.19 (1.06–1.34), I2 = 87.8% Unit change in PFOS associated with pooled OR not reported. Serious methodological limitations warrant cautious interpretations of results from this publication. These include missing studies, inclusion of studies with overlapping populations, lack of effect estimate with common unit change in exposure.

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Yu et al. (2023)	9	NR	Renal	**Hyperuricemia:** Pooled OR (6 studies) = 1.23 (1.01, 1.50), I^2 = 58% Change in PFOA associated with pooled OR not reported.
Zhang et al. (2023a)	13	Canada, China, Denmark, Norway, Spain, South Korea, Taiwan, United States,	Endocrine	**TSH during pregnancy:** Pooled β per ng/mL increase in PFOS (13 studies) = 0.010 (0.009, 0.011), I^2 = 26.0% No significant associations with other thyroid hormones (e.g., total T3, total T4, free T3, free T4)

Notes: PFOS = perfluorooctane sulfonic acid; PFAS = perfluoroalkyl substances; ln = natural log; OR = odds ratio; LBW = low birth weight; PTB = preterm birth; GDM = gestational diabetes mellitus; SGA = small for gestational age; ALT = alanine aminotransferase; GGT = γ-glutamyltransferase; AST = aspartate aminotransferase; CVD = cardiovascular disease; CIMT = carotid artery intima-media thickness (mm); TC = total cholesterol; LDL = low-density lipoproteins; TG = triglyceride; T4 = thyroxine; T3 = triiodothyronine; TSH = thyroid stimulating hormone; BMI = body mass index; ADHD = attention deficit hyperactivity disorder; GA = gestational age; T2DM = type 2 diabetes mellitus; HR = hazard ratio; NR = not rated.

[a] Results reported as effect estimate and 95% confidence interval (CI) unless otherwise stated.

[b] Toxicological study data included in these publications were not subject to meta-analysis.

[c] Preterm birth was defined as birth ≤ 37 weeks of gestation.

A.3 Studies Identified In Supplemental Literature Search Assessment

The EPA conducted a supplemental literature search in 2023. Consistent with the final IRIS handbook (U.S. EPA, 2022c), the studies identified after February 3, 2022, including studies recommended via public comment, were "considered for inclusion only if they [were] directly relevant to the assessment PECO criteria and [were] expected to potentially impact assessment conclusions or address key uncertainties" (U.S. EPA, 2022b). For the purposes of this assessment, EPA defined impacts on the assessment conclusions as data from a study (or studies) that, if incorporated into the assessment, have the potential to significantly affect (i.e., by an order of magnitude or more) the final toxicity values (i.e., RfDs and CSFs) for PFOS or alter the cancer classification.

The EPA has defined a systematic process for assessing the potential for a quantitative impact that is consistent with the final IRIS Handbook. First, EPA reviewed studies against two broad inclusion criteria for new relevant health effects studies: 1) the study met the pre-defined PECO criteria and 2) following the SAB PFAS Review Panel's recommendation, the health effect/endpoint described in the study was within the one of the five health outcomes determined to have the strongest weight of evidence (i.e., developmental, hepatic, immune, cardiovascular, and cancer) (U.S. EPA, 2022c). Second, for studies that met these two inclusion criteria, two or more subject matter experts (e.g., epidemiologists and/or toxicologists) independently reviewed the studies to determine whether the study conclusions potentially impacted assessment conclusions. Subject matter experts considered a variety of factors to determine this, including, but not limited to, whether the publication provided 1) information on a health effect (within the five priority health outcomes) that was not previously quantitatively considered for dose response; 2) information on health effects that were previously considered quantitatively and potentially indicated effects at lower doses than the critical studies selected for the draft points of departure (PODs), RfD, or CSF; or 3) information on health effects that were previously considered quantitively and may have improved study design or data analyses compared with those that were selected for POD, RfD, or CSF derivation. If the subject matter experts disagreed about a study's potential quantitative impact, an additional expert independently reviewed the rationale and made a final decision. The EPA provides the rationales for study inclusion decisions in Table A-46 and Table A-47. For PFOS, 52 epidemiological and 4 animal toxicity studies were identified after the updated literature search in 2022 and underwent title/abstract and full-text screening according to Section A.1.6. These studies are summarized below (Table A-46 and Table A-47). Studies that were selected for inclusion proceeded to study quality evaluation and were incorporated into the relevant evidence synthesis and dose-response analysis when the study was determined to be *medium* or *high* confidence.

Numerous studies identified in the supplemental literature search examined associations between elevated exposure to PFOS and the primary health outcomes described in the Toxicity Assessment (U.S. EPA, 2024) (i.e., cancer, hepatic, immune, cardiovascular, and developmental). Specifically, there were six studies examining the effects of exposure to PFOS on cancer, 11 studies examining the effects of exposure to PFOS on serum lipids, nine studies examining the effects of exposure to PFOS on birth weight, one study examining the effect of exposure to PFOS on antibody response in children, and four studies examined the effect of exposure to PFOS on ALT concentrations. Summaries of these studies and their potential impact

to the evidence base, as well as additional studies examining outcomes belonging to the five priority health outcomes, are provided in Table A-46.

Two studies (Cao et al., 2022; Goodrich et al., 2022) identified in the supplemental literature search evaluated the risk of liver cancer with elevated exposure to PFOS, and both studies reported increased risks of liver cancer. In the 2022 updated evidence base, there were no studies (0/2) that reported significantly increased risk of liver cancer. Considering both studies identified in the 2023 supplemental literature search reported significant positive associations, there are altogether two studies reporting significantly increased risk of liver cancer (2/4). Both studies went through study quality evaluation, extraction, were considered for deriving PODs for PFOS, and were moved forward and integrated into the PFOS MCLG syntheses for cancer. One study (Zhang et al., 2023c) examining immune effects was determined to impact assessment conclusions and proceeded through systematic review steps, including study quality evaluation, extraction, incorporation into the evidence synthesis, and considered for dose-response analysis. The study reported a decreased antibody response to rubella in adolescents associated with elevated exposure to PFOA. This effect was consistent with other studies reporting decreased antibody response to other pathogens (i.e., tetanus and diphtheria), but provided additional evidence for a different pathogen.

Table A-46. Studies Identified After Updated Literature Review (Published or Identified After February 2022)

Reference	Major Findings	Assessment Implications
	Cancer	
Cao et al. (2022)	Case-control study conducted in Zhejiang, China of 203 liver cancer cases and 203 healthy controls. The odds of liver cancer incidence were significantly elevated with increasing PFOS exposure (OR = 2.609, 95% CI: 1.179, 4.029, p for trend = 0.001).	*Liver Cancer:* Exposure to PFOS may be associated with increased risk of liver cancer in adults. In the updated evidence base, there were no studies (0/2) that reported significantly increased risk of liver cancer. Considering both studies post-dating the 2022 literature search which reported significant positive associations, there are altogether two studies reporting significantly increased risk of liver cancer (2/4). Both studies were considered for deriving PODs for PFOS and were moved forward and integrated into the MCLG synthesis for cancer (see Toxicity Assessment, (U.S. EPA, 2024)). Cao et al. (2022) was determined to be *low* confidence during study quality evaluation and was not modeled. For Goodrich et al. (2022), the study had a limited number of cases (n = 11) and controls (n = 4) in the highest exposure group and was not modeled due to low sensitivity.
Goodrich et al. (2022)	Nested case-control study within the MEC Study, including incident, non-viral HCC cases (n = 50) and healthy controls (n = 50). Significant increase in risk in in those with high exposure (>85th percentile; >54.9 µg/L) *vs.* low exposure (<85th percentile; <54.9 µg/L) (OR = 4.50, 95% CI: 1.20, 16.00).	
Feng et al. (2022b)	Case-cohort study within the Dongfeng-Tongji cohort, including incident breast cancer cases (n = 226) and a random sub-cohort (n = 990). No association with PFOS or with summed PFSAs.	*Breast Cancer:* Exposure to PFOS may be associated with increased risk of breast cancer. Evidence for breast cancer was mixed in the updated evidence base with four studies reporting an increased risk (4/10). Significant increases in risk were only observed in some subpopulations (e.g., stratified by genotype) and for some specific types of breast cancer (e.g., ER– and PR– breast cancers). A recent meta-analysis reported a nonsignificant positive association for breast cancer, although there were methodological imitations that warrant cautions interpretations of results (Jiang et al., 2022). Considering the studies post-dating the 2022 updated literature review, one study (1/3)
Li et al. (2022)	Case-control study of incident Chinese breast cancer cases (n = 373) and healthy controls (n = 657). An inverse relationship was observed between increasing PFOS exposure and incident breast cancer.	
Velarde et al. (2022)	Case-control study of 150 Filipino women (75 breast cancer cases and 75 controls). Serum PFOS levels were significantly higher in cases than in controls. PFOS was positively but not statistically significantly associated with breast cancer risk across quartiles of exposure after adjusting for potential confounders. Positive significant association observed in crude models only in the highest quartile of PFOS.	

Reference	Major Findings	Assessment Implications
		reported an indication of increased risk of breast cancer. Altogether, five studies (5/13) reported an increased risk of breast cancer, which provides an indication of a potentially increased risk of breast cancer with increasing PFOS exposure, but the overall evidence remains mixed. The studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Wen et al. (2022)	Population-based cohort study of 11,747 participants from 1999–2014 NHANES followed up to December 2015. PFOS was statistically significantly associated with an increased risk in cancer mortality (OR = 1.75; 95% CI: 1.10, 2.83), but only in the highest tertile (≥17.1 ng/mL) compared with the lowest tertile (<7.9 ng/mL).	*All-cause Cancer:* There was concern for the lack of specificity of all-cause cancer in this study. Neither study examining all-cause cancer (0/2) from the updated evidence base reported a significantly increased risk. Considering the study post-dating the 2022 updated literature did not observe associations, there was altogether mixed evidence for all-cause cancer (1/3). The studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
	Cardiovascular	
Batzella et al. (2022b)	Cross-sectional study of residents (n = 36,517; aged 20–64) of the Veneto Region, Italy, a high-exposure community. In single-pollutant models, PFOS was significantly associated with increased TC (β per 1-ln ng/mL increase in PFOS = 5.14, 95% CI: 4.56, 5.72), HDL-C (β = 1.34, 95% CI: 1.12, 1.56), and LDL-C (β = 4.11, 95% CI: 3.60, 4.62). Significant positive associations were observed for all three lipid measurements in PFAS mixture analyses (WQS), with PFOS identified as the primary contributor to the association between increased PFAS exposure and elevated TC (weight: 0.43), HDL-C (weight: 0.65), and LDL-C (0.61) in the overall population. Similar results were observed in BKMR and Q-Gcomp analyses.	*Total Cholesterol:* Eleven studies identified after the 2022 updated literature search evaluated changes in TC, and eight (8/11) reported significant increases in TC with elevated exposure to PFOS (Batzella et al., 2022b; Batzella et al., 2022a; Cakmak et al., 2022; Cheng et al., 2022; Maranhao Neto et al., 2022; Nilsson et al., 2022; Rosen et al., 2022; Schillemans et al., 2022). In the updated evidence base, there was evidence of increases in TC (18/23) associated with elevated PFOS exposure in studies of adults (see Toxicity Assessment, (U.S. EPA, 2024)). Considering the updated evidence base and studies post-dating the 2022 literature search together, there were 23 of 33 general population adult studies reporting positive associations for TC. Overall, these studies support EPA's conclusion of *evidence indicates* that elevated exposures to PFOS
Batzella et al. (2022a)	Cross-sectional occupational study of retired and former male workers (n = 232) at a PFAS production plant located in Veneto, Italy (2018–2020). TC, LDL-C, and SBP were significantly elevated in the highest quartile of PFOS exposure compared with the lowest (TC: β = 17.04, 95% CI: 2.8, 31.27; LDL-C: β = 16.79, 95% CI: 3.37, 30.21; SBP β = 4.51, 95% CI: 0.09, 8.93), and in analyses of continuous exposure (TC β per 1-ln-ng/mL increase in PFOS = 7.26, 95% CI: 2.04, 12.48; LDL-C β = 5.90, 95% CI: 0.97, 10.83; SBP β = 2.58, 95% CI: 0.97,	

Reference	Major Findings	Assessment Implications
	4.18). SBP was also significantly elevated in WQS regression analyses of PFAS mixture, with PFOS identified as a main contributor (PFOS weight: 0.56). No significant associations observed for HDL-C and DBP.	are associated with adverse cardiovascular effects, specifically serum lipids, as well as EPA's selection of increased total cholesterol in adults for dose-response modeling.
Cheng et al. (2022)	Cross-sectional study of 98 patients recruited from Shiyan Renmin Hospital (Hubei, China), 2018–2019. Plasma PFOS was significantly associated with increased TC (β per 1-ln-ng/mL increase in PFOS = 4.761, 95% CI: 0.863, 8.809) and LDL-C (β = 6.206, 95% CI: 1.832, 10.767). No significant associations were observed for HDL-C or TG. Associations were partly mediated by methylation of genes related to lipid metabolism.	*LDL-C, HDL-C, and TG:* Nine studies identified after the 2022 updated literature search evaluated changes in LDL-C, and four studies (4/9) reported significant increases in LDL-C with elevated exposure to PFOS (Batzella et al., 2022b; Batzella et al., 2022a; Cheng et al., 2022; Nilsson et al., 2022). In the updated evidence base, thirteen general population adult studies (13/18) reported positive associations for LDL-C. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were 17 general population adult studies (17/27) reporting positive associations for LDL-C. The findings for HDL-C and TG in these ten studies were mixed, similar to results provided in the updated evidence base. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward; however, these studies support EPA's conclusion of *evidence indicates* that elevated exposures to PFOS are associated with adverse cardiovascular effects, specifically serum lipids.
Cakmak et al. (2022)	Population-based cross-sectional study (Canadian Health Measures Survey) of 6,768 participants aged 3–79 yr old. Increases in PFOS were significantly associated with increased TC (percent change per GM [5.3 µg/L] increase in PFOS: 3.3, 95% CI: 0.7, 5.9) and the TC/HDL ratio (2.6, 95% CI: 0.8, 4.4). No significant associations observed for LDL-C, HDL-C, or TG.	
Maranhao Neto et al. (2022)	Cross-sectional study of 479 adult participants (aged 25–89) from the Kardiovize study, Czech Republic. Serum PFOS was significantly associated with increased SBP (β per 1-ln-ng/mL increase in PFOS = 1.18, SE = 0.48), TC (β = 0.13, SE = 0.05), and HDL-C (β = 0.04, SE = 0.01). No significant associations observed for DBP, LDL-C, or TG.	
Rosen et al. (2022)	Cross-sectional study of 326 participants in the GenX Exposure Study (2017–2018) in Wilmington, North Carolina. Serum PFOS was positively associated with total non-HDL-C (β per quartile increase in PFOS = 4.89, 95% CI: 0.10, 9.68) and with TC (β = 5.71, 95% CI: 0.38, 11.04). Associations for non-HDL cholesterol and TC were strongest among older participants aged 63–86 yr. No associations were observed between serum PFOS and other serum lipid outcomes (HDL-C, LDL-C, and TG).	
Schillemans et al. (2022)	Population-based nested case-control study of Swedish adults (n = 1,528) within two cohorts, the SMC-C and the Cohort of 60YO, including the first incident myocardial infarction (n = 345) and stroke (n = 354) cases. In cross-sectional analyses among 631 controls, baseline plasma PFOS was associated with increased TC (β per 1-SD-ln-ng/mL PFOS = 0.14, 95% CI: 0.06, 0.22), increased LDL-C (β = 0.13, 95% CI: 0.06, 0.20), increased HDL-C (β = 0.05, 95% CI: 0.01, 0.07), increased apolipoprotein A1 (β = 0.04, 95% CI: 0.02, 0.08), and decreased TG (β = −0.11, 95% CI: −0.17, −0.05). No significant association was observed for apolipoprotein B. In prospective analyses of the pooled cohorts, there were no significant associations between baseline PFOS and subsequent incidence of myocardial infarction, stroke, or CVD.	*Blood Pressure:* Measures of blood pressure and hypertension were examined in six studies identified after the updated 2022 literature search, and three studies (3/6) reported significant increases in systolic blood pressure or increased risk of hypertension (Batzella et al., 2022a; Ding et al., 2022; Maranhao Neto et al., 2022). One meta-

Reference	Major Findings	Assessment Implications
Papadopoulou et al. (2022)	Study of Norwegian adults ages 24–72 yr old (n = 127) in the EuroMix study. Serum PFOS was associated with significantly higher day 1 and day 2 HDL-C, apolipoprotein A1, and apolipoprotein A2 in women after adjustment for false discovery rate (HDL-C % change per IQR increase in PFOS = 10%, 95% CI: 4%, 18%; apolipoprotein A1 = 7%, 95% CI: 3%, 12%; apolipoprotein A2 = 8%, 95% CI: 3%, 12%). No significant associations were observed for TC, LDL-C, or TG.	analysis post-dating the 2022 literature search reported a significantly increased risk of hypertension in adults, but there were some methodological limitations which warrant cautious interpretations of results (Pan et al., 2023). In the updated evidence base, there was evidence of increases in systolic (7/9) and diastolic blood pressure (7/8), and increased risk of hypertension (4/7) in adults. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were nine general population adult studies (9/14) reporting increases in systolic blood pressure and diastolic blood pressure (9/17); and five (5/9) general population adult studies reporting increases in risk for hypertension. Evidence for changes in blood pressure and increases in risk for hypertension were supportive of a conclusion of *moderate* evidence for cardiovascular effects, specifically serum lipids, although blood pressure and hypertension were not selected as outcomes for modeling.
Nilsson et al. (2022)	Prospective occupational study of Australian firefighters who had used AFFF reporting cross-sectional (n = 783) and longitudinal (n = 130) analyses. PFOS was significantly associated with increased TC for those in the highest exposure quartile (β per doubling of PFOS = 0.273, 95% CI: 0.027, 0.52) and LDL-C (β = 0.100, 95% CI: 0.029, 0.17) in cross-sectional analyses. No significant associations were observed for serum lipids in longitudinal analyses.	
Linakis et al. (2022)	Cross-sectional study of 7,242 NHANES participants (cycles 2003–2016). Serum PFOS was positively associated with ln-TC and the magnitude of the association was not substantially altered by additional adjustment for energy intake-adjusted fiber.	
Dunder et al. (2022)	Prospective cohort study PIVUS of seniors at age 70 (n = 864), followed up at age 75 (n = 614) and age 80 (n = 404). Increases in PFOS over the 10-year follow-up were significantly associated with increases in HDL-C (β = 0.04, 95% CI: 0.02, 0.06, p = 0.001). No significant association between changes in PFOS and TC, LDL-C, or TG.	
Ding et al. (2022)	Cohort study of 1,058 women (ages 42–52) with no hypertension from the multiethnic and multiracial SWAN. There was significantly increased risk of hypertension per doubling of PFOS (HR = 1.18, 95% CI: 1.09, 1.28), and across tertiles of baseline serum PFOS (p-trend < 0.0001). In the mixture analysis, women in the highest tertile of PFAS concentrations had a significantly higher risk of hypertension compared with those in the lowest tertile (HR = 1.71, 95% CI: 1.15, 2.54; p-trend = 0.008).	*Cardiovascular disease:* A variety of cardiovascular diseases, including heart arrythmia, myocardial infarction, stroke, angina, heart disease, and acute coronary syndrome were examined in four studies identified after the 2022 updated literature search, and three studies (3/4) reported significant increases in risk for at least one type of cardiovascular disease (Li et al., 2023; Feng et al., 2022a; Wen et al., 2022). In the updated evidence base, evidence was limited for cardiovascular diseases with one study reporting increased risk of microvascular disease (1/1), myocardial infarction (1/1), and all cardiovascular disease (1/4). Other cardiovascular diseases were examined in single studies, and no associations were observed. Considering the
Yang et al. (2022a)	Prospective study of 826 pregnant women from the Jiashan Birth Cohort (enrollment 2016–2018), Jiashan, Zhejiang, China. Plasma PFOS measured within 16 wk gestation was inversely associated with gestational hypertension (OR per 1-ln ng/mL increase in PFOS = 0.62, 95% CI: 0.39, 0.99), and with SBP in the third trimester (β = −1.14, 95% CI: −2.10, −0.18). Similar associations were observed across PFOS quartiles. PFOS was not associated with SBP in other trimesters, or with DBP in any trimester.	
Tian et al. (2023)	Case-control study of pregnant women from Hangzhou, China, with (n = 82) and without (n = 169) preeclampsia. PFOS exposure measured 1–2 d before delivery was not significantly associated with SBP or DBP in pregnant women.	

Reference	Major Findings	Assessment Implications
Zhang et al. (2022e)	Prospective study of 1,080 participants in the Dongfeng-Tongji cohort of retired workers in China established in 2008 and followed for approximately 5 yr. Baseline serum PFOS concentrations were significantly inversely associated with risk of incident hypertension (RR per 1-log10-ng/mL increase in PFOS = 0.94, 95% CI: 0.88, 0.99) and with changes in SBP over the follow-up period (β = −1.48, 95% CI: −2.56, −0.51). Significantly inverse associations for risk of incident hypertension (p for trend = 0.016) and changes in SBP (p for trend = 0.032) persisted across PFOS quartiles. Associations with hypertension risk were observed among females but not males (p-value for interaction −0.44). Baseline serum PFOS concentrations were not significantly associated with changes in DBP.	updated evidence base and studies post-dating the 2022 literature search together, evidence was mixed for any cardiovascular disease (4/8). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Atherosclerotic changes:* One study identified after the updated 2022 literature search examined atherosclerotic changes in young adults, and the study reported significantly increased CIMT (Lin et al., 2022). In the updated evidence base, two studies in children and adolescents (2/3) observed significant changes in CIMT across exposure groups. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were three studies (3/4) reporting changes to CIMT in children and adolescents. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Feng et al. (2022a)	Population-based cross-sectional study (NHANES, 2003–2012) of 7,904 adults. In males, there was a significantly increased odds of heart attack (OR per 1-log-ng/mL increase in PFOS = 1.01, 95% CI: 1.00, 1.01, p = 0.040) and stroke (OR per 1-log-ng/mL increase in PFOS = 1.01, 95% CI: 1.00, 1.01, p = 0.008). No associations were observed between PFOS exposure and heart failure, coronary heart disease, angina, or total CVD in either males or females.	
Lin et al. (2022)	Cross-sectional study of participants from two prior studies in Taiwan: controls (aged 22–63; n = 601) from a CVD study (2008–2011) and participants (aged 12–30, n = 886) from the YOTA cohort (2006–2008). Serum PFOS was associated with significantly increased mean CIMT (β per 1-ln ng/mL increase = 9.240, SE: 2.077). Significantly increased CIMT was also observed when examining specific measurements such as the left and right common carotid artery, and the left and right carotid bulb. No associations were observed for the left and right internal carotid arteries.	
Li et al. (2023)	Hospital-based case-control study of adults with and without ACS (355 cases, 355 age- and sex-matched controls) recruited in 2022 in Shijiazhuang, Hebei, China. In single PFAS models, plasma PFOS was significantly associated with ACS (OR per 1-lm-ng/mL increase in PFOS = 1.65, 95% CI: 1.14, 2.38). The association between PFOS and ACS remained significant in multiple-PFAS models. No significant associations were observed with PFAS mixtures.	
Wen et al. (2022)	Population-based cohort study of 11,747 participants from 1999–2014 NHANES followed up to December 2015. PFOS was statistically significantly associated with an increased risk in heart disease mortality (OR = 1.75; 95% CI: 1.10, 2.83), but only in the highest tertile (≥17.1 ng/mL) compared with the lowest tertile (<7.9 ng/mL).	
	Developmental	

Reference	Major Findings	Assessment Implications
Sevelsted et al. (2022)	Prospective study of 738 maternal-child pairs enrolled in a population-based birth cohort study (COPSAC-2010) in Zealand, Denmark (2008–2010). Maternal plasma PFOS (measured at 24 wk GA and 1 wk postpartum) was associated with significantly lower birth BMI z-score (β per 1-ng/mL increase = −0.04, 95% CI: −0.08, −0.01), decreased birth weight z-score (β = −0.04, 95% CI: −0.07, −0.01), and decreased birth weight percentile for sex and GA (β = −1.07, 95% CI: −1.96, −0.19).	*Birth weight:* Nine studies identified after the updated literature search evaluated changes in birth weight (i.e., birth weight and birth weight for sex and GA), and four studies reported significant decreases. Studies reporting significant results examined changes in birth weight in relation to PFOS concentrations measured in later pregnancy (Jia et al., 2023; Tian et al., 2023; Hall et al., 2022; Sevelsted et al., 2022). Other studies not observing decreases in birth weight were generally smaller (i.e., <200 participants) (Wang et al., 2023a; Zhang et al., 2023a). In the updated evidence base, there were 27 studies reporting deficits in birth weight (27/39). Considering the updated evidence base and studies post-dating the 2022 literature search together, deficits in birth weight were observed in 33 studies (31/48). Overall, these studies support EPA's conclusion of evidence indicates that elevated exposures to PFOS are associated with adverse developmental effects, as well as EPA's selection of decreased birth weight for dose-response modeling. *Other FGR:* Regarding other fetal growth restriction outcomes, three studies identified after the updated literature search evaluated changes in other measures of fetal growth restriction (e.g., birth length, head circumference, and ponderal index) and no associations were observed. In the updated evidence base, there was some evidence of adverse effects for birth length (15/28) and head circumference (13/23), but the evidence was generally mixed. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Gestational duration and PTB:* Preterm birth was examined in three studies, and two studies reported
Tian et al. (2023)	Case-control study of pregnant women from Hangzhou, China, with (n = 82) and without (n = 169) preeclampsia. PFOS exposure measured 1–2 d before delivery was significantly associated with decrease in birth weight (β per 1-log10-unit increase in PFOS = −20.3, 95% CI: −33.2, −7.54).	
Jia et al. (2023)	Cross-sectional study of 66 infants born to women at a maternity hospital in Shijiazhuang, Hebei, China in 2022. Umbilical cord serum PFOS was inversely correlated with birth weight (Spearman correlation coefficient = −0.319, $p < 0.05$).	
Hall et al. (2022)	Prospective birth cohort study of 120 mother-child pairs enrolled in the HPHB cohort in Durham, North Carolina (enrollment 2010–2011). The highest tertile of placental PFOS exposure was significantly associated with decreased birth weight percentile in male infants (% change compared with lowest tertile = −13%, 95% CI: −23%, −1.6%), and significantly associated with increased birth weight in female infants (% change = 11%, 95% CI: 2.8%, 19%). No other associations with gestational age or birth weight for gestational age were observed.	
Shen et al. (2022)	Prospective study of 506 maternal-child pairs enrolled in a birth cohort study in Hangzhou, China (2020–2021). No significant associations were observed between maternal serum PFOS (GA at assessment not specified) and birth weight, Apgar scores, or preterm birth after adjustment for confounders.	
Wang et al. (2023a)	Prospective study of 180 maternal-child pairs enrolled in a birth cohort study in Tangshan City, Hebei province, China, 2013–2014. No associations were observed between placental PFOS and birth outcomes (birth weight, birth length, head circumference, and ponderal index).	
Wang et al. (2023b)	Prospective study of 1,405 maternal-child pairs enrolled in the Shanghai Birth Cohort in Shanghai, China (2013–2016). No significant associations were observed between first trimester PFOS and birth weight z-score in children of women with low or high fasting plasma third trimester glucose levels.	
Peterson et al. (2022)	Prospective study of pregnant women and their fetuses (n = 335 mother-fetus pairs) from the Maternal and Developmental Risks from Environmental and Social Stressors (MADRES) pregnancy cohort. No significant associations were observed between maternal serum PFOS measured during pregnancy	

Reference	Major Findings	Assessment Implications
	(median = 19 wk, range = 5.7–38.3 wk GA) and fetal growth parameters (head circumference, biparietal diameter, femur bone length, abdominal circumference, and estimated fetal weight).	significantly increased risks (Liao et al., 2022; Yu et al., 2022). Exposure sample timing differed between the two studies, with one cohort study colleting maternal samples in the first trimester (Liao et al., 2022) and one study colleting maternal samples in the third trimester (Yu et al., 2022). In the updated evidence base, there are ten studies (10/20) reporting increased risk of preterm birth. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Pregnancy loss:* Pregnancy loss was examined in two studies, and neither study reported significantly increased risks (Mi et al., 2022; Nian et al., 2022). Timing of exposure sample collection was reported in one case-control study analyzing pre-pregnancy plasma samples (Nian et al., 2022) and one nested case-control study did not report exposure sample timing (Mi et al., 2022). In the updated evidence base, there are four studies (4/7) reporting increased risk of pregnancy loss. Considering the updated evidence base and studies post-dating the 2022 literature search together, there are four studies reporting increased risk of pregnancy loss (4/9). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Postnatal growth:* Five studies examined postnatal growth in early childhood, and two studies reported an increased risk of following adverse BMI growth trajectories in early childhood (Zeng et al., 2023; Romano et al., 2022). No significant associations were reported from other studies examining postnatal growth from studies on birth cohorts such as the Shanghai Birth Cohort (Zhang et al., 2022d), the Flemish Environmental Health Study (Cai et al.,
Zhang et al. (2022a)	Cohort study of pregnant women and their children (n = 94 mother-child pairs) living near an e-waste recycling facility in Guangdong, China (2016). No significant associations observed between maternal serum PFOS and birth outcomes (i.e., birth weight, birth length, and head circumference).	
Liao et al. (2022)	Prospective study of 1,341 maternal-child pairs enrolled in the Guangxi Zhuang Birth Cohort study in Guangxi, China, 2015–2019. In single PFAS models, first trimester serum PFOS was associated with increased risk of preterm birth (RR per log10-ng/mL increase = 2.251, 95% CI: 1.307, 3.874). There was a significant trend across PFOS quartiles. PFAS mixture was associated with increased risk of preterm birth, with PFOS identified as one of the main contributors (weight: 31.8%).	
Yu et al. (2022)	Prospective study of 836 maternal-child pairs enrolled in the Maoming Cohort Study in Maoming, China, 2015–2018. Maternal third trimester serum PFOS was positively associated with preterm birth (OR per ln-ng/mL increase = 2.07, 95% CI: 1.70, 2.52); paternal serum PFOS was inversely associated with preterm birth (OR = 0.44, 95% CI: 0.36, 0.54). No association was observed with neonatal PFOS.	
Nian et al. (2022)	Case-control study of women with and without unexplained recurrent spontaneous abortion (URSA) (464 cases, 440 controls) in Shandong and Zhejiang provinces, China (2014–2016). No association was observed between prepregnancy plasma PFOS and URSA.	
Mi et al. (2022)	Nested case-control study of women with and without early pregnancy loss (41 cases, 47 controls) in Beijing, China (2018–2020). No association was observed between prenatal PFOS (GA at measurement not specified) and early pregnancy loss.	
Romano et al. (2022)	Prospective study of 481 maternal-child pairs enrolled in the NHBCS with at least four child anthropometric measurements in the first year of life, (2009–2018). Among girls, maternal second trimester PFOS was associated with an increased chance of following a growth trajectory in which BMI increases gradually over the first year of life compared with a growth trajectory in which BMI increases gradually and plateaus around 3 mo (relative risk ratio per doubling of PFOS = 2.5, 95% CI: 1.0, 6.1). Among girls, PFOS was also associated with an increased chance of following a growth trajectory in which BMI steeply increases in mo 1–3 of life (relative risk ratio per doubling = 2.8, 95% CI: 1.0, 7.6). At	

Reference	Major Findings	Assessment Implications
	6 mo, the estimated mean difference in BMI was significantly higher in girls in the highest tertile of PFOS exposure compared with the lowest (β = 0.54, 95% CI: 0.05, 1.03). No associations were observed with other growth trajectory groups or with BMI at other timepoints.	2023), or the Danish National Birth Cohort (Luo et al., 2022). In the updated evidence base, increased risk for adverse changes in postnatal weight changes in infancy were observed in eight (8/10) studies. Considering the updated evidence base and studies post-dating the 2022 literature search together, there are ten studies reporting increased risk adverse effects on postnatal growth (12/15). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Zeng et al. (2023)	Prospective study of mother-child pairs (n = 1,671) from the Shanghai Birth Cohort in China (2013–2016). Child anthropometric measures were taken at 6, 12, 24, and 48 mo. Maternal serum PFOS measured in early pregnancy (9–16 weeks GA) was associated with significantly increased odds of following a BMI-for-age z-score trajectory which increases steadily for the first 12 mo followed by steeper increases up to 40 mo compared with a trajectory with a steep increase in the first 12 mo but progressively reversed to a stable trajectory at 40 mo (OR = 2.36, 95% CI: 1.27, 4.40).	
Cai et al. (2023)	Prospective study of 207 mother-child pairs from two birth cohorts from the FLEHS: FLEHS I (2002–2004) and FLEHS II (2008–2009). No statistically significant associations were observed between cord blood PFOS and infant growth in single- or multi-pollutant models.	
Zhang et al. (2022d)	Prospective cohort study (the Shanghai Birth Cohort) of 2,395 mother-infant pairs. Prenatal PFOS exposure measured in early pregnancy (median, 15 gestational wk) was not associated with infant length, weight, and head circumference at birth, 42 d, 6 mo, and 12 mo.	
Luo et al. (2022)	Prospective study in the DNBC, 656 children. Prenatal exposure to PFOS was not associated with facial features (measures of palpebral fissure length, philtrum groove, and upper-lip thickness) in children at age 5.	
	Immune	
Zhang et al. (2023c)	Population-based cross-sectional study of adolescents aged 12–19 with detectable serum rubella and measles antibody levels (n = 819) from the NHANES 2009–2010 and 2013–2014 cycles. The study population was stratified in two groups of lower (n = 552) and upper (n = 267) folate levels based on the <66th percentile. Significant inverse associations were observed for rubella antibody response in the whole study population (% change per 2.7-fold increase in serum PFOS = −8.16, 95% CI: −13.67, −2.31) and in the lower folate group (% change = −11.00, 95% CI: −18.08, −3.31). No significant associations for rubella antibodies in the higher folate group, or for mumps and measles antibodies.	*Vaccine response:* Three studies identified after the updated literature search evaluated antibody responses to multiple pathogens in different populations, and two studies observed an effect (Zhang et al., 2023c; Porter et al., 2022). The only study examining rubella antibody response observed a significant decrease (Zhang et al., 2023c). In the updated evidence base, there was one study (1/2) which reported significant decreases in rubella antibody response in children and adolescents. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were two studies (2/3) in children and adolescents
Kaur et al. (2023)	Cross-sectional study of pregnant participants with past SARS-CoV-2 infection (n = 72) from the Generation C Study. No significant association was observed between maternal plasma PFOS and SARS-CoV-2 anti-spike IgG titers. In WQS regression analysis of a PFAS mixture index, maternal SARS-CoV-2 anti-spike	

Reference	Major Findings	Assessment Implications
	IgG titers were significantly decreased (β = −0.35, 95% CI: −0.52, −0.17, p-value = 0.0003), with PFOS accounting for greater than 10% of the effect.	reporting significantly decreased rubella antibody response. Zhang et al. (2023c) was considered for deriving PODs for PFOS and was moved forward and integrated into the MCLG synthesis for immune effects (see Toxicity Assessment, (U.S. EPA, 2024)). One study (1/2) examining SARS-CoV-2 antibody response reported significant inverse associations (Porter et al., 2022). There were a limited number of studies examining SARS-CoV-2 in the studies captured in updated 2022 evidence base, but these studies post-dating the 2022 updated literature search suggest there may be an association between exposure to PFOS and decreased SARS-CoV-2 antibody response, coherent with decreases in the antibody response for other pathogens. Overall, these studies provide additional evidence for decreased antibody response for multiple pathogens, including in populations located in the United States, and support EPA's conclusion of *evidence indicates* that elevated exposures to PFOS are associated with immunological effects in humans, as well as EPA's selection of decreased vaccine response in children for dose-response modeling. *Infectious disease:* One study identified after the updated 2022 literature search examined infectious disease in children and reported a significantly increased odds of a recent common cold (Zhang et al., 2022c). In the updated evidence base, results were mixed for infectious disease in children, with five studies (5/12) reporting positive associations or increased risk. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were five studies (6/13) reporting positive associations or increased risk of infectious disease in children. Overall, the study was judged to not quantitatively impact assessment conclusions and were not moved forward.
Porter et al. (2022)	Longitudinal study of current and retired workers (n = 415; 757 observations) of 3M facilities in Decatur, Alabama and Menomonie, Wisconsin (Spring 2021). Serum PFOS was associated with decreases in SARS-CoV-2 anti-spike IgG antibody and SARS-CoV-2 neutralizing antibody response after adjustment for age, gender, race, BMI, location, smoking, immunocompromising conditions or recent corticosteroid use, and time since antigenic stimulus. Associations were not significant after further adjustment for the antigenic stimulus group.	
Jones et al. (2022)	Cross-sectional analysis of infants (n = 3,448) from the Upstate KIDS Study Birth Cohort (2008–2010). PFOS and immunoglobulins were both quantified in infant heel stick blood spots. No significant associations were observed for IgA, IgE, IgG_1, IgG_2, IgG_3, IgG_4, or IgM.	
Zhang et al. (2022c)	Population-based cross-sectional study of children aged 3–11 (n = 517) and adolescents aged 12–19 (n = 2,732) from the NHANES 2013–2014 cycle and 2003–2016 cycles, respectively. The odds of a recent common cold (i.e., past 30 d) was significantly elevated in adolescents per doubling in serum PFOS after mutual adjustment for other PFAS (OR per 1-log2 increase = 1.26, 95% CI: 1.01, 1.56). The association was not significant in single-pollutant models, and no associations were observed in children.	
Qu et al. (2022)	Case-control study from the Second Affiliated Hospital of Zhejiang University School of Medicine (2019–2020), including rheumatoid arthritis patients (n = 156) and healthy controls (n = 156). The odds of rheumatoid arthritis were non-significantly elevated with increasing serum PFOS (OR = 1.381, 95% CI: 0.972, 1.658, p = 0.06).	
Zhao et al. (2022b)	Case-control study of rheumatoid arthritis (RA) patients (n = 294) and volunteer controls (n = 280) in Hangzhou, China from January 2018–December 2020. A significant positive association was observed between serum PFOS and RF, an indicator of RA (β per 1-ln ng/mL increase = 0.52, 95% CI: 0.28, 0.77), and ACPA (β = 0.48, 95% CI: 0.23, 0.73). A significant inverse association was observed for IgM (β = −0.24, 95% CI: −0.64, 0.15). No significant associations observed for C-RP, IgA, IgG, C4, C3, KAP, and LAM.	
Zhao et al. (2022a)	Case-control study from the Second Affiliated Hospital of Zhejiang University School of Medicine (2019–2020), including RA patients (n = 155) and healthy controls (n = 145). Serum PFOS concentrations were higher in cases than in controls (p < 0.0001). In a cross-sectional analysis of cases only, cases were categorized by their DAS28; inactivity, low activity, moderate activity, and high activity). Significant differences (p = 0.0001) in median serum PFOS	

Reference	Major Findings	Assessment Implications
	concentrations were observed between the four groups of DAS28, with the highest median PFOS concentrations observed among cases with the highest DAS28 score (≥5.1). Comparing cases categorized as with leukopenia (WBC < 4.0×10^9/L) to those without leukopenia (WBC ≥ 4.0×10^9/L), serum PFOS levels were higher in the non-leukopenia group. No significant associations were observed between PFOA exposure and interstitial lung disease in cases.	*Immunoglobulins:* Two studies identified after the updated 2022 literature search examined immunoglobulins, and one study (1/2) observed an effect (Zhang et al., 2022c). In the updated evidence base, four studies examined immunoglobulins in a variety of populations, with mixed evidence. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Autoimmune disease:* Three studies examining RA were identified after the updated 2022 literature search, and two studies (2/3) observed significantly increased RA biomarkers (Zhao et al., 2022b) and increased RA severity scores (Zhao et al., 2022a). While both studies observed increases in risk or evidence of increased biomarkers related to RA, the methods of examination differed between the studies, limiting comparability of the results. No studies in the updated evidence base examined RA. Evidence for other autoimmune diseases in the updated evidence base was mixed and limited to a small number of studies. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
	Hepatic	
Liu et al. (2022)	Community-based cross-sectional study of adults (n = 1,303) living in Guangzhou, China. Positive dose-response relationships between PFOS and liver enzymes, except for ALP. Significant associations were observed for the 50th compared with the 25th percentile of PFOs for liver function biomarkers (percentage differences): ALB (4.80, 95% CI: 4.47, 5.13), ALT (7.01, 95% CI: 4.69, 9.37), AST (2.76, 95% CI: 1.29, 4.25), GGT (6.74, 95% CI: 4.01, 9.55), and DBIL (3.72, 95% CI: 5.41). Associations remained significant for other comparisons (75th percentile vs. 25th percentile and 95th percentile vs. 25th percentile). No significant association observed for ALP.	*ALT:* Four studies identified after the updated 2022 literature search examined ALT, and one (1/4) reported a significant increase (Liu et al., 2022). In the updated evidence base, there were six *medium* confidence studies (6/8) reporting increased ALT in adults. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were eleven (7/12) studies reporting increases in ALT in adults. Overall, the studies support EPA's conclusion that *evidence indicates* that PFOS exposure is likely to cause hepatotoxicity
Borghese et al. (2022)	Population-based cross-sectional study of adults (n = 4,657) from three cycles of the CHMS. A twofold increase in serum PFOS was associated with significantly	

Reference	Major Findings	Assessment Implications
	elevated liver enzymes (percent difference): GGT (7.6, 95% CI: 3.0, 12.4), ALP (4.1, 95% CI: 2.4, 5.9), and AST in men (7.6, 95% CI: 4.6, 10.8) and women (3.3, 1.2, 5.5). No significant associations observed for ALT and total bilirubin.	in humans, specifically increased ALT in adults; however, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Other liver enzymes:* Three studies identified after the updated 2022 literature search examined liver enzymes besides ALT, and all three studies (3/3) observed effects (Borghese et al., 2022; Cakmak et al., 2022; Liu et al., 2022). In the updated evidence base, there were three studies (3/11) reporting increases in GGT in adults. Results for other liver enzymes in adults were generally mixed. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were six studies (6/14) reporting increases in GGT in adults. Overall, the studies support EPA's conclusion that *evidence indicates* that PFOS exposure is likely to cause hepatotoxicity in humans, specifically increased ALT in adults; however, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Liver disease:* Two studies identified after the updated 2022 literature search examined liver disease, and none reported increased risk of any liver disease (0/2). In the updated evidence base, there was one study examining all liver disease and did not observe an association (0/1). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Cakmak et al. (2022)	Population-based cross-sectional study (CHMS) of 6,768 participants aged 3–79 yr old. Increases in PFOS were significantly associated with decreased ALP (percent change per GM [5.3 μg/L] increase in PFOS: −2.1, 95% CI: −3.7, −0.5). Significant increases were observed for GGT (11.6, 95% CI: 1.8, 22.3), and bilirubin (4.7, 95% CI: 3.8, 5.6). No significant associations observed for AST or ALT.	
Zhang et al. (2022a)	Cohort study of pregnant women and their children (n = 94 mother-child pairs) living near an e-waste recycling facility in Guangdong, China (2016). Cross-sectional analyses of maternal liver enzymes observed significantly decreased AST (β per 1-ln ng/mL increase in PFOS = −0.236, 95% CI: −0.429, −0.043), but no association for ALT.	
Nilsson et al. (2022)	Cross-sectional occupational study of Australian firefighters who had used AFFF (n = 783). No significant associations were observed for ALT or self-reported liver problems.	
E et al. (2023)	Population-based cross-sectional study of adults (n = 3,464) from NHANES (2005–2018). The relative risk of NAFLD was decreased in men (RR per 1-log ng/mL increase in PFOS = 0.878, 95% CI: 0.778, 0.991). No significant associations were observed in all participants or in women only. No significant monotonic trends across qualities of PFOS were observed.	

Notes: OR = odds ratio; CI = confidence interval; PFOS = perfluorooctane sulfonic acid; POD = point of departure; MEC = Multiethnic Cohort Study; HCC = hepatocellular carcinoma; PFSAs = perfluorinated sulfonic acids; NHANES = National Health and Nutrition Examination Survey; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PFAS = perfluoroalkyl substances; WQS = weighted quantile sum; BKMR = Bayesian kernel machine regression; Q-Gcomp = quantile-g computation; ln = natural log; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglycerides; GM = geometric mean; SE = standard error; SMC-C = Swedish Mammography Cohort-Clinical; 60YO = 60-year-olds; SD = standard deviation; CVD = cardiovascular disease; MI = myocardial infarction; IQR = interquartile range; AFFF = aqueous film forming foams; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors Study; SWAN = Study of Women's

Health Across the Nation; HR = hazard ratio; GA = gestational age; BMI = body mass index; RR = relative risk; YOTA = Young Taiwanese Cohort Study; CIMT = carotid artery intima-media thickness; ACS = acute coronary syndrome; COPSAC-2010 = Copenhagen Prospective Studies of Asthma in Childhood 2010; HPHB = Healthy Pregnancy, Healthy Baby; MADRES = Maternal and Developmental Risks from Environmental and Social Stressors; URSA = unexplained recurrent spontaneous abortion; NHBCS = New Hampshire Birth Cohort Study; FLEHS = Flemish Environment and Health Studies; DNBC = Danish National Birth Cohort; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; IgG = immunoglobulin G; IgA = immunoglobulin A; IgE = immunoglobulin E; IgG_1 = immunoglobulin G subclass 1; IgG_2 = immunoglobulin G subclass 2; IgG_3 = immunoglobulin G subclass 3; IgG_4 = immunoglobulin G subclass 4; IgM = immunoglobulin M; RA = rheumatoid arthritis; RF = rheumatoid factor; ACPA = anti-citrullinated protein antibodies; C-RP = c-reactive protein; C4 = complement 4; C3 = complement 3; KAP = light chain kappa isotype; LAM = light chain lambda isotype; DAS28 = Disease Activity Score28; WBC = white blood cell; ALP = alkaline phosphatase; ALB = albumin; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; DBIL = direct bilirubin; CHMS = Canadian Health Measures Survey; AST = aspartate transaminase; NAFLD = non-alcoholic fatty liver disease.

Table A-47. Animal Studies Identified After Updated Literature Review (Published or Identified After February 2022)

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Narizzano et al. (2022)	Cardiovascular, Developmental, Hepatic, Immune	PFOS (0.2, 1, or 5 mg/kg/day) was administered via oral gavage for 28 d prior to gestation and continued throughout gestation and weaning (until PND25) to parental white-footed mice (*Peromyscus leucopus*). PFOS exposure led to neonatal mortality and total litter loss at high doses. Both sexes of parental animals exhibited increased liver:body weight, decreased serum thyroxine, and increased hepatocyte cytoplasmic vacuolization.	General Notes: Study uses a slightly lower dose level (0.2 mg/kg/day) than many other studies and a new rodent model (*Peromyscus*, a wild species with observable differences to traditional laboratory strains). Cardiovascular: No change in heart weight in parental generation, database currently has mixed results, and decreases seen only at levels around highest dose of this study. Developmental: Effects on developmental endpoints (e.g., stillbirth, live pups born) are consistent with results from Luebker et al. (2005a), which used similar dose range in rats with a similar study design but more dose groups. There was a lack of effect on pup and fetal weight not consistent with other data but could have been confounded by fetal mortality. Developmental delays are not apparent but could also be confounded by increased fetal mortality. This animal model has a small litter size compared with traditional laboratory mouse models. Hepatic: Increased organ weight in parental generation consistent with current database. Effects are not observed at the lowest dose. The study does demonstrate histopathological evidence of cytoplasmic vacuolization. Immune: The study measures spleen and thymus weights only. Overall Assessment Conclusion: Effects are generally consistent with current database, using generally similar dose levels (though low dose is lower than many studies). Endpoints measured for hepatic, developmental,

Reference	Health Outcome(s)	Major Findings	Assessment Implications
			cardiovascular, and immune were not endpoints that were brought forward previously for dose-response. Generally, data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.
Dangudubiyyam et al. (2022)	Cardiovascular, Developmental	PFOS exposure (0.005, 0.05, 0.5, 5, 10, or 50 μg/mL) via drinking water during gestation in rats (GD4-GD20) increased vascular resistance and increased blood pressure in dams. Decreased fetal and placental weight were also observed. No effect (qualitative) was observed on the number of living fetuses.	Cardiovascular: Increased maternal mean arterial blood pressure on GD 20 (0.5–50 μg/mL); however, this endpoint was not previously modeled. Developmental: Decreased fetal body weight (0.5–50 μg/mL) and placental weight (10–50 μg/mL) is consistent with PFOS database. Overall Assessment Conclusion: Effects are consistent with current database. Fetal body weight, an endpoint previously brought forward for dose-response modeling, was decreased with increased dose levels. Study moved forward to study quality evaluation. Maternal body weight could significantly impact fetal body weights and be a main driver of the results. Correspondence with the author did not provide additional information on maternal toxicity. Doses provided in drinking water in μg/mL were difficult to accurately equate to mg/kg/day without maternal body weight and water consumption data. Not modeled due to *low* confidence study quality evaluation for developmental endpoints.
Conley et al. (2022a)	Developmental, Hepatic	Pregnant rats were exposed to PFOS (0.1, 0.3, 1, 2, or 5 mg/kg/day) via oral gavage from GD8 to PND2. Maternal gestational and postnatal body weights were decreased, and relative liver weight was increased. Offspring effects were also observed and included decreased pup body weight, survival, absolute and relative liver weight, and liver glycogen.	General Notes: A large number of dose groups at levels that are relatively low compared with other studies in the PFOS database. Developmental: Decreased maternal body weight in the high dose group is consistent with PFOS database; however, other studies found this effect at lower dose levels. Decreased pup weight in the two highest dose groups is consistent with PFOS database; however, other studies such as the Luebker et al. (2005a) critical study found this effect at lower dose levels. Pup survival decrease in the high dose group is consistent with PFOS database; however, other studies found this effect at lower dose levels.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
			Hepatic: Maternal liver weight was increased in the high dose group while pup liver weight displayed a non-monotonic decrease. Serum enzyme levels were all nonsignificant. Overall Assessment Conclusion: Effects are generally consistent with current database, although other studies investigating developmental endpoints tended to use lower dose levels. Endpoints measured for hepatic (e.g., liver weight, serum enzymes, bilirubin) were not endpoints that were brought forward previously for dose-response (e.g., individual cell necrosis in the liver). Generally, data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.
Shi et al. (2022)	Developmental, Hepatic	Gestational PFOS exposure (1 or 3 mg/kg/day) via oral gavage from GD4.5–17.5 in mice. At GD17.5, male fetuses exposed to PFOA had lower body weights and higher relative liver weights. At PND21, male offspring in the 3 mg/kg/day group had increased body weights.	General Notes: Only two dose groups, both of which are relatively high dose levels compared with other studies in the PFOS database. Developmental: Reduced fetal body weight is consistent with PFOS database, although other studies use lower dose levels and more dose groups. Alterations in pup weight are inconsistent depending on diet. Hepatic: Increased relative liver weight and lipid accumulation are consistent with PFOS database. Overall Assessment Conclusion: Effects are generally consistent with the current database, although other studies tended to use lower dose levels and more dose groups. Males were the only sex studied in this paper, and the exposure window was not for the entirety of gestation. Generally, data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.

Notes: PFOS = perfluorooctane sulfonic acid; PND = postnatal day; GD = gestational day.

A.4 Studies Identified After Assessment Literature Searches

Studies identified after the updated literature review (February 2023) did not undergo the systematic review protocol. Studies were reviewed for major findings and how those findings may affect the assessment**.** For PFOS, 17 epidemiological studies were identified after the updated literature search in 2023 and are summarized below (Table A-48).

Table A-48. Human Studies Identified After 2023 Updated Literature Search (Published or Identified After February 2023)

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		Primary Epidemiologic Studies	
Purdue et al. (2023)	Cancer	Nested case-control study of 530 matched pairs of U.S. Air Force Servicemen conducted using serum samples from the DoD Serum Repository and the DoD Cancer Registry (1990–2018). Sera was collected as a part of routine screening and was collected every 2 yr starting in 2004. Using the earliest pre-diagnosis sample for all Servicemen, a nonsignificant increase in risk of TGCT was observed comparing the fourth quartile to the first quartile of PFOS exposure (OR = 1.8, 95% CI: 0.9, 3.6, p-trend = 0.15), after adjustment for other PFAS. For those with multiple PFOS samples, significant increases in risk of TGCT were observed when comparing the third quartile of PFOS exposure (OR = 2.8, 95% CI: 1.1, 7.0) and the fourth quartile (OR = 4.6, 95% CI: 1.4, 15.1) to the lowest quartile, after adjustment for other PFAS. The trend across quartiles was significant before and after adjustment for other PFAS (p-trend = 0.009).	Exposure to PFOS may be associated with TGCT. Supports determination of carcinogenicity for PFOS.
Kang et al. (2023)	Cardiovascular	Prospective study of 1,130 women from the Study of Women's Health Across the Nation 45–56 yr old at baseline (1999–2000) followed through 2016. Serum lipids were collected at multiple timepoints over the course of 17 yr, and high, medium, and low trajectories for serum lipids were identified using a latent class growth model. Exposure to branched PFOS at baseline was associated with an increased risk of belonging to the high trajectory class for TC compared with the low trajectory class (OR per doubling of Sm-PFOS = 1.20, 95% CI: 1.00, 1.44). A similar positive association was observed for total PFOS and belonging to the high	Supports an association between exposure to PFOS and trajectories of total cholesterol. Exposure to PFOS may be associated with trajectories of LDL cholesterol.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		trajectory class of TC compared with the low trajectory class (OR = 1.21, 95% CI: 0.99, 1.49). In categorical analyses, significant increases in risk for belonging to the high trajectory class for TC were observed for both the second and third tertiles of PFOS exposure compared with the first. Serum concentrations of total PFOS were associated with significantly increased risk of belonging to the high trajectory class for LDL-C compared with the low trajectory class (OR = 1.28, 95% CI: 1.04, 1.56). No associations were observed between PFOS and risk of belonging to medium or high trajectories classes of HDL-C or TG compared with the low trajectory classes. In PFAS mixture analyses, significant increases in risk were observed for belonging to the medium or high trajectory class for TC and LDL-C. In cross-sectional analysis of baseline PFOS concentrations and serum lipid concentrations, Sm-PFOS was associated with increased LDL-C (β per doubling in Sm-PFOS = 2.47, 95% CI: 0.53, 4.42), and n-PFOS was associated with decreased TG. No associations were observed for TC, HDL-C, or TG in cross-sectional analyses of baseline data.	
Tan et al. (2023)	Immune	Prospective study of 425 pregnant women from the Atlanta African American Maternal-Child Cohort. The association between serum PFAS mixture, collected at 8–14 wk gestation, and serum inflammatory biomarkers was analyzed using mixture modeling approaches, including quantile g-computation, BKMR, BWS, and WQS. Serum PFAS mixture was associated with significantly increased serum concentrations of multiple cytokines and inflammatory markers (i.e., IFN-γ IL-10, and TNF-α) in both cross-sectional analyses (i.e., 8–14 wk gestation) and at a later follow-up visit at 24–30 wk gestation. PFOS was noted to be consistently the main driver of overall mixture effects across the four methods, and the effect was reported to be stronger for inflammatory biomarkers measured at the 24–30 wk visit.	Exposure to PFAS mixture may be associated with increased cytokine and inflammatory markers. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Andersson et al. (2023)	Immune	Prospective study of adults (20–60 yr old) from Ronneby, Sweden comparing a group of 309 adults with high exposure (median PFOS concentration = 47 ng/mL) and 47 adults with background exposure (median PFOS concentration = 4 ng/mL). No significant association was observed between baseline serum PFOS concentrations and SARS-CoV-2 anti-spike antibody levels at 5 wk post-vaccination or 6 mo post-vaccination. Similarly, no association was observed at 5 wk or 6 mo post-vaccination for PFAS mixture (summed PFOA, PFOS, PFHxS, and PFNA).	No change.
Siwakoti et al. (2023)	Developmental	Nested case-control study of 128 preterm cases and 373 term controls from the LIFECODES cohort (2006–2008). PFOS was measured in samples collected in early pregnancy. No significant association was observed for preterm birth.	No change.
Zheng et al. (2023)	Developmental	Cohort study of 97 pregnant women enrolled in the Collaborative Perinatal Project (CPP) Study (1960–1966). Sample collection timing was not reported. Birth weight was significantly reduced for mothers above the median PFOS exposure level compared with mothers below the median PFOS exposure level ($\beta = -0.323$, $p = 0.006$). No significant association for birth height or ponderal index.	Supports an association between elevated PFOS exposure and reduced birth weight. No change.
Ma et al. (2023)	Hepatic	Cross-sectional study of 11,794 participants from NHANES (2003–2016). PFOS was inversely associated with ALP concentrations, but the trend was not significant. Total bilirubin was significantly increased in participants in the highest quartile of PFOS exposure compared with the lowest (OR = 1.57, 95% CI: 1.01, 2.43, p for trend = 0.02). No associations were observed for ALT, AST, or GGT.	Exposure to PFOS may be associated with changes to ALP and bilirubin. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Gump et al. (2023)	Cardiovascular	Cross-sectional study of 291 children (9–11 yr old) from the EECHO study located in upstate New York (2013–2017). Elevated exposure to PFOS was associated with significantly increased resting HR at baseline (β per ln-ng/mL = 0.17, 95% CI: 0.02, 0.32). Blood pressure reactivity to acute stress was examined by measuring blood pressure after three acute stress computer tasks. Elevated exposure to PFOS was associated with significantly decreased PEP reactivity (β = −0.27, 95% CI: −0.42, −0.12), which was also significant in BKMR analyses. No associations were observed for CIMT, cfPVW, LV mass index, resting SBP, DBP, PEP, and PP; or SBP, DBP, HR, and PP reactivity.	Exposure to PFOS may be associated with increased resting HR and changes to PEP reactivity. No change.
Xu et al. (2023)	Cardiovascular	Prospective study of 129 mother-child pairs from the Shanghai Birth Cohort (SBC) (recruitment: 2013–2016). Exposure to PFOS was measured in cord blood at birth, and blood pressure was measured at a follow-up visit at 4 yr of age (2018–2021). Elevated exposure to PFOS was significantly associated with decreased SBP (β per ln-ng/mL increase = –3.10, 95% CI: –5.20, –0.89), decreased DBP (β = –2.15, 95% CI: –4.04, –0.33), and decreased mean artery pressure (β = –1.96, 95% CI: –3.72, –0.24). In sex-stratified analyses, all associations were inverse for both boys and girls, but were only significant for one sex for SBP (male), DBP (female), and mean artery pressure (male). Exposure to PFAS mixture was significantly associated with decreased SBP, DBP, and mean artery pressure in BKMR and WQS regression analyses. No significant association observed for pulse pressure.	Exposure to PFOS may be associated with changes in blood pressure in children. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Pumarega et al. (2023)	Immune	Prospective study of 240 adults from Barcelona, Spain (2016–2021). Exposure to PFOS was measured in blood collected in 2016–2017, and SARS-CoV-2 infection was detected in nasopharyngeal swabs or blood samples collected in 2020–2021. No association was observed for PFOS or PFAS mixture and SARS-CoV-2 seropositivity or COVID-19 disease.	No change.
Rhee et al. (2023)	Cancer	Nested case-control study of 428 matched pairs of renal cell carcinoma (RCC) cases and healthy controls from the Multiethnic Cohort (MEC) Study. No significant association was observed between elevated exposure to PFOS and increased risk of RCC.	No change.
Zhang et al. (2023b)	Cancer	Two individual nested case-control studies conducted on 251 matched pairs from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) and 360 matched pairs from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). No significant association was observed between elevated exposure to PFOS and risk of PDAC in 50–69-year-old Finnish men from ATBC (1985–1988) or 50–74-year-old American men and women (1993–2001) from PLCO.	No change.
van Gerwen et al. (2023)	Cancer	Nested case-control study of 88 matched pairs of thyroid cancer patients and healthy controls from the Bio*Me* Biobank, medical record-linked biobank of participants from New York City (2008–2021). Elevated exposure to n-PFOS was associated with a significant increase in risk of thyroid cancer (OR per doubling of PFOS = 1.56. 95% CI: 1.17, 2.15). A borderline significant increased risk for thyroid cancer was observed with elevated exposure to branched PFOS (OR = 1.32, 95% CI: 0.99, 1.81). In sensitivity analyses, the association between elevated exposure to n-PFOS remained significant when restricting the	No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		analysis to papillary thyroid cancer (n = 74 matched pairs). Additional sensitivity analyses stratified the sample by longitudinal cases (i.e., diagnosed ≥1 yr after sample collection; n = 31 matched pairs) and cross-sectional cases (i.e., diagnosed <1 yr after sample collection; n = 57 matched pairs), and significant increases in risk for thyroid cancer were observed for n-PFOS in for both longitudinal and cross-sectional case analyses.	
Kim et al. (2023)	Hepatic	Cross-sectional study of 1,404 adults from the Korean National Environmental Health Survey (KoNEHS), Cycle 3 (2015–2017). Significant positive associations were observed between serum PFOS concentrations and levels of ALT, AST, and GGT in single-pollutant models. In sex-stratified analyses, associations remained significant for men and women for ALT and AST. For GGT, the association was only significant in women. In analyses stratified by BMI status, significant positive associations were observed for all three liver enzymes in individuals with a BMI <25. For individuals with a BMI of 25 or greater, the association was significant for AST only. PFAS mixture was analyzed using quantile g-computation, and significant positive associations were observed for ALT, AST, and GGT. Partial effects (weights) from quantile g-computation were reported and demonstrated PFOS contributing to the positive effects for ALT (PFOS weight: 0.25), AST (0.36), and GGT (0.10).	No change.
Zell-Baran et al. (2023)	Immune	Prospective cohort study of 145 mother-child pairs from the Healthy Start cohort study (enrollment: 2009–2014) with antibody levels measured at a follow-up visit at a mean age of 5 yr old (2015–2019). An increased risk of having a low antibody titer for measles and mumps was observed, including a significantly increased risk for low antibody titer for mumps (OR per 1-ln ng/mL increase in PFOS = 1.72, 95% CI: 1.00, 2.97). In quantile g-computation analyses, an increased risk of having a low antibody was observed for both measles	Supports an association between elevated exposure to PFOS and increased risk of decreased antibody response in children. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		and mumps, and a positive weight was observed for PFOS for measles (weight: 0.13) and an inverse weight for mumps (–0.24). In linear regression analyses, no associations were observed for varicella or rubella antibody titers. In quantile g-computation analyses, a positive association was observed for PFAS mixture and rubella antibody titer, however, the weight for PFOS was inverse (weight: –0.14). PFAS mixture was not associated with changes in varicella antibody titers.	
Winquist et al. (2023)	Cancer	Case-cohort study of 999 participants without cancer at enrollment and 3,762 incident cancer cases within the American Cancer Society's prospective Cancer Prevention Study II (CPS-II) (1998–2001). A decreased risk of hematological malignancies was observed with elevated PFOS exposure in females, as well as decreased risks for B-cell non-Hodgkin leukemia/lymphoma in females and multiple myeloma in females, males, and both sexes in analyses of histological subtypes. Associations for bladder, kidney, and pancreatic cancer were all nonsignificant in analyses of the total population and sex-stratified analyses.	No change.
		Meta-analysis and Pooled Analysis Studies	
Padula et al. (2023)	Developmental	Pooled analysis of 3,339 mother-child pairs from 1 prospective birth cohort in the ECHO program across the United States. Prenatal PFOS concentrations were significantly associated with decreases in birthweight-for-gestational-age z-score (β per ln-ng/mL increase in PFOS = −0.14, 95% CI: −0.28, −0.002). Results were similar in sex-stratified analyses. Nonsignificant associations were observed for term low birth weight (OR per ln-ng/mL increase in PFOS = 1.21, 95% CI: 0.43, 3.39) and preterm birth (OR = 1.29, 95% CI: 0.76, 2.18), and for gestational age at birth (β: −0.16, 95% CI: −0.40, 0.09). Associations were stronger between increased PFOS in the first trimester and lower birthweight-for-gestational-age z-score and increased risk of term low birth weight and SGA. PFAS mixture	Supports an association between exposure to PFOS and decreased birthweight. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		was inversely associated with birthweight-for-gestational-age z-score (PFOS weight: 0.12) and gestational age at birth (PFOS weight: 0.20), and the association was not significant for gestational age at birth. No associations were observed for SGA or LGA.	

Notes: DoD = Department of Defense; TGCT = testicular germ cell tumors; PFOS = perfluorooctane sulfonic acid; PFAS = per- and polyfluoroalkyl substances; TC = total cholesterol; OR = odds ratio; CI = confidence interval; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; BKMR = Bayesian Kernel Machine Regression; BWS = Bayesian Weighted Sums; WQS = weighted quantile sum regression; IL-γ = interleukin gamma; IL-10 = interleukin 10; TNF-α = tumor necrosis factor alpha; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; PFOA = perfluorooctanoic acid; PFHxS = perfluorohexanesulfonic acid; PFNA = perfluorononanoic acid; CPP = Collaborative Perinatal Project; NHANES = National Health and Nutrition Examination Survey; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyltransferase; EECHO = Environmental Exposures and Child Health Outcomes; HR = heart rate; PEP = pre-ejection period; CIMT = carotid intima-media thickness; cfPVW = carotid-femoral pulse wave velocity; LV = left ventricular; SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; SBC = Shanghai Birth Cohort; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; COVID-19 = coronavirus disease 2019; ATBC = Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; PDAC = pancreatic ductal adenocarcinoma; CPS-II = Cancer Prevention Study II; ECHO = Environmental influences on Child Health Outcomes; ln = natural log; CI = confidence interval; OR = odds ratio; SGA = small for gestational age; LGA = large-for-gestational-age

Appendix B. Detailed Toxicokinetics

B.1 Absorption

A summary of studies that provide information on perfluorooctane sulfonic acid (PFOS) absorption from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS Health Effects Support Document (HESD) is shown in Figure B-1.

Evidence Stream	Grand Total
Animal	5
Human	2
In Vitro	1
Grand Total	8

Figure B-1. Summary of PFOS Absorption Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOS HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.1.1 Cellular Uptake

Lipid binding may influence PFOS accumulation in various cell types relevant to absorption as well as distribution. Sanchez Garcia et al. (2018) compared PFOA and PFOS in their ability to accumulate and be retained in cells including lung epithelial cells (NCI-H292). Cellular accumulation and retention of PFOS was observed in lung cells at levels higher than azithromycin-dihydrate (AZI), a lysosomotropic cationic amphiphilic drug used as a reference compound. In contrast, PFOA only accumulated to very low levels (Table B-1). Phospholipid binding was assessed by measuring the relative affinity for a phosphatidylcholine (PC)-coated column at pH 7.4 to calculate a chromatographic index (CHIIAM7.4). Lipid binding (LogD7.4) was determined by measuring the relative affinity of compounds for a C18-coated liquid chromatography column at pH 7.4. LogP values obtained from the PubChem database were used as a comparative lipophilicity measure. Phospholipophilicity correlated ($r^2 = 0.75$) to cellular accumulation better than other lipophilicity measures. The extent to which PFOS phospholipophilicity influences absorption through the GI tract, lungs, or skin is unknown.

Table B-1. Cellular Accumulation and Retention Relative to Lipophilicity and Phospholipophilicity as Reported by Sanchez Garcia et al. (2018)

Chemical	Cellular Accumulation and Retention		Lipophilicity		
	Accumulation in Lung Epithelium (% AZI)	Retention in Lung Epithelium	Phospholipid Binding (CHIIAM7.4)	Lipid Binding (LogD7.4)	LogP
PFOS	313 ± 101*	26 ± 4	39 ± 3*	2.33 ± 0.11*	5
PFOA	15 ± 3	ND	29 ± 1	1.29 ± 0.02	4.9

Notes: AZI = azithromycin-dihydrate; ND = not determined.
*Statistically significant at $p \leq 0.05$ from PFOA.

The study by Sanchez Garcia et al. (2018) raises the possibility of passive update of PFOS into cells. This is consistent with observations that cells transfected with vector only, could take up PFOS, albeit at lower levels than cells transfected with PFOS-specific transporters (discussed further in Section B.4.2.1). Ebert et al. (2020) determined membrane/water partition coefficients ($K_{mem/w}$) for PFOS and examined passible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. Membrane permeability and partition coefficients were predicted using an approach developed to model molecules in micellar systems and biomembranes (COSMOmic and related tools, Klamt et al., 2008). The predicted log ($K_{mem/w}$/[L/kg]) for PFOS was 4.69, similar to the experimentally determined value of 4.89 ± 0.30. $K_{mem/w}$ values increase with increasing chain length, reflecting increased surface area for van der Waals interactions. The authors observed that perfluoroalkanesulfonic acids (PFSAs) adsorb about 1.2 log units more strongly to the membrane than perfluorocarboxylates (PFCAs) with the same number of perfluorinated carbons. Permeability showed the same chain-length dependence as $K_{mem/w}$ values. The predicted anionic permeability (logP_{ion}/[cm/s]) for PFOS ranged from −4.74 to −3.58, considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes. The extent to which passive uptake influences absorption in vivo remains to be determined.

B.1.2 Oral Exposure

Chang et al. (2012) administered a single oral dose of 4.2 mg/kg of [^{14}C]PFOSin solution to three male Sprague-Dawley rats. At 48 hours after dosing, only 9.08 ± 0.51% of the total [^{14}C]PFOS dose was recovered across digestive tract, feces, or urine, while the carcass retained 94.2 ± 5.1%, indicating that the PFOS was largely absorbed.

B.1.3 Inhalation Exposure

An acute median lethal concentration (LC_{50}) study in rats indicates that PFOS absorption occurs after inhalation exposures; however, pharmacokinetic data were not included in the published report (Rusch, 1979). The analytical methods for measuring PFOS in animals were limited at the time the study was conducted. More recent data on PFOS absorption following inhalation exposure are not available.

B.1.4 Dermal Exposure

The literature contains no studies on the dermal absorption of PFOS.

B.1.5 Developmental Exposure

The literature contains no studies on PFOS absorption following developmental exposure. Additional information on PFOS distribution during reproduction and development is found in Section B.2.3.

B.1.6 Bioavailability

Toxicokinetic parameters informing absorption were derived by comparing oral to intravenous (IV) dosing in rats (Kim et al., 2016b). Sprague-Dawley rats were administered 2 mg/kg by

either the IV or oral route. Urine and feces were collected weekly, and blood was collected at 10 time points over the first day and then up to 70 days after exposure. In contrast to the sex differences observed for PFOA, the time to reach the maximum PFOS plasma concentration (T_{max}) following oral exposure was similar in males and females (10.8 hours and 11.5 hours, respectively). In a similar study (Huang et al., 2019a), male and female Sprague-Dawley rats were administered a single dose of 2 mg/kg by IV injection or a single dose of 2 mg/kg or 20 mg/kg by oral gavage and observed from 5 minutes to 20 weeks after dosing. The maximal plasma concentrations (C_{max}) were similar for oral gavage and IV administration of 2 mg/kg, and T_{max} values were consistent with those observed by Kim and colleagues (14.3 hours and 12.2 hours in males and females, respectively).

The results from these studies are compared in Table B-2. Both studies found very high (≥100%) bioavailability in rats (calculated by dividing the dose-adjusted gavage area under the curve (AUC) by the IV AUC). Huang and colleagues speculate that the ≥100% bioavailability after oral dosing is due to enterohepatic circulation that occurs after gavage but not IV administration. The T_{max} values ranged from 10.8 to 14.3 hours and was slightly longer in the Huang study for both males and females. Neither bioavailability nor T_{max} exhibited sex-specific differences. However, Huang et al. did observe slightly higher C_{max} concentrations in females relative to males.

Table B-2. PFOS Parameters From Toxicokinetic Studies Informing Bioavailability in Sprague-Dawley Rats

Study	Dose (mg/kg)	Route	Sex	C_{max} (µg/mL)[a]	T_{max} (hours)[b]
Kim et al. (2016b)	2	Oral	Male	6.71 ± 0.30	10.8 ± 0.96
		IV	Male	5.23 ± 0.24	NA
		Oral	Female	6.66 ± 0.29	11.52 ± 1.2
		IV	Female	5.69 ± 0.33	NA
Huang et al. (2019a)	2	Oral	Male	5.00 ± 5.00	14.3 ± 2.7
		IV	Male	5.00 ± 5.00	NA
		Oral	Female	10.00 ± 5.00	12.2 ± 5.2
		IV	Female	5.00 ± 5.00	NA

Notes: C_{max} = maximum serum concentration, IV = intravenous, NA = not applicable, T_{max} = time to C_{max}.
[a] Converted published C_{max} (mM) to C_{max} (µg/mL) for Huang et al. (2019a).
[b] Converted published T_{max} (days) to T_{max} (hours) for Kim et al. (2016b).

B.2 Distribution

A summary of studies that provide information on PFOS distribution from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD is shown in Figure B-2.

Evidence Stream	Grand Total
Animal	20
Human	70
In Vitro	13
Grand Total	97

Figure B-2. Summary of PFOS Distribution Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOS HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.2.1 Protein Binding

Kerstner-Wood et al. (2003) examined the in vitro protein binding of PFOS in rat, monkey, and human plasma at concentrations of 1 ppm to 500 ppm and found that PFOS was bound to plasma protein in all three species. When incubated with separate human-derived plasma protein fractions, PFOS was highly bound (99.8%) to albumin and showed affinity for low-density lipoproteins (95.6%) with some binding to alpha-globulins (59.4%) and gamma-globulins (24.1%). Low levels of binding to alpha-2-macroglobulin and transferrin were measured when the protein concentrations were approximately 10% of physiological concentration.

Zhang et al. (2009) conducted an in vitro study using equilibrium dialysis, fluorophotometry, isothermal titration calorimetry, and circular dichroism to characterize interactions between PFOS with serum albumin and DNA. The authors reported that serum albumin could bind up to 45 moles of PFOS/mole of protein and 0.36 moles/base pair of DNA. The binding ratio increased with increasing PFOS concentrations and decreasing solution pH. The authors concluded that the interactions between serum albumin and PFOS were the results of surface electrostatic interactions between the sulfonate functional group and the positively charged side chains of lysine and arginine. Hydrogen binding interactions between the negative dipoles (fluorine) of the PFOS carbon-fluorine bonds could also play a role in the noncovalent bonding of PFOS with serum albumin.

Chen and Guo (2009) investigated the binding of PFOS to human serum albumin using site-specific fluorescence and found that PFOS induced fluorescence quenching indicative of binding. A binding constant of 2.2×10^4 M^{-1} and a binding ratio of PFOS to human albumin of 14 moles PFOS/mole albumin were calculated. Fluorescence displacement measurements were used to study the interaction between PFOS and two high-affinity drug binding sites on human serum albumin known as Sudlow's drug Site I and Site II. The findings indicated that PFOS has binding sites that are similar to those identified for fatty acids.

Salvalaglio et al. (2010) used molecular modeling to determine the structure and energy of PFOS binding sites for human serum albumin. The binding sites impacted were ones identified as human serum albumin fatty acid binding sites. The most populated albumin binding site for PFOS was dominated by van der Waals interactions. The PFOS binding site with the highest energy (−8.8 kcal/mole) was located near the tip of the tryptophan 214 binding site, and the maximum number of ligands that could bind to human serum albumin for PFOS was 11.

D'Alessandro et al. (2013) used electrospray ionization mass spectrometry to evaluate PFOS binding to bovine serum albumin. Using this approach, the maximum number of PFOS binding sites was estimated as 11, but the data on collision-induced PFOS removal was more consistent with 7 binding sites. This study also showed that PFOS competes with ibuprofen for its site when the PFOS:ibuprofen ratio is ≥0.5 moles:1 mole. In addition, when the binding site is occupied by PFOS, ibuprofen is unable to bind. Zhang et al. (2009) conducted a similar study of the impact of PFOS on the ability of serum albumin to bind vitamin B_2 (riboflavin) and found that, under normal physiological conditions, PFOS decreased the binding ratio of serum albumin for

riboflavin in vitro. These data suggest that PFOS can alter the pharmacokinetics and pharmacodynamics of medicinal and natural substances that share a common site on albumin.

Beesoon and Martin (2015) examined differences in the binding of linear and branched chain isomers of PFOS to calf serum albumin and human serum proteins. The linear PFOS molecule was found to bind more strongly to calf serum albumin than the branched chain isomers. When arranged in order of increasing binding, the order was 3m < 4m < 1m < 5m < 6m (iso) < linear. In the isomer-specific binding to spiked total human serum protein, the 1m branched PFOS isomer bound most strongly and the 4m branched PFOS isomer the least.

Liu et al. (2017) used spectroscopy, molecular modeling, and calorimetry techniques to evaluate the mechanism by which PFOS interacts with human serum albumin through hydrogen bonds and electrostatic interactions. PFOS binding to albumin is a spontaneous exothermic process driven by electrostatic interactions. This study observed that the backbone and secondary structure of albumin did not significantly change after exposure to PFOS; however, results suggest the interaction with PFOS changed the local structure around the esterase active site. A molecular docking study indicated that PFOS binds to the active center Arg 410 residue in albumin. This corresponded to a 28.6% decrease in esterase activity. By examining multiple PFAS, esterase activity of albumin was found to decrease with the shortening of the carbon chain and the authors suggest this may correlate with toxicity.

Sheng et al. (2020) measured uptake of PFOS in human placental choriocarcinoma (JAr) cells in the presence or absence of human serum albumin for 48 hours. PFOS concentrations in the culture medium decreased by 21.4%, 78.1%, and 92.8% with the addition of 0.5 μM, 10 μM, and 200 μM albumin, respectively. This result supports a paradigm in which binding of albumin to PFOS in the culture medium blocked their entrance into the cells. The binding affinity (K_d) of PFOS to human serum albumin was calculated to be 30.7 μM. Using a limited proteolysis technique, the authors identified the core albumin peptides that bind to PFOS as residues 189–457.

Binding to albumin and other serum proteins may affect transfer of PFOS from maternal blood to the fetus. Gao et al. (2019) correlated placental transfer with experimentally measured dissociation constants (K_d) to human serum binding proteins, serum albumin, and L-FABP. For PFOS, K_d values were calculated to be 49 ± 8 μM for serum binding proteins, 38 ± 5 μM for albumin, and 81 ± 7 μM for L-FABP. These K_d values significantly correlated with placental transfer efficiencies measured in 132 maternal blood–cord blood pairs from subjects in Beijing, China, suggesting serum and binding proteins, especially albumin, play an important role in placental transfer efficiency. The authors suggested that lower cord blood albumin levels compared with maternal blood albumin levels may set up a competition for PFOS binding on either side of the placenta.

Since there is effectively a competition between PFOS binding in maternal serum versus cord blood, lower cord blood albumin levels compared with maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017) found that the concentration of cord serum albumin was associated with higher transfer efficiencies (increase of 4.1% (CI: 2.7, 5.4) per 1 g/L albumin). However, maternal serum albumin concentration was associated with reduced transfer efficiency (decrease of 3.4% (CI: −5.0, −1.8) per 1 g/L albumin). Because albumin cannot cross the placental barrier, the

authors speculate that binding of PFOS to maternal serum albumin can reduce the free PFOS available to move across the barrier through passive diffusion. Similarly, higher fetal albumin levels will lead to less free PFOS in cord blood, which may facilitate the rate of placental transfer via passive diffusion.

PFOS also binds to intracellular proteins. Luebker et al. (2002), Zhang et al. (2013a), and Yang et al. (2020a) conducted in vitro studies that examined the binding of PFOS and other PFAS to the liver fatty acid binding protein (L-FABP). L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators (Erol et al., 2004) and constitutes 2%–5% of the cytosolic protein in hepatocytes. Luebker et al. (2002) evaluated the ability of perfluorinated chemicals to displace a fluorescent substrate from L-FABP and reported that PFOA exhibited some binding to L-FABP, but its binding potential was about 50% less than that of PFOS and far less than that of oleic acid. Zhang et al. (2013a) cloned the human L-FABP gene and used it to produce purified protein for evaluation of the binding of PFOA and PFOS. The median inhibiting concentration (IC_{50}) values for PFOA and PFOS were 9.0 ± 0.7 μmol and 3.3 ± 0.1 μmol, respectively, suggesting that PFOA has a lower binding affinity than PFOS. PFOA was bound to the carrier protein in a 1:1 ratio, and the interaction was mediated by electrostatic interactions and hydrogen binding with the fatty acid binding site. Using size-exclusion column coelution and nontarget analysis to identify additional PFAS ligands from contaminated environmental sources, Yang et al. (2020a) also found that that both polar and hydrophobic interactions are crucial for binding affinities to L-FABP for PFOA and PFOS.

A computational modeling approach that combined molecular docking and molecular dynamics simulation techniques was used to estimate the relative binding of affinity of PFOS for human and rat L-FABP (Cheng and Ng, 2018). The authors found that predicted free energies correlated well with binding affinities measured in three previous studies (Sheng et al., 2018; Zhang et al., 2013a; Woodcroft et al., 2010). Key residues contributing to free binding energies (ΔG_{bind}) for L-FABP include ARG 122, SER 124, and ILE 52 (human) and TYR 120, ARG 122, ILE 60, and ILE 53 (rat).

B.2.2 Tissue Distribution

B.2.2.1 Human Studies

Human blood is a known site of PFOS accumulation. A recent example measured PFAS in blood samples from 344 Wilmington, NC residents (289 adults and 55 children) exposed to contaminated drinking water from release of PFAS chemicals into the Cape Fear River between 1980 and 2017. The mean serum PFOS concentration was 9.4 ng/mL in adults and 5.1 ng/mL in children (Kotlarz et al., 2020). In an analysis of Faroese children (ages 5 to 14) from three birth cohorts, PFOS accounted for the largest fraction (54%–74%) of the PFAS in serum, followed by PFOA (11%–24%) (Dassuncao et al., 2018). A mean serum PFOS concentration of 6.9 ng/mL was measured in 41 Norwegian women (Haug et al., 2011). Using adjusted multiple linear regression models, PFOS serum concentrations were significantly correlated to the number of months since breastfeeding ended and consumption of fish, but not age or weight of participants.

PFOS accumulation in blood impacts distribution to various tissues and organs, but few studies have examined PFOS partitioning to human blood fractions. Forsthuber et al., (2020) measured

the distribution of PFOS in blood fractions including plasma, albumin, and lipoprotein fractions (e.g., very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL)). Blood from four young healthy volunteers (two women, two men, 23–31 years old) were separated into fractions using size fractionation (for proteins) and serial ultracentrifugation. Results found that albumin was the most important carrier for PFOS with 4.3 ± 2.2 ng/mL present in this fraction. In contrast, the amount of PFOS associated with VLDL, LDL and HDL fractions was below the limit of quantification (LOQ), 0.1 ± 0.1 ng/mL, and 0.16 ± 0.06 ng/mL, respectively.

Jin et al. (2016) analyzed 60 blood samples from a Chinese population, and three whole blood samples from an exposed Canadian family to investigate the partitioning of PFAS of different chain lengths and their major isomers between human blood and plasma. Increasing chain length for PFAS correlated with an increased mass fraction in human plasma (F_p) from C6 (mean 0.24) to C11 (0.87). The PFOS plasma:whole blood ratio in the Jin et al. (2016) study was lower (1.5 ± 0.42) compared with the mean plasma:whole blood (2.2–2.3) (Ehresman et al., 2007) and serum:whole blood (1.2–2.3) (Hanssen et al., 2013; Kärrman et al., 2006) ratios previously reported. Linear isomers of PFOS had lower mean F_p than their corresponding total branched isomers. In blood samples obtained from three highly exposed Canadian subjects, the highest levels of PFOS were measured in plasma (0.14 ng/mL) compared with red blood cells (RBCs, 0.04 ng/mL) and in washed RBCs (0.04 ng/mL). The authors suggested that these values could be used as more accurate conversion factors when converting concentrations between whole blood and plasma.

Fractionation to blood fractions was also examined in 61 male and female participants from Oslo, Norway in 2013–2014 (Poothong et al., 2017). The median relative PFAS compositions in serum, plasma, and whole blood were dominated by PFOS, followed by PFOA (representing 60%–70% of blood PFAS), relative to the other 23 PFAS chemicals analyzed. Median PFOS concentrations in plasma, serum, and whole blood were 5.24 ng/mL, 4.77 ng/mL, and 2.85 ng/mL, respectively. Similar to other studies, PFOS preferentially accumulated in plasma relative to serum and whole blood; this result suggests that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum or plasma will not provide accurate estimates for PFOS.

B.2.2.1.1 Distribution in Tissues

No clinical studies are available that examined tissue distribution in humans following administration of a controlled dose of PFOS. However, samples collected in biomonitoring and epidemiological studies provide data showing distribution of PFOS.

In humans, PFOS distributes primarily to the liver and blood. Olsen et al. (2003b) sampled both liver and serum from cadavers for PFOS and found a good correlation between samples from the same subject. There were no sex- or age group-specific differences in PFOS concentrations. In another study, Kärrman et al. (2010) identified PFOS in postmortem liver samples (n = 12; 6 males, 6 females, aged 27–79 years) with a mean concentration of 26.6 ng/g tissue.

Pérez et al. (2013) collected tissue samples (liver, kidney, brain, lung, and bone) in the first 24 hours after death from 20 adult subjects (aged 28–83 years) who had been living in Catalonia, Spain. PFOS was present in 90% of the samples but could be quantified in only 20% (median 1.9 ng/g). PFOS accumulated primarily in the liver (104 ng/g), kidney (75.6 ng/g), and lung

(29.1 ng/g), and brain (4.9 ng/g), with levels below the limit of detection (LOD) in the bone. Maestri et al. (2006) examined pooled postmortem tissues from five males and two females from northern Italy ranging in age from 12 to 83 years. Of the 12 tissues analyzed, the highest PFOS levels were detected in liver, lung, and pituitary gland (13.6, 7.9, and 7.6 ng/g, respectively) and the lowest levels were detected in skeletal muscle, brain, and basal ganglia (1.0, 1.3, and 1.2 ng/g, respectively). Both linear and branched PFOS were observed in these tissues, with linear:branched PFOS peak area ratios ranging from 1.6 (in blood) to 4.8 (in basal ganglia). PFOS was also detected in cranium, rib bone, and tibia bone marrow samples from a cadaver of a 46-year-old, and from biopsies from live subjects in a bone bank in Finland (Koskela et al., 2017). However, PFOS was below the detection limit in other bone tissues (e.g., humerus, femur, fibula) but was detected in soft tissues including brain, liver, and lung.

PFOS also accumulates in follicular fluid (Kang et al., 2020) and gonads (Maestri et al., 2006). Kang et al. (Kang et al., 2020) measured a concentration of 4.82 ± 3.07 ng/mL (geometric mean) in follicular fluid samples from 28 women undergoing oocyte retrieval for in vitro fertilization procedures. A positive correlation was found between paired serum and follicular fluid samples for PFOS ($r^2 = 0.78$, $p < 0.001$), though PFOA correlations were even stronger ($r^2 = 0.93$, $p < 0.001$). Maestri et al. (2006) measured a mean concentration of 3.4 ng/g of the linear PFOS isoform in pooled gonads collected postmortem from subjects in northern Italy (five males and two females aged from 12 to 83 years). Exposure of oocytes and gonads to PFOS raises the possibility of reproductive toxicity in humans.

Stein et al. (2012) compared PFAS levels in paired samples of maternal serum and amniotic fluid from 28 females in their second trimester of pregnancy. PFOS was detected in all serum samples (0.0036–0.0287 µg/mL) and in nine amniotic fluid samples (0.0002–0.0018 µg/mL). The Spearman correlation coefficient between the serum and amniotic fluid levels was 0.76 ($p = 0.01$), indicating a direct relationship between PFOS levels in blood and amniotic fluid. The median ratio of maternal serum:amniotic fluid concentration was 25.5.

Two studies examined accumulation of PFOS in cerebrospinal fluid and serum (Wang et al., 2018b; Harada et al., 2007). In both studies, PFOS levels in cerebrospinal fluid were two orders of magnitude lower than in the serum. These results indicate that PFOS does not easily cross the adult blood-brain barrier.

PFOS has been detected in both umbilical cord blood and breast milk indicating that maternal transfer occurs (von Ehrenstein et al., 2009; Völkel et al., 2008; Apelberg et al., 2007a). Kärrman et al. (2010) identified PFOS in breast milk samples from healthy females (n = 10; aged 30–39 years), and the levels in milk (mean 0.12 ng/mL) were low compared with levels in the liver.

Balk et al. (2019) developed a one-compartment PBPK model to analyze intake in children from 1 to 10.5 years of age. Measured serum concentrations were derived from a subgroup of a longitudinal child study (LUKAS 2) (Koponen et al., 2018). Estimated daily intakes ranged between 0.16 and 0.55 ng/kg bw/day for low and high exposure scenarios. Measured PFOS serum concentrations (5th–95th percentile) ranged from 1.2–4.1 ng/mL (age 6) to 0.84–2.8 ng/mL (age 10.5). The model reconstructed median PFOS serum concentrations compared with corresponding measured median serum concentrations and predicted that growth dilution contributed from 63% to 77% of total PFOS loss, with elimination pathways accounting for the remaining PFOS loss in children.

B.2.2.2 Animal Studies

Studies of tissue distribution are available for several species of animals including nonhuman primates, rats, and, to a lesser extent, mice. Studies of nonhuman primates indicate that levels of PFOS in serum accumulate in a dose-dependent manner. While data are limited on liver accumulation of PFOS in monkeys, PFOS accumulation in the liver appears to be similar to that of serum, if not slightly lower. Several rodent studies identified the liver as a major site of accumulation, and that PFOS distributes to a wide range of tissues including kidney, heart, and lungs, and spleen. Interestingly, PFOS has been measured in moderate quantities in both the brain and testicles of rodents, indicating that it does cross the blood-brain barrier and blood-testis barrier. While monkeys had nearly a 1:1 liver-to-serum ratio, rodent models were observed to contain far more PFOS in liver than serum.

B.2.2.2.1 Nonhuman Primates

Two long-term studies in monkeys examined PFOS accumulation in the serum and liver. Seacat et al. (2002) administered 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day PFOS orally in a capsule by intragastric intubation to young-adult to adult cynomolgus monkeys for 26 weeks. Serum and tissues were collected at necropsy. The dosing was followed by a 52-week recovery period in two animals in the control, 0.15 mg/kg/day, and 0.75 mg/kg/day groups. Serum PFOS measurements demonstrated a linear increase with dosing duration in the 0.03 mg/kg/day and 0.15 mg/kg/day groups and a nonlinear increase in the 0.75 mg/kg/day group. Levels in the high-dose group appeared to plateau after about 100 days (14 weeks) but began to decline sometime after week 37. The average percent of the cumulative dose of PFOS in the liver at the end of treatment ranged from 4.4% to 8.7% with no difference by dose group or sex. At the two lower doses, serum levels were comparable in the males and females, whereas at 0.75 mg/kg/day, levels were generally elevated in the males compared with females. Only the highest dose group appeared to reach a serum steady state at week 16. In the 0.03 mg/kg/day groups, the serum levels continued to increase temporally until week 27 when serum sampling stopped for that cohort. Once dosing ceased, serum levels declined in all animals that continued in the study.

In the second study conducted in cynomolgus monkeys (Chang et al., 2017), animals were given PFOS doses to reach target serum concentrations of 70 µg/mL or 100 µg/mL that were chosen to match levels of the medium- and high-dose groups from Seacat et al. (2002). The control group (n = 6/sex) was dosed with vehicle, the low-dose group (n = 6/sex) received a single dose of 9 mg/kg PFOS on day 106 of the study, and the high-dose group (n = 4–6/sex) received three separate PFOS doses (11–17.2 mg/kg) on days 43, 288, and 358. Measurements of serum PFOS indicate that male and female monkeys reached the target dose of 70 µg/mL and 100 µg/mL on day 113 and 50, respectively. Male and female animals in the high-dose group reached peak PFOS serum levels of 160–165 µg/mL on day 365. Consistent with the previous study, no sex differences were found. At the end of the experiment, the animals were reported to have a 1:1 PFOS liver:serum ratio, while the previous Seacat et al. (2002) study reported a ratio closer to 2:1. Chang et al. (2017) attributed these differences in findings to the dosing approaches and regimens used in the two studies (gelatin capsule vs. gastric intubation).

B.2.2.2.2 Rats

Numerous studies have been performed on models of PFOS distribution in rats. These studies range from acute (hours) to longer-term studies (20 weeks) and include various levels of dosing. Distribution is measured primarily in serum, liver, and lungs, but approaches were used to measure brain distribution as well.

Martin et al. (2007) administered PFOS (10 mg/kg/day) to adult male Sprague-Dawley rats for 1, 3, or 5 days by gavage and determined the liver and serum levels. Mean liver PFOS levels were 83 ± 5 μg/g, 229 ± 10 μg/g, and 401 ± 21 μg/g after 1, 3, or 5 daily doses, respectively. Mean serum concentrations were 23 ± 2.8 μg/g and 87.7 ± 4.1 μg/mL after 1 and 3 days of dosing, respectively. Day 5 serum levels were not available through the publication. This study observed a liver:serum ratio of nearly 3:1. Liver PFOS concentrations also exhibit a dose-dependency in male Sprague-Dawley (SD) rats administered 1 or 10 mg/kg/day PFOS by oral gavage for 28 days with PFOS concentrations 27- and 54-fold higher than those of control rats (Han et al., 2018a).

In another acute study performed by Yu et al. (2011), female Wistar rats were administered doses of PFOS (0, 0.2, 1.0, or 3.0 mg/kg/day) dissolved in 0.5% Tween 20 for 5 consecutive days. Blood and bile were collected 24 hours after the last dose was given. Data indicate that there is a linear dose-dependent increase in both serum and bile, which likely reflects levels in liver.

A 28-day toxicity study by NTP exemplifies patterns of PFOS accumulation in blood and liver (NTP, 2019). Male and female Sprague-Dawley rats were administered daily doses of 0 mg/kg/day, 0.312 mg/kg/day, 0.625 mg/kg/day, 1.25 mg/kg/day, 2.5 mg/kg/day, or 5 mg/kg/day of PFOS by oral gavage. Plasma and liver concentrations were analyzed approximately 24 hours after the last dose. A dose-dependent increase in plasma concentrations of PFOS was observed in both males and females. In contrast to studies with PFOA, plasma PFOS concentrations in females were generally similar to males, and dose-normalized plasma concentrations (μM/mmol/kg/day) in males and females were within 1.5-fold across the dose groups. The lowest dose-normalized concentration was observed in the highest dose group in both sexes. In males, PFOS concentrations in plasma were 23.73 ± 1.11 μg/mL and 318.2 ± 8.87 μg/mL at the lowest and highest doses, respectively. In females, these values were 30.53 ± 0.92 μg/mL and 413.56 ± 8.07 μg/mL at the lowest and highest doses, respectively. However, there were quantifiable levels of PFOS in female controls that were 562 times lower than the lowest dose administered and required caution in interpreting these findings. Concentrations in livers of males increased with increasing dose, but when normalized with dose, there was a steady decrease as dose increased. This corresponded with a decreasing liver:plasma ratio as dose increased. Liver:plasma ratios, measured only in males, were 3.76 ± 0.24 at the lowest dose and 2.74 ± 0.08 at the highest dose.

Additional studies have been performed that expand on PFOS dosing, time of treatment, and organ distribution. Cui et al. (2009) delivered 5 or 20 mg/kg/day of PFOS via oral gavage to 3-month-old Sprague-Dawley rats. At the end of dosing (28 days), serum and organ concentrations were measured (Table B-3). No blood samples were available at the 20 mg/kg/day dose due to animal deaths in this group. The liver appeared to have by far the highest concentration of PFOS at both 5 mg/kg/day and 20 mg/kg/day. Levels in the heart were approximately half the

concentration observed in liver followed by the kidney, serum, and lungs. Of the organs examined, testicles and spleen exhibited the lowest PFOS levels. Of note was the differential accumulation by organ and dose. For liver, kidney, and heart, 2–3-fold increases in PFOS concentrations were observed between the low and high doses even though the high dose was 4 times higher than the low dose. Interestingly, the brain and lungs were most susceptible to the increase in dose by accumulating 10- and 5-fold more PFOS, respectively.

Table B-3. Concentrations of PFOS in Various Tissues of Male Sprague-Dawley Rats Exposed to PFOS by Gavage for 28 Days as Reported by Cui et al. (2009)

Tissue[a]	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day
Blood (μg/mL)	ND	72.0 ± 25.7	No sample[b]
Liver (μg/g)	ND	345 ± 40	648 ± 17
Kidney (μg/g)	ND	93.9 ± 13.6	248 ± 26
Lung (μg/g)	ND	46.6 ± 17.8	228 ± 122
Heart (μg/g)	ND	168 ± 17	497 ± 64
Spleen (μg/g)	ND	38.5 ± 11.8	167 ± 64
Testicle (μg/g)	ND	39.5 ± 10.0	127 ± 11
Brain (μg/g)	ND	13.6 ± 1.0	146 ± 34

Notes: ND = not detected.
[a] Data are presented as mean ± standard deviation.
[b] Animal deaths in this group precluded blood measurements.

In a similar study conducted by Curran et al. (2008), male and female Sprague-Dawley rats were administered 0 mg/kg/day, 2 mg/kg/day, 20 mg/kg/day, 50 mg/kg/day, or 100 mg/kg/day via feed for 28 days (Table B-4). The highest PFOS concentration was found in the liver at all doses, accounting for 70%–80% of total distribution measured in males and 65%– 80% of total distribution in females. The spleen and heart also contained notable levels of PFOS, however, accumulation in the heart was approximately 25% less than the amount in spleen. PFOS in animal livers followed a linear dose-dependent distribution between 2 mg/kg/day and 20 mg/kg/day; however, this linearity was lost between the 20 mg/kg/day, 50 mg/kg/day, and 100 mg/kg/day dose escalation. This could be due to an increase in excretion or changes in distribution to other organs that were not measured in this study. No consistent differences between the sexes were found, however, female rats generally had higher levels of PFOS in the heart and spleen at all doses.

Table B-4. Concentrations of PFOS in Various Tissues of Male and Female Sprague-Dawley Rats Exposed to PFOS by Feed for 28 Days as Reported by Curran et al. (2008)

Parameter	0 mg/kg/day		2 mg/kg/day		20 mg/kg/day		50 mg/kg/day		100 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
PFOS consumption (mg/kg bw/day)	0	0	0.14 ± 0.02	0.15 ± 0.02	1.33 ± 0.24	1.43 ± 0.24	3.21 ± 0.57	3.73 ± 0.57	6.34 ± 1.35	7.58 ± 0.68
Spleen (µg/g)	0.27 ± 0.36	2.08 ± 4.17	6.07 ± 1.85	7.94 ± 3.76	45.27 ± 2.16	70.03 ± 36.66	122.51 ± 7.83	139.45 ± 15.44	230.73 ± 11.47	294.96 ± 26.66
Heart (µg/g)	0.10 ± 0.14	1.42 ± 2.91	4.67 ± 1.73	6.54 ± 3.07	33.00 ± 3.44	54.65 ± 30.89	90.28 ± 4.95	107.53 ± 6.24	154.13 ± 11.78	214.45 ± 17.58
Serum (µg/g)	0.47 ± 0.27	0.95 ± 0.51	0.95 ± 0.13	1.50 ± 0.23	13.45 ± 1.48	15.40 ± 1.56	20.93 ± 2.36	31.93 ± 3.59	29.88 ± 3.53	43.20 ± 3.95
Liver (µg/g)	0.79 ± 0.49	0.89 ± 0.44	48.28 ± 5.81	43.44 ± 6.79	560.23 ± 104.43	716.55 ± 59.15	856.90 ± 353.83	596.75 ± 158.01	1030.40 ± 162.80	1008.59 ± 49.41
Liver:Serum Ratio	2.04 ± 1.39	1.30 ± 1.32	51.34 ± 9.20	29.99 ± 8.11	42.10 ± 9.20	46.81 ± 5.26	41.42 ± 16.95	20.23 ± 7.50	35.23 ± 8.50	23.48 ± 1.98

Notes:
[a] Data are presented as mean ± standard deviation.

Iwabuchi et al. (2017) exposed male Wistar rats to PFOS in drinking water at 0 µg/kg/day, 0.077 µg/kg/day, 0.38 µg/kg/day, or 1.8 µg/kg/day for 1 or 3 months. Animals were necropsied at the end of the 1- or 3-month study, and serum, whole blood, and organ levels of PFOS were measured (Table B-5). Similar to previous studies, the liver was found to contain the highest levels of PFOS; however, distribution to other organs (kidney, spleen, and heart) and serum were remarkably lower when compared with other studies.

Table B-5. Distribution of PFOS in Male Wistar Rats Exposed via Drinking Water for 1 or 3 Months as Reported by Iwabuchi et al. (2017)

Tissue[a]	1-Month Exposure			3-Month Exposure		
	0.077 µg/kg/day	0.38 µg/kg/day	1.8 µg/kg/day	0.077 µg/kg/day	0.38 µg/kg/day	1.8 µg/kg/day
Brain (µg/kg)	0.95	0.14	0.081	0.35	0.3	0.43
Heart (µg/kg)	0.17	0.23	0.12	0.6	0.57	0.7
Liver (µg/kg)	44	45	25	110	100	100
Spleen (µg/kg)	0.366	0.36	0.21	0.96	0.91	1.3
Kidney (µg/kg)	1.1	1.1	0.57	3.6	2.6	3.5
Whole Blood (µg/L)	0.69	0.77	0.46	1.5	1.4	2.1
Serum (µg/L)	1.1	1.3	0.73	2.7	2.5	3.1

Notes:
[a] Data are presented as mean values.

A combined chronic toxicity/carcinogenicity good laboratory practice (GLP) study was performed in male and female Sprague-Dawley Crl:CD (SD)IGS BR rats administered 0 ppm, 0.5 ppm, 2 ppm, 5 ppm, or 20 ppm PFOS (equivalent to 0 mg/kg/day, 0.018–0.023 mg/kg/day, 0.072–0.099 mg/kg/day, 0.184–0.247 mg/kg/day, and 0.765–1.1 mg/kg/day, respectively) for 104 weeks (Butenhoff et al., 2012; Thomford, 2002). A recovery group was administered the test substance at 20 ppm for 52 weeks and observed until necropsy at 106 weeks. Serum and liver samples were obtained during and at the end of the study to determine the concentration of PFOS (Table B-6). The findings were in opposition to the Iwabuchi et al. (2017) study as dose-dependent increases in the PFOS level in the serum and liver were observed in both male and female rats, with values slightly higher in females after the 5 ppm and 20 ppm doses.

Table B-6. PFOS Levels in the Serum and Liver of Male and Female Sprague-Dawley Rats Exposed to PFOS in Feed for 2 Years as Reported by Thomford (2002)

Timepoint (weeks)	0 ppm		0.5 ppm		2 ppm		5 ppm		20 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
	Serum PFOS levels (µg/mL)									
0	<LOQ[a]	0.0259	0.907	1.61	4.33	6.62	7.57	12.6	41.8	54.0
14	<LOQ[b]	2.67	4.04	6.96	17.1	27.3	43.9	64.4	148	223
53	0.0249	0.395	–	–	–	–	–	–	146	220
105	0.0118	0.0836	1.31	4.35	7.60	–	22.5	75.0	69.3	233
106[c]	–	–	–	–	–	–	–	–	2.42	9.51

Timepoint (weeks)	0 ppm		0.5 ppm		2 ppm		5 ppm		20 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Liver PFOS levels (µg/g)										
0	0.104	0.107	11.0	8.71	31.3	25.0	47.6	83.0	282	373
10	0.459	12.0	23.8	19.2	74.0	69.2	358	370	568	635
53	0.635	0.932	–	–	–	–	–	–	435	560
105	0.114	0.185	7.83	12.9	26.4		70.5	131	189	381
106[c]	–	–	–	–	–	–	–	–	3.12	12.9

Notes: LOQ = limit of quantification.
[a] LOQ = 0.00910 pg/mL.
[b] LOQ = 0.0457 pg/mL.
[c] Samples were obtained from the recovery group administered 20 ppm for 52 weeks and then observed until necropsy at 106 weeks.

Wang et al. (2015c) compared PFOS levels in serum and brain (hippocampus) tissue in Wistar rat dams and pups exposed continuously, only prenatally, and only postnatally. Animals were administered either 5 or 15 mg/L PFOS in drinking water. Tissues from dams were analyzed on PND 7 and 35, and pup tissues were analyzed on PND 1, 7, and 35. In dams, hippocampal PFOS concentrations were lower than the respective serum PFOS concentrations, but both serum and hippocampal levels exhibited dose- and duration-dependent increases. In serum of pups, the highest levels were observed in pups continuously exposed for 35 days (37.8 ± 2.9 µg/mL and 121.0 ± 7.1 µg/mL in the 5 and 15 g/L exposure groups). In prenatally exposed pups, serum levels decreased over time (21.7 ± 1.5 µg/mL on PND 7 compared with 2.7 ± 0.5 µg/mL on PND 35) in the high-dose group, as did levels in the hippocampus (10.8 ± 0.5 µg/mL on PND 7 compared with 0.3 ± 0.0 µg/mL on PND 35). The authors suggest the lower hippocampal PFOS concentrations in the prenatally exposed groups was primarily attributable to PFOS elimination through feces and urine. In contrast, serum levels increased over time in postnatally exposed pups (8.7 ± 1.4 µg/mL on PND 7 compared with 61.3 ± 1.1 µg/mL on PND 35) and increased in the hippocampus (3.5 ± 0.5 µg/mL on PND 7 compared with 5.7 ± 0.7 µg/mL on PND 35) in the high-dose group. Notably, increases in PFOS levels over time in the hippocampus were not observed in continuously exposed rats, where levels decreased from 32.30 ± 1.8 µg/mL on PND 7 to 14.66 ± 1.0 µg/mL on PND 35 in the high-dose group. The authors suggest that this observation may be related to maturation of the blood-brain barrier after PND 24 and/or brain growth and PFOS redistribution. Strikingly, in the continuously exposed groups for which data were available on PND 1, hippocampal levels exceeded serum levels (55.9 ± 8.1 µg/mL in serum compared with 373.4 ± 1.8 µg/mL in the hippocampus) in the high-dose group, suggesting that prenatal exposure poses a high risk to the neural system.

B.2.2.2.3 Mice

Few studies have evaluated PFOS exposure in mice. Findings within these studies focus primarily on serum and liver concentrations after dosing. Lai et al. (2018) observed that distribution from serum to liver exhibited dose-dependency after long-term (7 weeks) PFOS administration in female CD-1 mice. At the lower dose (0.3 mg/kg/day), liver and serum concentrations were similar (32,942 ng/g and 33,781 ng/g, respectively). At the higher dose (3 mg/kg/day) liver concentrations were higher (503,817 ng/g) than those observed in serum (109,526 ng/g). Studies in C57BL/6 mice suggest there are limited dose-, sex-, and age-specific

differences in PFOS levels in serum of mice exposed in utero. Zhong and colleagues (Zhong et al., 2016) administered PFOS to pregnant females (0.1, 1.0, and 5.0 mg/kg/day) by gavage from GD 1–17. PFOS serum levels were measured in male and female offspring at 4 and 8 weeks after birth. At 4 weeks only, males had significantly higher serum PFOS levels compared with females at the 1.0 mg/kg/day dose (47.03 ± 3.23 mg/L vs. 41.81 ± 3.62 mg/L) and at the 5.0 mg/kg/day dose (118.40 mg/L ± 6.27 vs. 107.53 ± 4.51 mg/L).

Bogdanska et al. (2011) performed a radioisotope distribution study in adult C57BL/6 male mice using ^{35}S-PFOS feed at a low and high dose for 1, 3, and 5 days. Doses were equivalent to 0.031 mg/kg/day in the low-dose group and 23 mg/kg/day in the high-dose group. At both doses and at all timepoints, the liver contained the highest amount of PFOS. At the low dose, the liver PFOS level relative to blood concentration increased with time, whereas at the high dose, the ratio plateaued after 3 days. The autoradiography indicated that the distribution within the liver did not appear to favor one area to a greater extent than any other. The liver contained 40%–50% of the recovered PFOS at the high dose. The authors hypothesized that this could possibly reflect high levels of binding to tissue proteins. After the liver, lungs accumulated PFOS at the next highest level in the high-dose group. Distribution was fairly uniform with some favoring of specific surface areas. The tissue:blood ratio for the lung was greater than that for all other tissues except the liver. The lowest PFOS levels were in the brain and fat deposits. Levels for the kidney roughly equaled those values observed in the blood at both concentrations and all timepoints. For the bone measurements, a whole-body autoradiogram of a mouse 48 hours after a single oral dose of ^{35}S-PFOS (12.5 mg/kg) indicated that most PFOS was found in the bone marrow and not the calcified bone.

Recently, the spatial distribution of PFOS in the kidney was investigated using imaging mass spectrometry (IMS) based on matrix-assisted laser desorption/ionization (MALDI) (Yang et al., 2019). This methodology can provide spatial information (defined as pixel-to-pixel) with a unique mass to charge ratio (m/z) for a specified compound in the same tissue section without extra labeling. The authors first determined that α-Cyano-4-hydroxycinnamic acid (CHCA) was the optimal matrix for detection of PFOS. Next, male BALB/c mice were administered PFOS by oral gavage at 10 mg/kg/day for 14 days, at which time kidneys were harvested and frozen. Continued tissue sections were cut. One section was used for the analysis by MALDI-IMS while the other two sections were homogenized and used to quantitate PFOS using HPLC-MS/MS. The average concentration of two sections in the PFOS-exposed kidney was 2.56 ± 0.193 μg/mL, almost 1,000-fold higher than the 3.25 ± 0.274 ng/mL measured in control sections. PFOS was mainly distributed in the kidney cortex region, which was consistent with the PFOS-induced glomerular atrophy observed in hematoxylin and eosin-stained sections. The authors conclude that the average concentration of the whole kidney fails to reflect the spatial accumulation of PFOS within the kidney, which can be measured and correlated to pathogenetic changes using MALDI-IMS.

In an immunotoxicity study conducted by Qazi et al. (2009), C57BL/6 male mice were administered diets with 0% to 0.02% PFOS for 10 days and PFOS levels in serum were measured. The authors found that PFOS levels in the serum increased as the dietary level of PFOS increased. While this study does not assess PFOS levels over time, it does demonstrate dose-dependent increases in serum concentrations.

Wimsatt et al. (2016) dosed male (0 mg/kg, 10 mg/kg, 50 mg/kg, or 200 mg/kg single dose) and female (0 mg/kg, 20 mg/kg, or 250 mg/kg single dose) mice with PFOS via drinking water. After 8 weeks for males and 9 weeks for females, serum PFOS levels were found to be dose-dependent.

Similar to rats (Cui et al., 2009), PFOS exposure is found to cross the blood-brain barrier. In Yu et al. (2019), male ICR mice were dosed with 0 mg/kg/day, 0.25 mg/kg/day, 2.5 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day for 28 days via oral gavage, and measurements of PFOS in serum and in brain deposits were collected. Mean serum PFOS levels were approximately 0 μg/mL, 5 μg/mL, 40 μg/mL, 240 μg/mL, and 300 μg/mL and PFOS levels in the brain were approximately 0 μg/g, 2 μg/g, 5 μg/g, 30 μg/g, and 70 μg/g for the 0 mg/kg/day, 0.25 mg/kg/day, 2.5 mg/kg/day, 25 mg/kg/day, and 50 mg/kg/day dose groups, respectively. These data indicated that PFOS levels in serum and in brain deposits are dose-dependent and that brain levels were much lower (100-fold less than that observed in blood and liver). These authors also conducted in vitro studies showing that PFOS significantly decreased the expression of tight junction-related proteins (e.g., ZO-1, Claudin-5, Claudin-11, Occludin) in endothelial cells. These findings suggest that exposure to PFOS may also disrupt the blood-brain barrier, that in turn could lead to increased accumulation of PFOS in brain. Qui et al. (2013) exposed ICR mice orally to PFOS at 0 mg/kg/day, 0.25 mg/kg/day, 2.5 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day for 28 days via gavage and examined the testicular deposition of PFOS. The study found a positive correlation between the linear dose-dependent increases in serum concentration and testicle deposition, indicating that PFOS can cross the blood-testis barrier in mice.

B.2.2.3 Tissue Transporters

PFOS entry from serum into tissues appears to be controlled by several families of membrane transporters based on PFOA studies. Yu et al. (2011) administered PFOS to rats and extracted the messenger ribonucleic acids (mRNAs) for OATp1, OATp2, and MRP2 from the liver to determine if changes in expression of transport molecules correlated with hepatic uptake. Female Wistar rats were administered PFOS at 0 mg/kg/day, 0.2 mg/kg/day, 1 mg/kg/day, or 3 mg/kg/day via gavage for 5 consecutive days. Blood, bile, and liver tissue were collected 24 hours after the last dose. Exposure to 3.0 mg/kg/day of PFOS increased hepatic OATp2 mRNA expression (1.43-fold) while MRP2 was increased approximately 1.80-fold and 1.69-fold in the 1 mg/kg/day and 3 mg/kg/day groups, respectively. No effect with treatment was observed on OATp1.

Transporters responsible for PFOS transport across the placenta are not well understood. Kummu et al. (2015) used placentas donated from healthy mothers to investigate the role of OAT4 and ATP-binding cassette transporter G2 (ABCG2) proteins. Using an *ex vivo* perfusion system, the authors administered concentrations of PFOA and PFOS (1,000 ng/mL) by perfusing through the maternal circulation. The fetal:maternal ratios of PFOA and PFOS were 0.20 ± 0.04 and 0.26 ± 0.09, which corresponded to transfer index percentages (TI%) of 12.9 ± 1.5% and 14.4 ± 3.9%, respectively. Immunoblot analysis of OAT4 and AGCG2 in perfused placentas indicated a linear negative correlation between the expression of OAT4 protein (but not ABCG2) and PFOA ($r^2 = 0.92$, $p = 0.043$) and PFOS ($r^2 = 0.99$, $p = 0.007$) transfer at 120 min. The authors speculated that OAT4 may play a role in decreasing placental passage of PFAS and intrauterine exposure to these compounds; however, the low number of placentas examined and

lack of direct evidence for uptake via OAT4 indicates further studies are needed to understand what, if any, role transporters play in placental transfer of PFOA and PFOS.

To further elucidate the role of placental transporters in facilitating the transfer of maternal PFAS into the fetus, Li et al. (2020a) compared gene expression of selected transporters in preterm and full-term placentas and determined whether the differences in expression could influence the transplacental transfer efficiencies (TTEs). The authors selected nine placental genes with known xenobiotic activity on the maternal side of the placenta: organic cation/carnitine transporter 2 (OCTN2), reduced folate carrier 1 (RFC-1), equilibrative nucleoside transporter (ENT1), folate receptor alpha (FRα), heme carrier protein 1 (PCFT), serotonin transporter (SERT), p-glycoprotein (MDR1), multi-drug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP). MDR1 expression levels were significantly associated with TTEs of branched PFOS and iso-PFOS, (3 + 4 + 5)m-PFOS, but not linear PFOS or PFOA. MRP2 expression was associated with total PFOS, linear PFOS, branched PFOS, and iso-PFOS, (3 + 4 + 5)m-PFOS, but not PFOA. BCRP expression levels did not significantly change with PFOA or PFOS. Interestingly, the pattern of expression of MDR1, MRP2 and BCRP were only observed in full-term placentas. Preterm placentas showed significant expression levels of ENT1, FRα, and SERT and were associated with 1m-PFOS and iso-PFOS. Thus, the expression of transporters and TTEs appear to differ between preterm and full-term placentas. Authors noted that the three transporters that were significantly associated with PFOS (MDR1, MRP2, and BCRP) are also ATP-binding cassette (ABC) transporters, which play a protective role for the placenta tissue and the fetus by effluxing xenobiotics across the placental barrier thereby reducing exposure to PFOS. It is unclear why there were no correlations with PFOA although this may be related to the fact that gene expression associations with TTE were not confirmed using protein expression data of the candidate genes.

More research is needed to explain how different transporters respond to PFAS and whether physiochemical properties such as chain length and branching may influence the substrate binding capacity of these transplacental transporters.

B.2.3 *Distribution During Reproduction and Development*

The availability of distribution data from pregnant females plus animal pups and neonates is a strength of the PFOS pharmacokinetic database because it helps to identify those tissues receiving the highest concentration of PFOS during development. For this reason, the information on tissue levels during reproduction and development are presented separately from those that are representative of other lifestages.

B.2.3.1 Human Studies

Zhang et al. (2013b) recruited 32 pregnant females (aged 21–39 years; gestational period 35–47 weeks) from Tianjin, China, for a study to examine the distribution of PFOS between maternal blood, cord blood, the placenta, and amniotic fluid. Samples were collected at time of delivery (31 maternal whole blood samples, 30 cord blood samples, 29 amniotic fluid samples, and 29 placentas). The maternal blood contained variable levels of 10 PFAS, and the mean maternal blood concentration was highest for PFOS (14.6 ng/mL), followed by PFOA (3.35 ng/mL). In both cases, the mean was greater than the median, indicating a distribution skewed toward the higher concentrations. PFOS was found in all fluids/tissues sampled. It was

transferred to the amniotic fluid to a lesser extent than PFOA based on their relative proportions in the maternal blood and cord blood (21% vs. 58%, respectively). Compared with the mean PFOS value in maternal blood, the mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.14% of the mean levels in the mother's blood, respectively. The correlation coefficients between the maternal PFOS blood levels and placenta, cord blood, and amniotic fluid levels ranged from 0.7 to 0.9 ($p < 0.001$).

B.2.3.1.1 Partitioning to Placenta

The placenta serves as an important link between the mother and the growing fetus throughout gestation. It forms a physiological barrier that facilitates the exchange of nutrients, gases, xenobiotics, and several biological components between maternal and fetal circulation. Several PFAS compounds including PFOA and PFOS have been identified in amniotic fluid, cord blood, and fetal tissue, indicating that these chemicals cross the transplacental barrier and influence PFAS distribution to the fetus and elimination during pregnancy.

The role of the placenta in facilitating the transport of PFAS compounds to the fetal compartment during gestation is informed by the ratio of placental concentration and matched maternal serum concentration, or RPM. RPM is a quantitative measure of the placenta's ability to retain or accumulate compounds. To determine the transplacental transfer of PFOS, Chen et al. (2017a, b) examined the distribution of PFAS in maternal serum, cord serum, and placentas from 32 pregnant women and their matched infants in Wuhan, China. Mean maternal age for the population was 27.1 years, with average pre-pregnancy BMI of 20.4 and gestational age of 38.9 weeks. In Chen et al. (2017b), mean concentrations of total PFOS in the placentas, cord serum, and maternal serum were 2.842 ng/g, 3.668 ng/mL, and 8.670 ng/mL, respectively, and the mean RPM was 0.330. The PFOS concentrations in all three matrices from Chen et al. (2017a) followed a similar pattern, however, the PFOS accumulation in the placenta was approximately 14.5% less in Chen et al. (2017a) than in Chen et al. (2017b).

Zhang et al. (2013b) (described above) recorded mean PFOS concentrations of 8.18 ng/g in the placenta, 3.09 ng/mL in cord blood, and 14.6 ng/mL in maternal blood. These concentrations were significantly higher than the PFOA concentrations in all three compartments. On the basis of RPM, 59% of maternal PFOS is accumulated in the placenta. This study and the Chen et al. (2017a, b) studies had similar maternal characteristics (sample size, geographical location (China), gestational age, maternal age), yet placental PFOS accumulation significantly varied across studies, ranging from 4.8% to 59%. One distinguishing characteristic that may account for increased PFOS accumulation in Zhang et al. (2013b) is parity. About 82% of the mothers in Zhang et al. (2013b) were primiparous whereas only 46.8% were primiparous in Chen et al. (2017a, b), which may explain the higher PFOS concentrations in maternal serum and placenta found in the Zhang et al. (2013b) study. Primiparous mothers also tend to have higher levels of PFAS in breast milk than women who have had multiple children (Lee et al., 2017), adding to the evidence that pregnancy and lactation durations are critical for PFAS distribution.

Mamsen et al. (2019) demonstrated that factors such as gestational age can affect PFOS concentrations in maternal serum and placentas. Using a linear graph of normalized percentage placenta accumulation as a function of gestational age, the authors observed a steady increase of placenta accumulation of PFOS during gestation days 50 to 300, with male and female placentas showing similar trends. However, accumulation was significantly higher in males than in

females. Authors estimated a placenta PFOS accumulation rate of 0.13% increase per day during gestation.

Zhang et al. (2015b) determined that branched PFOS makes up 18% of total PFOS in placenta, suggesting that branched and linear PFOS accumulate in the placenta at different proportions. Among branched isomers of the same compound, RPM seemed to differ by functional groups and branching. Particularly, RPM of branched PFOS isomers seem to increase as the branching points away from the sulfonate group: iso-PFOS < 4m-PFOS < (3 + 5)m-PFOS < 1m-PFOS. In contrast, the RPM of PFHxS showed a different pattern: branched PFHxS < linear PFHxS (Chen et al., 2017b). Moreover, RPM of linear and branched PFOA (3m-PFOA) did not significantly differ from each other. The variation in RPM between the branched isomers of PFOS, PFHxS, PFOA and their corresponding linear isomers suggest that their capacity to accumulate in the placenta is partly influenced by structure, functional group, and isomerization.

Umbilical cord blood is a known tissue for PFOS distribution during pregnancy. Kato et al. (2014) collected blood samples from 71 mothers and their infants in a prospective birth cohort in the Cincinnati, Ohio metropolitan area. They quantified PFAS in maternal blood at 16 weeks of gestation and at delivery, evaluated the correlation between maternal PFAS levels in maternal serum and matched cord blood. Maternal serum levels at 16 weeks of gestation and at the time of delivery were higher for PFOS (12.7 μg/L and 8.50 μg/L, respectively) than PFOA (4.8 μg/L and 3.3 μg/L, respectively). Authors reported a positive correlation between maternal serum PFOS levels during gestation and cord serum (correlation coefficient = 0.87). Similarly, the correlation between maternal serum at the time of delivery and cord serum was also positive (correlation coefficient = 0.82). A strong correlation between PFOS levels in maternal serum (collected within 1 week of delivery) and cord serum (collected at delivery) was also observed in a cohort of 50 mother-infant pairs from the Jiangsu province of China (correlation coefficient = 0.882, $p < 0.001$) (Yang et al., 2016b). In another study conducted in China, 157 paired maternal and cord serum samples collected in Beijing around delivery (Yang et al., 2016a). PFOS, followed by PFOA, was the dominant PFAS contaminant in these samples. Mean PFOS levels were 5.08 ± 3.26 ng/mL and 1.52 ± 1.01 ng/mL in maternal and cord serum, respectively (mean cord:maternal serum ratio was 0.36 ± 0.35:1).

Porpora et al. (2013) quantified PFOS levels in maternal serum and cord blood from 38 mother-infant pairs in Rome, Italy. The women were Italian Caucasian between the ages of 26 and 45 (mean age, 34.5 years). The average gestational age for participants in this study was 39 weeks. Maternal and cord serum PFOS concentrations were 3.2 ng/g and 1.4 ng/g, respectively. A strong positive correlation was observed between maternal and cord serum concentrations ($r = 0.74$, $p < 0.001$). These values suggest a cord to maternal serum ratio of 0.44.

Fromme et al. (2010) measured PFOS in mothers and infants in Munich, Germany. Maternal blood was sampled during pregnancy, at delivery, and 6 months after delivery in mothers aged 21–43 years. PFOS was also measured in cord blood and in infant blood at 6 and 19 months after birth. Maternal PFOS serum concentrations ranged from 0.8 to 9.4 μg/L (38 samples) and cord serum concentrations ranged from 0.3 to 2.8 μg/L (33 samples). The cord to maternal serum mean ratio was 0.3.

Wang et al. (2019c) measured the levels of 10 PFAS chemicals, including PFOS, in paired maternal and umbilical cord serum from a prospective birth cohort in Shandong, China. PFOS

was detected in all maternal and umbilical cord serum samples with a geometric mean of 4.25 ng/mL (range of 0.55–29.85 ng/mL) in maternal serum and 1.33 ng/mL (range 0.12–5.89 ng/mL) in cord serum. PFOS concentrations in maternal serum were strongly correlated to concentrations in cord blood (r = 0.745).

Linear and branched PFOS have been detected in both maternal and cord serum (Cai et al., 2020; Li et al., 2020a). Branched PFOS levels in cord blood are consistently lower than linear PFOS levels. Branched PFOS isomers contributed approximately 19.5% of total PFOS in cord blood (Cai et al., 2020). Similarly, Li et al. (2020a) showed that branched PFOS makes up 17% of total PFOS in cord blood from preterm births and 19.2% from full-term births (Table B-7). Together, these studies suggest that branched PFOS is likely less accumulative in cord blood than linear isomers. It is worth noting that other factors, such as differential binding affinities in serum and type of chemical exposure (branched vs. linear PFOS), may also influence the proportions in serum.

Similar to PFOA, differential TTEs were observed for linear PFOS isomers. Cai et al. (2020) found an 8% increase in branched PFOS accumulation compared with linear PFOS isomers. Similarly, Li et al. (2020a) showed a 6% increase in branched PFOS accumulation compared with linear PFOS isomers. Zhao et al. (2017b) observed higher TTEs for 1m, 4m, 3 + 5m, and m2 compared with n-PFOS. Moreover, the TTEs of branched PFOS isomers increased as the branching point moved closer to the sulfonate moiety. Together, these findings indicate that branched isomers of PFOS transfer more efficiently from maternal blood to cord blood compared with linear isomers.

In summary, these studies suggest that maternal serum levels of PFOS are positively correlated with cord blood and is a direct determinant of in utero exposure regardless of gestational age or location of exposure. Maternal serum PFOS levels are consistently higher than cord serum levels across all studies. PFOS concentrations in both maternal and cord serum varied substantially across studies, and factors such as exposure sources, parity, and other maternal demographics may account for these variations. For example, in Eryasa et al. (2019), authors noted that seafood diet (including high consumption of pilot whale) and consumer products as main sources of exposure. This may likely explain why maternal and cord serum PFOS concentrations are higher than all other studies listed in Table B-7. Additionally, linear PFOS are detected at higher frequency and at higher levels in blood than branched PFOS but are less transferable across compartments from maternal serum to cord serum.

Table B-7. PFOS Concentrations in Human Cord Blood, Maternal Blood, and Transplacental Transfer Ratios (RCM)

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOS Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)[c]	Cord: Maternal Serum Ratios (RCM)[d]
Manzano-Salgado et al. (2015)	Sabadell and Valencia, Spain	53	NR	total PFOS	1.86	6.99	0.30
	Note: Serum concentrations reported as p50. whereas geometric mean concentrations were used by authors to calculate cord:maternal serum ratios. Reported concentrations from 66 maternal plasma samples, and 66 cord blood samples, and 53 maternal serum samples.						
Chen et al. (2017a) and Chen et al. (2017b)	Wuhan, China	32	38.9 ± 1.6	total PFOS	3.67 ± 2.51	8.67 ± 5.27	0.431
				n-PFOS	2.713	6.971	0.384
				iso-PFOS	0.203	0.49	0.388
				(3 + 5)m-PFOS	0.506	0.466	0.684
				4m-PFOS	1.8	0.157	0.695
				1m-PFOS	0.226	0.136	0.835
	Note: PFOS detected in 100% of maternal and cord samples except for m-PFOS in cord samples, where the detection rate of 96.87%. PFOS isomers were reported in Chen (2017a) and total PFOS was reported in Chen (2017b).						
Cariou et al. (2015)	Toulouse, France	94	NR	total PFOS	1.28	3.67	0.38
	Note: Concentrations represent mean values from 100 pairs. Semi-quantified values below LOD were taken into account for mean calculation.						
Eryasa et al. (2019)	Faroese Birth Cohort, Denmark (cohort 3)	100	39.9 ± 1.3	total PFOS	9.5 (6.34–13.89)	23.8 (15.8–36.9)	0.38[e]
				n-PFOS	5.98 (3.97–8.71)	15.6 (10.5–22.96)	0.37
				branched PFOS	3.50 (2.38–4.94)	8.15(5.22–12.58)	0.42
	Faroese Birth Cohort, Denmark (cohort 5)	51	39.7 ± 1.1	total PFOS	3.09 (2.31–4.42)	8.82 (6.94–11.6)	0.36[e]
				n-PFOS	1.89 (1.46–2.84)	5.55 (4.16–7.45)	0.35
				branched PFOS	1.17 (0.88–1.73)	3.18(2.35–4.33)	0.37
	Note: Cohort 3 included 100 singleton births from 1999 to 2001 and cohort 5 included 51 singleton births from 2008 to 2005. Both cohorts had the same source of exposure and are similar in maternal characteristics. Ratios were reported as median p50. Serum concentrations reported here geometric mean and interquartile ranges (IQR).						
Cai et al. (2020)	Maoming Birth Cohort, China	424	39.3 ± 1.1	total PFOS	2.66 ± 4.80	6.71 ± 19.57	0.51
				linear PFOS	2.14 ± 4.42	5.62 ± 17.33	0.5
				branched PFOS	0.52 ± 0.49	1.09 ± 2.35	0.58

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOS Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)[c]	Cord: Maternal Serum Ratios (RCM)[d]
	Note: Values represented as mean concentrations ± SD. Ratios were calculated from matched maternal and infant pairs for which all cord blood samples were >LOD. Percent detect rates were 100% for total PFOS, 99.76% for linear PFOS, and 99.53% for branched PFOS.						
Li et al. (2020a)	Maoming Birth Cohort, China (preterm infants)	86	33.8 ± 3.0	total PFOS	1.93	5.87	0.32
				linear PFOS	1.6	4.85	0.3
				branched PFOS	0.33	1.01	0.36
				iso-PFOS	0.08	0.35	0.26
				(3 + 4+5)m-PFOS	0.2	0.57	0.35
				1m-PFOS	0.06	0.09	0.65
	Maoming Birth Cohort, China (full-term infants)	187	39.5 ± 1.1	total PFOS	2.6	4.44	0.58
				linear PFOS	2.1	3.76	0.57
				branched PFOS	0.5	0.68	0.68
				iso-PFOS	0.11	0.2	0.51
				(3 + 4+5)m-PFOS	0.32	0.41	0.73
				1m-PFOS	0.08	0.07	1.07
	Note: 273 mother-infant pairs were analyzed, including 86 preterm deliveries and 187 full-term deliveries. Only PFAS substances quantifiable in >50% of maternal and cord sera are included in generating mean concentration values.						
Li et al. (2020b)	Beijing, China	112	39.0 ± 1.2	total PFOS	2.31	6.74	0.482
	Note: PFOA detection rate was 97.44% in maternal serum and 95.73% in cord serum. For PFOS, 112 of 117 matched cord and maternal serum samples were used to generate $R_{CM.}$						
Wang et al. (2019c)	Shandong, China	369	39.4 ± 1.3	total PFOS	1.33	4.25	0.30
	Note: PFOS detected in 100% of maternal and cord samples.						
Pan et al. (2017)	Wuhan, China	100	39.4 ± 1.3	total PFOS	4.33	12.7	0.34
	Note: Maternal blood collected in third trimester (38.4 ± 1.6 weeks) used for R_{CM} calculation and PFOS was detected in 100% of maternal and cord samples.						
Zhao et al. (2017b)	People's Hospital of Hong'an County, China	63	39.3 ± 0.82	n-PFOS	3.86	16.8	0.21
		59	39.3 ± 0.82	iso-PFOS	0.229	1.08	0.22
		63	39.3 ± 0.82	3 + 5m-PFOS	0.417	1.44	0.29
		38	39.3 ± 0.82	4m-PFOS	0.142	0.536	0.51
		61	39.3 ± 0.82	1m-PFOS	0.716	1.25	0.48
		19	39.3 ± 0.82	m2-PFOS	0.043	0.099	0.3
		63	39.3 ± 0.82	Total PFOS	5.41	21.2	0.22

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOS Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)[c]	Cord: Maternal Serum Ratios (RCM)[d]
	Note: Authors reported that samples <LOD were not included in RCM analysis. Mean ratios reported for matched pairs.						
Beeson et al. (2011)	Chemicals, Health and Pregnancy (CHirP) cohort, Vancouver, Canada	20	NR	total PFOS	1.8	5.5	0.33
		20	NR	n-PFOS	NR	NR	0.33
		20	NR	Iso-PFOS	NR	NR	0.36
		20	NR	5m-PFOS	NR	NR	0.53
		20	NR	4m-PFOS	NR	NR	0.53
		20	NR	3m-PFOS	NR	NR	0.67
		20	NR	1m-PFOS	NR	NR	0.87
	Note: Ratios were derived from PFOA concentrations in cord serum at delivery by maternal serum concentration at 15 weeks of gestation for each mother-cord pair.						
Fei et al. (2007)[i]	Danish National Birth Cohort, maternal blood obtained in first trimester	50	40.06 ± 1.57	total PFOS	11.0 ± 4.7	35.3 ± 13.0	0.29
	Danish National Birth Cohort, maternal blood obtained in second trimester	50	40.06 ± 1.57	total PFOS	11.0 ± 4.7	29.9 ± 11.0	0.34
	Note: First trimester samples collected between gestation weeks 4 and 14. Timing of second trimester blood collection was not reported. Ratios and concentrations were generated from blood samples collected from 50 randomly selected matched maternal-cord pairs that met study criteria (from a total of = 80,678 maternal participants in the cohort).						
Hanssen et al. (2010)	Johannesburg, South Africa	71 maternal samples, 58 cord samples	NR	total PFOS	0.7	1.6	0.45
	Note: Authors did not specify if matched maternal and cord blood samples were used to derive ratios.						
Inoue et al. (2004)	Hokkaido, Japan	15	39.7 ± 1.05	total PFOS	1.6–5.3	4.9–17.6	0.32
	Note: Authors collected maternal and cord blood from 15 matched pairs. Authors report individual concentrations, but not mean concentrations for this population.						
Kim et al. (2011)	Seoul, Cheongju and Gumi, South Korea	44 maternal samples, 43 cord samples	39 ± 1.6	total PFOS	1.26 (0.81–1.82)	2.93 (2.0–4.36)	0.48

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOS Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)[c]	Cord: Maternal Serum Ratios (RCM)[d]
	Note: Median serum concentrations reported. Values in parentheses are 25%–75% IQRs.						
Fromme et al. (2010)	Germany	38 maternal samples, 33 cord samples	NR	total PFOS	1	2.9	0.3
	Note: Maternal and cord blood samples taken at time of delivery.						
Needham et al. (2011)	Faroe Islands	12	NR	total PFOS	6.6	19.7	0.34
	Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.						
Liu et al. (2011)	Jinhu, China	50 (all)	NR	total PFOS	1.686	3.184	0.57
		26 (male infants)	NR	total PFOS	NR	NR	0.55
		24 (female infants)	NR	total PFOS	NR	NR	0.58
	Note: Maternal samples collected in the first weeks after delivery.						
Midasch et al. (2007)	NR	11	NR	total PFOS	7.3	13	0.6
	Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.						
Verner et al. (2016)	NA	NA	NA	NA	NA	NA	0.45
	Note: Authors developed a two-compartment, two-generation pharmacokinetic model of prenatal and postnatal exposure to PFOA and PFOS. R_{CMs} applied in model were derived from an average of ratios reported in Aylward et al. (2014).						

Notes: CHirP = Chemicals, Health and Pregnancy; IQR = interquartile range; LOD = level of detection; NA = not applicable, NR = not reported; SD = standard deviation.

[a] Number represents number of matched pairs used for RCM calculation unless otherwise noted in comments.

[b] Gestational age reported as mean ± SD, represents gestational age at the time of cord blood sampling (delivery) and may not be the same as age at the time of maternal blood sampling.

[c] Concentrations in cord or maternal samples are reported as means with or without SD or IQR unless otherwise noted in comments. Note that several studies, the mean serum concentrations may be derived from more subjects than values used for RCM calculation, which typically included only matched pairs for which both cord and maternal serum concentrations were above the limit of detection.

[d] Data are presented as a ratio of cord serum to maternal serum concentrations unless otherwise noted in comments.

B.2.3.1.2 Partitioning to Amniotic Fluid

Zhang et al. (2013b) measured the levels of 11 PFAS chemicals in maternal blood, cord blood and placenta. All 11 PFAS were detected in their respective biological tissues at different concentrations. The mean concentration ratio between amniotic fluid and maternal blood (AF:MB) was higher in PFOA (0.13) than in PFOS (0.0014). Similarly, the mean concentration ratio between amniotic fluid and cord blood (AF:CB) was higher in PFOA (0.023) than in PFOS (0.0065). Authors attributed the differences in ratios between the two compartments to the solubility of PFOS and PFOA and their respective binding protein binding capacities in the two matrices. PFOA is highly soluble in water relative to PFOS (solubilities of 3.4 g/L and 0.68 g/L, respectively). Since amniotic fluid is 94% water, the solubility properties may account for the observation that the PFOA concentration (0.044 ng/mL) was twice as much as PFOS (0.02 ng/mL) in this matrix.

Table B-8 presents means or medians and ranges of measured and estimated PFOS concentrations in maternal blood from recent studies (2013 to present) that also measured fetal indicators of exposure (cord blood, placenta, and/or amniotic fluid). These studies demonstrate the variability of PFOS accumulation in these tissues across geographic regions. Maternal serum values ranged from 0.062 ng/mL in Rome, Italy (Porpora et al., 2013) to 183 ng/mL in Hubei, China (Zhao et al., 2017a). Cord serum values ranged from <LOD in Wuhan, China (Chen et al., 2017b) and Toulouse, France (Cariou et al., 2015) to 13.89 ng/mL in Faroe Islands, Denmark (Eryasa et al., 2019). Fewer studies measured PFOS in placentas and amniotic fluid. Placenta values were lower than maternal and cord blood values and ranged from 0.06 ng/g in Wuhan, China (Chen et al., 2017a) to 21.4 ng/g in Tianjin, China (Zhang et al., 2013b). Only two studies from Tianjin, China measured PFOS in amniotic fluid, which showed lower levels than those observed in other matrices. Values ranged from <LOD (Zhang and Qin, 2014) to 0.121 ng/mL (Zhang et al., 2013b). The very wide concentration ranges observed across these geographic locations and matrices highlight the challenges of comparing partitioning of PFOS from mother to fetus across studies.

In addition to geographic variation, inter-individual variability likely plays an important role in the range of concentrations observed in maternal and fetal tissues and matrices. Variability was examined by Brochot et al. (2019) using a PBPK model calibrated in a population framework to provide quantitative estimates for the PFOA and PFOS placental transfers in humans. The measured values of maternal plasma:cord serum inputted in their model were, on average, close to 1 but showed a variability of close to tenfold. The measured transfer rates of PFOA and PFOS used were also quite variable, indicating that PFOA crosses the placental barrier at a 3-times higher rate than PFOS. The coefficients of variation of the maximal transfer rate across subjects were estimated at 75% for PFOA and 55% for PFOS, Variation was also observed in the ranking of PFOA and PFOS when comparing exposure levels to fetal indicators of exposure. Maternal daily intake estimates were then used as inputs to the PBPK model to simulate the fetal exposure in several target organs over the whole pregnancy. The PFOA and PFOS fetal plasma concentrations are quite similar at the end of pregnancy for the whole cohort. This similarity was also predicted for the brain, but not in the kidneys and liver. When examined at the individual level, the ranking of PFOA and PFOS exposure exhibited a wide range of variability. Interestingly, the model estimated that approximately one-third of the population has levels of one compound always higher than levels of the other compound, whereas the remaining two-thirds exhibited different patterns of accumulation for PFOA and PFOS. The majority, however,

were predicted to accumulate PFOA at higher levels than PFOS levels for most of the fetal indicators of exposure. The authors concluded that differences in fetal exposure are not predicted by the measurement of the maternal concentration during pregnancy.

Table B-8. Summary of PFOS Concentrations in Human Maternal Blood, Cord Blood, Placenta and Amniotic Fluid Studies

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
Porpora et al. (2013) (Rome, Italy)	Maternal serum Mean: 3.2 ng/g Median: 2.9 ng/g Range: 0.062–13 ng/g	Cord serum Mean: 1.4 ng/g Median: 1.1 Range: 0.23–3.7 ng/g	NR	NR	NR
Zhang et al. (2014) (Tianjin, China)	NR	NR	NR	Mean: 8.18 ng/g Median: 7.32 ng/g	Mean: 0.020 ng/mL Median: <LOQ ng/mL
Yang et al. (2016a) (Jiangsu, China)	Maternal serum Mean: 3.10 ng/mL SD: 1.44 ng/mL Median 2.98 ng/mL Range: 0.76–9.47 ng/mL	Cord serum Mean: 1.41 ng/mL SD: 0.93 ng/mL Median: 1.23 ng/mL Range: 0.25–5.60 ng/mL	NR	NR	NR
Manzano-Salgado et al. (2015) (Sabadell and Valencia, Spain)	Maternal plasma Median: 6.18 ng/mL Range: 1.46–38.58 ng/mL IQR: 4.44–12.63 ng/mL Maternal serum Median: 6.99 ng/mL Range: 1.17–23.14 ng/mL IQR: 4.47–11.12 ng/mL	Cord serum Median: 1.86 ng/mL Range: 0.53–4.71 ng/mL IQR: 1.40–3.07 ng/mL	NR	NR	NR
Chen et al. (2017b) (Wuhan, China)	Mean: 8.670 ng/mL, Range: 1.72–22.857 ng/mL	Mean: 0.331 ng/mL, Range: LOD–1.070 ng/mL	NR	Mean: 0.216 ng/mL, range: LOD–0.531 ng/g	NR
Chen et al. (2017b) (Wuhan, China)	Maternal serum Mean: 8.670 ng/mL SD: 5.27 ng/mL Median: 7.01 ng/mL Range: 1.72–22.9 ng/mL	Cord serum Mean: 3.67 ng/mL SD: 2.51 ng/mL Median: 3.64 ng/mL Range: 0.54–12.7 ng/mL	NR	Mean: 0.42 ng/g SD: 0.30 ng/g Median: 0.35 ng/g range: 0.06–0.138 ng/g	NR
Pan et al. (2017) (Wuhan, China)[a]	Maternal serum T1 Mean: 14.1 ng/mL Median: 14.23 ng/mL IQR: 7.99–21.68 ng/mL	Cord serum Mean: 4.38 ng/mL Median: 4.38 ng/mL IQR: 2.68–6.19 ng/mL	NR	NR	NR
	Maternal serum T2 Mean: 13.0 ng/mL		NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
	Median: 13.20 ng/mL IQR: 7.62– 20.38 ng/mL Maternal serum T3 Mean: 12.7 ng/mL Median: 12.32 ng/mL IQR: 7.61– 20.03 ng/mL		NR	NR	NR
Caserta et al. (2018) (Rome, Italy)	Mean: 1.54 ng/mL SD: 1.28 ng/mL Range: 0.018– 4.7 ng/mL	Mean: 1.75 ng/mL SD: 1.70 ng/mL Range: 0.018– 6.00 ng/mL	NR	NR	NR
Wang et al. (2019c) (Shandong, China)	Maternal serum GM: 4.25 ng/mL Median: 4.55 ng/mL Range: 0.55– 29.85 ng/mL	Cord serum Mean: 1.33 ng/mL Median: 1.39 ng/mL Range: 0.12– 5.89 ng/mL	NR	NR	NR
Zhao et al. (2017b) (Hong'an, China)	Maternal blood Mean: 21.2 ng/mL Median: 6.59 ng/mL Range: 1.51– 582 ng/mL	Cord Blood Mean: 5.41 ng/mL Median: 1.35 ng/mL Range: 0.346– 183 ng/mL	NR	NR	NR
Brochot et al. (2019) (INMA Prospective birth cohort, Spain)[b]	Group 1 mean (plasma): 7.14 ± 5.35 (0.69–38.58) ng/mL Group 2 mean (plasma): 5.70 ± 3.45 (0.26–25.98) ng/mL	Mean: 2.08 ± 1.00 Range: 0.53– 4.71 ng/mL	NR	NR	NR
Gao et al. (2019) (Beijing, China)	Mean: 4.64 ng/mL median: 4.07 ng/mL range: 0.07– 22.6 ng/mL	Mean: 2.35 ng/mL Median: 1.8 ng/mL Range: 0.04– 8.01 ng/mL	NR	NR	NR
Eryasa et al. (2019) (Faroese Birth Cohort, Denmark)[c]	Cohort 3 Maternal serum Mean: 23.8 ng/mL SD: 1.2 ng/mL IQR: 15.8– 36.9 ng/mL	Cohort 3 Cord serum: Mean: 9.50 ng/mL SD: 0.49 ng/mL IQR: 6.34– 13.89 ng/mL Whole cord blood: Mean: 4.90 ng/mL SD: 0.26 ng/mL IQR: 3.33–6.94 ng/mL	NR	NR	NR
	Cohort 5 mean: 8.82 ng/mL SD: 0.51 ng/mL IQR: 6.94– 11.6 ng/mL	Cohort 5 Cord serum: mean: 3.09 ng/mL SD: 0.22 ng/mL IQR: 2.31–4.42 ng/mL Whole cord blood: mean: 1.60 ng/mL	NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
		SD: 0.11 ng/mL IQR: 1.18–2.32 ng/mL			
Cai et al. (2020) (Maoming Birth Cohort, China)	Maternal serum Mean: 6.71 ng/mL SD: 19.57 ng/mL Median: 4.32 ng/mL IQR: 2.94–6.34 ng/mL	Cord serum Mean: 2.66 ng/mL SD: 4.80 ng/mL Median: 1.93 ng/mL IQR: 1.23–2.66 ng/mL	NR	NR	NR
Li et al. (2020a) (Maoming Birth Cohort, China)[d]	Total PFOS: Preterm delivery: Mean: 5.87 ng/mL Median: 3.53 ng/mL IQR: 2.36–5.93 Full-term delivery: Mean: 4.44 ng/mL Median: 3.54 ng/mL IQR 2.25–5.98	Total PFOS: Preterm delivery: Mean: 1.93 ng/mL Median: 1.47 ng/mL IQR: 0.83–1.97 Full-term delivery: Mean: 2.60 ng/mL Median: 2.08 ng/mL IQR 1.28– 3.06	NR	NR	NR
Li et al. (2020b) (Maoming Birth Cohort, China)	Mean: 6.74 ng/mL (95% CI: 6.27, 8.95) Median: 5.99 ng/mL	Mean: 2.31 ng/mL (95% CI: 2.9, 3.4) Median: 1.65 ng/mL	NR	NR	NR
Zhang et al. (2013d) (Tiajin, China)	Mean: 14.6 ng/mL RSD: 4.98 Range: 7.39–36.1 ng/mL	Mean: 3.09 ng/mL RSD: 1.84 Range: 0.14–10.2 ng/mL	NR	Mean: 8.18 ng/g RSD: 3.03 Range: 3.25–21.4 ng/g	Mean: 0.020 ng/mL RSD: 0.032 Range: <LOQ–0.121 ng/mL
Cariou et al. (2015) (Toulouse, France)	Maternal serum Mean: 3.67 ng/mL Median: 3.065 ng/mL Range: 0.316–24.5 ng/mL	Cord serum Mean: 1.28 ng/mL Median: 1.115 ng/mL Range: <LOD–8.04 ng/mL LOQ = 0.300 ng/mL	NR	NR	NR
Hanssen et al. (2013) (Norilsk, Russia)[e]	Plasma Median: 11.0 ng/mL Mean: 10.7 ng/mL Range: 5.56–14.5 ng/mL	Plasma Median: 4.11 ng/mL Mean: 3.93 ng/mL Range: 1.75–6.27 ng/mL	NR	NR	NR
	Whole blood Median: 5.79 ng/mL Mean: 6.11 ng/mL Range: 3.61–8.38 ng/mL	Whole blood Median: 1.88 ng/mL Mean: 1.92 ng/mL Range: 0.49–3.89 ng/mL	NR	NR	NR
Hanssen et al. (2013) (Uzbekistan, Russia)	Whole blood Median: 0.24 ng/mL AM: 0.40 ng/mL range: 0.11–1.20 ng/mL	NR	NR	NR	NR
	Plasma median: 0.23 ng/mL mean: 0.33 ng/mL range: <0.08–0.89 ng/mL	NR	NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
Mamsen et al. (2017) (Denmark)	Mean: 8.2 ng/g, Range: 2.5–16.7 ng/g	NR	NR	Mean: 1.3 ng/ Range: 0.3–3.1 ng/g	NR
Mamsen et al. (2019) (Denmark)[a]	T1 serum Mean: 8.14 ng/mL SD: 3.82 ng/mL Median: 6.76 ng/mL Range: 2.49–16.66 ng/mL	NR	NR	Mean: 1.43 ng/g SD: 0.63 ng/g Median: 1.35 ng/g Range: 0.65–3.09 ng/g	NR
	T2 serum Mean: 3.87 ng/mL SD: 1.99 ng/mL Median: 3.43 ng/mL Range: 1.04–8.19 ng/mL	NR	NR	Mean: 1.23 ng/g SD: 0.60 ng/g Median: 1.08 ng/g Range: 0.63–2.33 ng/g	NR
	T3 serum Mean: 3.58 ng/mL SD: 1.85 ng/mL Median: 3.26 ng/mL Range: 1.07–9.66 ng/mL	NR	NR	Mean: 1.53 ng/g SD: 0.90 ng/g Median: 1.42 ng/g Range: 0.45–3.87 ng/g	NR
Kato et al. (2014) (Ohio, USA)[f]	Maternal Serum at 16 wk Median: 12.70 µg/L Maternal serum at delivery Median: 8.50 µg/L	Cord serum at delivery Median: 3.50 µg/L			

Notes: AM = arithmetic mean; CI = confidence interval; GM = geometric mean; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; SD = standard deviation; NR = not reported; RSD = relative standard deviation; T1 = trimester 1; T2 = trimester 2; T3 = trimester 3; USA = United States of America.

[a] PFOS was collected at different timepoints during gestation: first trimester (T1), second trimester (T2) and third trimester (T3).

[b] Brochot et al. (2019) collected samples from women in two cohorts: Group 1 consist of 52 mother-child pairs that had available samples of maternal blood during pregnancy and cord serum. Group 2 consists of 355 mothers who provided maternal blood during pregnancy. Cord blood was not collected for Group 2.

[c] Eryasa et al. (2019) collected serum and whole blood from participants in two birth cohorts: Cohort 3 (100 Singleton births from 1999 to 2001), and cohort 5 (50 singleton births from 2008 to 2005). Both cohorts had the same source of exposure and are similar in maternal characteristics.

[d] Li et al. (2020a) measured PFOS in matched maternal-cord serum pairs with preterm deliveries and full-term deliveries.

[e] Hanssen et al. (2013) collected whole blood and plasma from women in two geographical locations: Norilsk (n = 7) and Uzbekistan (n = 10). Cord blood and cord plasma from infants born to the Norilsk mothers only.

[f] Kato et al. (2014) measured PFOS in 71 matched maternal and cord serum pairs. Maternal serum samples were collected at 16 weeks of gestation and at the time of delivery.

B.2.3.1.3 Distribution in Fetal Tissues

Mamsen et al. (2017) measured the concentrations of 5 PFAS chemicals in human fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark, who legally terminated their pregnancies before gestational week 12 for reasons other than fetal abnormality. The samples collected included 24 maternal blood, 34 placenta, and 108 fetal organs. The participants were healthy women ages 18–46 years with an average BMI of 22.7. About 51% of the mothers smoked during pregnancy at an average of 10 cigarettes per day or were exposed to

secondhand cigarette smoke for an average of 1.8 hours per day. Mean concentrations of PFOS in maternal serum, placenta, and fetal organs were reported as 8.2 (2.5–16.7) ng/g, 1.0 (0.3–2.6) ng/g, and 0.3 (0–0.7) ng/g, respectively. The concentrations of PFOS in all three matrices were significantly higher than all four PFAS chemicals including PFOA. For 21 of the samples where all three specimens (maternal plasma, placenta, and fetal tissues) were collected from the same women, the concentration of PFOS decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. The relative concentration of PFOS in the placenta was 14% of the concentrations found in maternal plasma and were further reduced to 5% in fetal tissues. Although PFOS concentrations in all three matrices were higher than the remaining PFAS chemicals, PFOS had the lowest relative concentrations in fetal tissues. In general, a positive trend was observed between gestational age and fetal/maternal plasma ratio. Although the gestational age reported in this study is short (37–68 days post conception), the results suggest that PFOA and PFOS accumulate in the fetus and may potentially continue to accumulate across gestation.

To determine whether PFOS accumulation in fetal organs changes across trimesters during gestation, Mamsen et al. (2019) quantified PFAS levels in embryos and fetuses at gestational weeks 7–42 and serum from their matched maternal pairs. Like Mamsen et al. (2017), participants were similar in age (18–46 years) and BMI (22.8 (first trimester)). However, the smoking status of the women in this study was not reported and the majority of the pregnancies were terminated due to intrauterine fetal death (IUFD) caused by placental insufficiency and intrauterine growth restriction (58%), and infection (13%). A total of 78 pregnant women were enrolled in the study. Fetal tissues (placenta, liver, lung, heart, CNS, and adipose) were collected from 38 first trimester pregnancies, 18 second trimester pregnancies, and 22 third trimester pregnancies. In all fetal tissues examined and across trimesters, PFOS concentrations were highest compared with other PFAS. The concentration of PFAS in fetal tissues fluctuated across trimesters and did not follow any particular trend. For example, PFOS concentration in the liver was higher in the second trimester compared with the third trimester, and lowest in the lung in the second trimester compared with the first and third trimesters. Interestingly, PFOA concentration in the liver was also highest in the second trimester compared with the first and third trimesters. Authors attributed this phenomenon to the unique architecture of the fetal liver during early gestation when authors attributed this phenomenon to the unique architecture of the fetal liver during early gestation when oxygenated cord venous blood bypasses the liver into the heart through the ductus venosus and is then delivered throughout the fetus. This pattern of blood distribution changes between week 20 and 26 of gestation (late second trimester). The amount of blood shunted from the liver is reduced from 60% to 30% in the second trimester Pennati et al. (2003). This reduction results in increased flow of cord blood through the liver, thus increasing levels of PFOA and PFOS during the second trimester. Furthermore, Mamsen et al. (2019) observed that PFOA and PFOS levels were lowest in the CNS than any of the tissues examined, suggesting that the CNS has less PFAS exposure and may be protected by the blood-brain barrier. When interpreting these results, it is important to note that second and third trimester fetal tissues were obtained from patients with IUFD and may not be comparable to normal pregnancies as the fetus died in utero of placental insufficiency and intrauterine growth restriction. Placental insufficiency can potentially reduce the amount of PFAS crossing the placenta. In addition, the PFAS exposure level in this cohort may vary due to different geographical locations of the participants. The first trimester participants were from Denmark and the second and third trimester participants came from Sweden.

B.2.3.1.4 Partitioning to Infants

Four studies shown in Table B-9 analyzed PFOS levels in maternal serum and levels in breast milk and/or infant blood. Maternal and infant serum PFOS levels were substantially higher in subjects in the United States exposed to contaminated drinking water (Mondal et al., 2014) compared with subjects analyzed in France, Denmark (Faroe Islands), or Sweden (Gyllenhammar et al., 2018a; Cariou et al., 2015; Mogensen et al., 2015b). In the Mondal study, geometric mean (GM) maternal serum PFOS concentrations were lower in breastfeeding mothers (11.63 ng/mL) versus non-breastfeeding mothers (13.48 ng/mL). Conversely, breastfed infants had higher GM serum PFOS (13.54 ng/mL) than infants who were never breastfed (12.65 ng/mL).

Cariou et al. (2015) reported that PFOS levels in breastmilk were approximately 66-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOS was 0.38 ± 0.16 (n = 19). The authors noted that the transfer rates from serum to breastmilk of PFAAs were lower compared with other lipophilic persistent organic pollutants such as polychlorinated biphenyls. In this study, four PFAS compounds were analyzed (PFOA, PFOS, PFNA, and PFHxS), and the individual patterns for these compounds exhibited important interindividual variability. While PFOS was the main contributor in serum, PFOA and PFOS were found to be the main contributors in breastmilk. Interestingly, while the number of pregnancies was inversely correlated with maternal serum levels, after adjustment, the correlation with parity did not reach significance for PFOS, although it did reach significance for PFHxS.

Mogensen et al. (2015b) relied on maternal serum concentrations measured at 32 weeks of pregnancy to assess prenatal exposure and measured concentrations in the serum of children at 11 and 18 months of age. They applied linear mixed models to estimate age-dependent serum concentrations for up to 5 years after birth. The only other exposure source adjusted for in this study was the eating whale meat by the infants. As shown in Table B-9, the increases in infant blood PFOS concentrations over time, with the greatest increases found at the end of the breastfeeding period, suggest that breastfeeding is the primary exposure source during infancy.

Gyllenhammar et al. (2018a) used multiple linear regression and general linear model analysis to investigate associations between serum PFOS concentrations in 2−4-month-old infants and maternal PFOS concentrations close to delivery, duration of in utero exposure (gestational age at delivery), duration of breastfeeding, and other parameters. The authors examined PFAAs of various chain lengths and observed decreased strength of association between maternal and infant concentrations with increased PFAA carbon chain length among breastfed infants. PFOS showed the highest median in both infants and mothers (order among measured PFAAs was PFOS > PFOA > PFHxS > PFNA > PFDA > PFUnDA). The infant:maternal serum ratios were similar for total, linear, and branched PFOS (0.69 (0.14–1.5), 0.66 (0.095–1.4), and 0.72 (0.19–1.7), respectively). Despite similar ratios, the authors observed that branched PFOS isomer concentrations increased on average 1% per day of gestational age, whereas linear isomer concentrations increased 0.75% per day of gestational age, supporting a higher efficiency of placental transfer of branched as opposed to linear isomers during gestation.

Table B-9. Summary of Human PFOS Concentrations in Maternal Serum, Breast Milk, and Infant Serum

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Mondal et al. (2014)	Subjects were a subcohort of the C8 Science Panel Study (exposed to contaminated drinking water in six water districts near Parkersburg, West Virginia) who had a child <3.5 yr of age and who provided blood samples and reported detailed information on breastfeeding at the time of survey (633 mothers and 49 infants included). PFAA serum concentrations were available for all mothers and 8% (n = 49) of the infants. Maternal and infant serum concentrations were regressed on duration of breastfeeding.	Maternal serum Breastfed and not breastfed GM: 12.33 ng/mL 95% CI: 11.77, 12.92 Breastfed: GM: 11.63 ng/mL 95% CI: 10.98, 12.31 Not breastfed GM: 13.48 ng/mL 95% CI: 12.45, 14.58	NR	Infant serum Breastfed and not breastfed GM: 13.21 ng/mL 95% CI: 11.17, 15.61 Breastfed GM:13.54 ng/mL 95% CI: 10.79, 17.00 Not breastfed GM: 12.65 ng/mL 95% CI: 9.74, 16.43
Mogensen et al. (2015b)[a]	80 singleton children in Faroese birth cohort born between 1997 and 2000. The children were breastfed exclusively for a median of 4.5 months, followed by partial breastfeeding with supplementary baby food for a median of 4 months.	NR	NR	Birth: median: 6.0 ng/mL (IQR 5.2,7.2) 11 months: median: 23.2 ng/mL (IQR 14.9, 34.7) 18 months: median: 24.0 ng/mL (IQR 20.2, 29.1) 60 months: median: 13.3 ng/mL (IQR 10.6, 16.6)
Cariou et al. (2015)	Female volunteers hospitalized between June 2010 and January 2013 for planned cesarean delivery in France. Maternal blood samples (n = 100) were collected during cesarean delivery and breast milk samples (61) were collected between the 4th and 5th day after delivery.	Maternal serum Mean: 3.67 ng/mL Median: 3.065 ng/mL Range: 0.316–24.5 ng/mL	Mean: 0.040 ng/mL Median: <LOQ LOQ = 0.040 ng/mL Range: <LOD–0.376 ng/mL	NR

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Gyllenhammar et al. (2018a)	Primaparae mother/child pairs in 1996–1999 recruited in Sweden. 101 maternal and 107 infant samples were available for PFAA analyses. Serum concentrations were determined in mothers 3 weeks after delivery and in 2–4-month-old infants.	Maternal serum Mean: 20 ng/g SD: 8.9 ng/g Median: 18 ng/g Range: 7.7–61 ng/g	NR	Infant serum Mean: 14 ng/g SD: 6.7 ng/g Median: 13 ng/g Range: 2.2–44 ng/g
Haug et al. (2011)	41 female volunteers from Oslo, Norway, of which 19 submitted breast milk samples. The timing of serum or milk samples obtained from breastfeeding women was not reported.	Maternal serum Mean: 6.9 ng/mL Range: 2.3–15 ng/mL	Mean: 0.093 ng/mL Range: 0.040– 0.35 ng/mL	NR

Notes: CI = confidence interval; GM = geometric mean; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; PFAA = perfluoroalkyl acid; NR = not reported; SD = standard deviation.

[a] Neonatal serum-PFAS concentrations was calculated based on PFAS ratios between cord and maternal pregnancy serum concentrations previously estimated for the same cohort (0.34 for PFOA) from Needham et al. (2011).

Mondal et al. (2014) also examined the change in maternal and infant PFOS levels with duration of breastfeeding (Table B-10). Maternal serum concentrations decreased with each month of breastfeeding (−3%; 95% CI: −5%, −2%) with the greatest decrease observed after 12 months of breastfeeding (−39%). Correspondingly, the infant PFOS serum concentrations increased by 4% (95% CI: 1%, 7%) with each month of breastfeeding. Using mixed linear model regression (Table B-11), Mogensen et al. (2015b) calculated more dramatic increases in infants during months with exclusive breastfeeding of 29.2% and 30.2% per month at 18 and 60 months, respectively. Increases were less striking for months with partial breastfeeding and small or none for months without breastfeeding. The Gyllenhammar et al. (2018a) study included only five exclusively bottle-fed infants. In this group, they observed a higher percentage of branched PFOS compared with exclusively breastfed infants, which may be the result of the higher efficiency of placental transfer of branched PFOS isomers versus linear isomers. Haug et al. (2011) reported a significant positive correlation between maternal serum and breast milk ($r = 0.71$, $n = 19$) and an average breast milk concentration of 1.4% of the corresponding serum concentration. The mean relative proportions of branched PFOS isomers were higher in serum (22%) compared with breast milk (17%), suggesting differential partitioning of branched isomers between placenta and breast milk. Altogether, these findings support breastfeeding as the primary source of infant PFOS accumulation and that distribution to the infant correlates with the length of breastfeeding.

Table B-10. Percent Change in PFOS Ratios in Human Maternal Serum and Breast Milk and Breast Milk and Infant Serum by Infant Age as Reported by Mondal et al. (2014)

Infant Age	Maternal Serum: Breast Milk	Breastmilk: Infant Serum
≤6 months	−9% (−18%, 1%)	−31% (−53%, 1%)
7–12 months	−24% (−34%, −13%)	40% (−9%, 115%)
>12 months	−39% (−52%, −23%)	71% (9%, 167%)
Continuous (per month)	−3% (−3%, −2%)	4% (1%, 7%)

Table B-11. Percent Change in Human PFOS Serum Concentration by Exclusive, Mixed or No Breastfeeding Per Month as Reported by Mogensen et al. (2015b)

Breastfeeding Status	Mixed Model up to 18 Months		Mixed model up to 60 Months	
	Percent Change	P value	Percent Change	P value
Exclusive	29.2 (25.3, 33.1)	<0.0001	30.2 (26.2, 34.3)	<0.0001
Partial	4.4 (1.0, 7.8)	0.0108	1 (−1.2, 3.2)	0.3762
None	0.7 (−0.5, 1.9)	0.2693	−0.9 (−1.2, −0.6)	<0.0001

The contributions of placental transfer, breastfeeding, and ingestion of PFAA-contaminated drinking water to early life PFOS levels in children were analyzed (Gyllenhammar et al., 2019). This study measured PFOS concentrations in children aged 4, 8, and 12 years ($n = 57$, 55, and 119, respectively) between 2008 and 2015 as part of the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study in Sweden. Mixed linear regression (MLR) models were used to ascertain associations with PFOS for these exposure sources. PFOS concentrations increased 1.3% per unit (ng/g serum) of increase in the maternal serum level at delivery. PFOS

significantly increased 3.8% per month of nursing. Maternal serum and nursing duration showed the strongest correlations in 4-year-old children. PFOS increased 0.93% per month of cumulative drinking water exposure. The authors suggested that, in addition to exposure *in utero* and through lactation, drinking water with low-to-moderate PFOS contamination is an important source of exposure for children.

B.2.3.2 Animal Studies

B.2.3.2.1 Rats

To determine the dose-response curve for neonatal mortality in rat pups born to PFOS-exposed dams and to investigate associated biochemical and pharmacokinetic parameters, five groups of 16 female Sprague-Dawley Crl:CD(SD)IGS VAF/Plus rats were administered 0, 0.1, 0.4, 1.6, or 3.2 mg PFOS/kg bw/day by oral gavage beginning 42 days prior to cohabitation and continuing through gestation day (GD) 14 or GD 20 (Luebker et al., 2005a). PFOS levels were analyzed in serum, liver, urine, and feces samples in dams and fetuses as indicated in Table B-12. The urine, feces, and liver of the control animals all contained PFOS at small concentrations. In treated rats, the highest concentration of PFOS was in the liver. Serum levels in the dams for each dose were consistent between GD 1 and GD 15, indicating achievement of steady state prior to conception. The GD 21 levels in the dams had dropped below those observed earlier in the pregnancy. Serum levels in the GD 21 fetuses were higher than those in the dams. In contrast, PFOS levels in the livers of dams on GD 21 were about 3 times higher than in the fetuses. Fecal excretion was greater than urinary excretion by the dams.

Table B-12. Liver, Serum, Urine, and Feces PFOS Concentrations in Pregnant Sprague-Dawley Dams and Fetuses (Luebker et al., 2005a)

Parameter	Dose (mg/kg/day)	GD 1	GD 7	GD 15	GD 21	
		Dams	Dams	Dams	Dams	Fetuses
Serum[a]	0.1	8.90 ± 1.10	7.83 ± 1.11	8.81 ± 1.47	4.52 ± 1.15	9.08
	0.4	40.7 ± 4.46	40.9 ± 5.89	41.4 ± 4.80	26.2 ± 16.1	34.3
	1.6	160 ± 12.5	154 ± 14.0	156 ± 25.9	136 ± 86.5	101
	3.2	318 ± 21.1	306 ± 32.1	275 ± 26.7	155 ± 39.3	164
Liver[b]	0.1	–	–	–	29.2 ± 10.5	7.92
	0.4	–	–	–	107 ± 22.7	30.6
	1.6	–	–	–	388 ± 167	86.5
	3.2	–	–	–	610 ± 142	230
Urine[a]	0.1	0.05 ± 0.02	0.06 ± 0.03	0.07 ± 0.04	0.06 ± 0.01	–
	0.4	0.28 ± 0.19	0.31 ± 0.20	0.53 ± 0.23	0.55 ± 0.16	–
	1.6	0.96 ± 0.39	1.10 ± 0.57	0.36 ± 0.35	2.71 ± 2.07	–
	3.2	1.53 ± 0.87	1.60 ± 0.97	0.52 ± 0.28	1.61 ± 0.53	–
Feces[b]	0.1	0.50 ± 0.14	0.49 ± 0.11	0.66 ± 0.10	0.42 ± 0.10	–
	0.4	2.42 ± 0.49	2.16 ± 0.43	2.93 ±0.62	2.39 ± 1.21	–
	1.6	10.3 ± 3.01	9.20 ± 2.68	11.1 ± 3.28	9.94 ± 4.51	–
	3.2	23.9 ± 4.16	33.0 ± 10.0	29.5 ± 8.92	20.1 ± 4.21	–

Notes: GD = gestation day.

[a] Data presented in mean ± standard deviation (μg/mL).
[b] Data presented in mean ± standard deviation (μg/g).

This same study also included a subset of dams allowed to litter naturally and dosed through lactation day (LD) 4. Liver and serum samples were collected from dams and pups on LD 5. In this sampling, serum PFOS levels were similar between the dam and offspring, but the liver values were now higher in the neonates than in the respective dams.

Twenty-five female Sprague-Dawley rats/group were administered 0 mg/kg/day, 0.1 mg/kg/day, 0.3 mg/kg/day, or 1.0 mg/kg/day potassium PFOS by gavage from GD 0 through PND 20. An additional 10 mated females served as satellite rats to each of the four groups and were used to collect additional blood and tissue samples. Further details from this study are provided in the Toxicity Assessment (U.S. EPA, 2024) as reported in Butenhoff et al. (2009). Samples were taken from the dams, fetuses, and pups for serum and tissue PFOS concentrations and the results were reported by Chang et al. (2009) (Table B-13).

Table B-13. Serum, Liver, and Brain Tissue PFOS Concentrations of Sprague-Dawley Dams and Offspring as Reported by Chang et al. (2009)

Time	Dose (mg/kg)	Serum PFOS[a]		Liver PFOS[b]		Brain PFOS[b]	
		Dam	Offspring	Dam	Offspring	Dam	Offspring
GD 20[c]	Control	<LLOQ	0.009 ± 0.001	<LLOQ	<LLOQ	<LLOQ	<LLOQ
	0.1	1.722 ± 0.068	3.906 ± 0.096	8.349 ± 0.344	3.205 ± 0.217	0.151 ± 0.012	1.233 ± 0.067
	0.3	6.245 ± 0.901	10.446 ± 0.291	21.725 ± 0.721	5.814 ± 0.245	0.368 ± 0.043	3.126 ± 0.238
	1.0	26.630 ± 3.943	31.463 ± 1.032	48.875 ± 72.733	20.025 ± 2.021	0.999 ± 0.083	12.984 ± 1.122
PND 4[c]	Control	0.008 ± 0.000	<LLOQ	NS	<LLOQ	NS	<LLOQ
	0.1	3.307 ± 0.080	2.236 ± 0.070	NS	9.463 ± 0.512	NS	0.680 ± 0.033
	0.3	10.449 ± 0.234	6.960 ± 0.163	NS	20.130 ± 0.963	NS	1.910 ± 0.074
	1.0	34.320 ± 31.154	22.440 ± 0.723	NS	50.180 ± 1.124	NS	6.683 ± 0.428
PND 21	Control	0.007 ± 0.000	<LLOQ (M/F)	NS	<LLOQ (M/F)	NS	<LLOQ (M/F)
	0.1	3.159 ± 0.081	1.729 ± 0.079 (M) 1.771 ± 0.076 (F)	NS	5.980 ± 0.614 (M) 5.278 ± 0.174 (F)	NS	0.220 ± 0.014 (M) 0.229 ± 0.011 (F)
	0.3	8.981 ± 0.275	5.048 ± 0.108 (M) 5.246 ± 0.138 (F)	NS	14.780 ± 0.832 (M) 13.550 ± 0.298 (F)	NS	0.649 ± 0.053 (M) 0.735 ± 0.039 (F)
	1.0	30.480 ± 1.294	18.611 ± 1.011 (M) 18.010 ± 0.744 (F)	NS	44.890 ± 2.637 (M) 41.230 ± 2.295 (F)	NS	2.619 ± 0.165 (M) 2.700 ± 0.187 (F)
PND 72	Control	NA	<LLOQ (M/F)	NA	<LLOQ (M/F)	NA	NS (M/F)
	0.1	NA	0.042 ± 0.004 (M) 0.207 ± 0.042 (F)	NA	0.981 ± 0.091 (M) 0.801 ± 0.082 (F)	NA	NS (M/F)
	0.3	NA	0.120 ± 0.009 (M) 0.556 ± 0.062 (F)	NA	2.464 ± 0.073 (M) 2.252 ± 0.095 (F)	NA	NS (M/F)
	1.0	NA	0.560 ± 0.105 (M) 1.993 ± 0.293 (F)	NA	7.170 ± 0.382 (M) 7.204 ± 0.414 (F)	NA	NS–M/F

Notes: F = female; GD = gestation day; <LLOQ = sample less than lower limit of quantification; M = male; NA = not applicable; NS = no sample obtained; PND = postnatal day.
[a] Data presented as mean ± standard deviation (μg/mL).
[b] Data presented as mean ± standard deviation (μg/g).
[c] Data are from samples pooled by litters in the fetuses/pups.

On GD 20, PFOS concentrations in maternal serum, liver, and brain correlated with the daily doses administered. Maternal liver-to-serum PFOS ratios ranged from 1.8 to 4.9, while the maternal brain-to-serum ratios were 0.04 to 0.09 (Chang et al., 2009). The concentrations in the brains of fetuses was about 10 times higher than in their dams for all doses. Given the maternal and offspring data on GD 20, there is placental transfer of PFOS from rat dams to developing fetuses. Serum values were approximately 1–2 times greater in the fetuses than in the dams at GD 20. The concentration of PFOS in fetal liver was less than that of dams, and the brain values were much higher; this is possibly due to the lack of development of the blood-brain barrier at this stage of offspring development. PFOS serum concentrations in the offspring were lower than those for the dams on postnatal day (PND) 4 and continued to drop through PND 72. However, based on the concentrations still present in the neonate serum, lactational transfer of PFOS was occurring. At PND 72, the males appeared to be eliminating PFOS more quickly as the serum values were lower than those in the females; this difference was not observed at earlier timepoints. In the liver, PFOS was the greatest in the offspring at PND 4 and decreased significantly by PND 72. Liver values were similar at all timepoints between males and females. On GD 20, the brain levels for the pups were tenfold higher than those for the dam. The levels in pup brains gradually declined between PND 4 and PND 21.

Ishida et al. (2017) also examined distribution to livers and brains in Wistar rat dams and pups on PND 4. Tissue-to-plasma partition coefficients (K_ps) for brain/plasma decreased with increasing dose in dams (0.92 in dams at 1 mg/kg and 0.87 in dams at 2 mg/kg). In pups, the brain/plasma K_p values were 0.447 and 0.408 at 1 mg/kg and 2 mg/kg, respectively. Liver/plasma K_p values were 4.13 and 3.85 in dams and 3.30 and 2.07 in pups at the lower and higher doses, respectively. Thus, the brain-plasma ratio of PFOS in pups is approximately 5 times higher than that in dams despite very similar liver/plasma ratios in pups and dams, indicating an age-dependent accumulation of PFOS in the CNS.

In a study by Zeng et al. (2011), 10 pregnant Sprague-Dawley rats/group were administered 0 mg/kg/day, 0.1 mg/kg/day, 0.6 mg/kg/day, or 2.0 mg/kg/day of PFOS by oral gavage in 0.5% Tween 80 from GD 2 to GD 21. On GD 21, dams were monitored for parturition, and the day of delivery was designated PND 0. On PND 0, five pups/litter were sacrificed, and the trunk blood, cortex, and hippocampus were collected for examination. The other pups were randomly redistributed to dams within the dosage groups and allowed to nurse until PND 21, when they were sacrificed with the same tissues collected as previously described. PFOS concentrations in the hippocampus, cortex, and serum increased in a dose-dependent manner but overall was lower in all tissues on PND 21 compared with PND 0 (Table B-14).

Table B-14. Serum, Hippocampus, and Cortex PFOS Concentrations of Sprague-Dawley Rat Pups as Reported by Zeng et al. (2011)

Time	Dose (mg/kg/day)	Serum[a]	Hippocampus[b]	Cortex[b]
PND0	Control	ND	ND	ND
	0.1	1.50 ± 0.43*	0.63 ± 0.19*	0.39 ± 0.09*
	0.6	24.60 ± 3.02**	7.43 ± 1.62*	5.23 ± 1.58**
	2.0	45.69 ± 4.77**	17.44 ± 4.12*	13.43 ± 3.89**
PND21	Control	ND	ND	ND
	0.1	0.37 ± 1.12*	0.25 ± 0.14*	0.06 ± 0.04*

Time	Dose (mg/kg/day)	Serum[a]	Hippocampus[b]	Cortex[b]
	0.6	1.86 ± 0.35**	1.59 ± 0.78**	1.03 ± 0.59**
	2.0	4.26 ± 1.73***	6.09 ± 1.30***	3.69 ± 0.95***

Notes: ND = not detected; PND = postnatal day.
* $p < 0.05$ compared with control in the same day.
** $p < 0.05$ compared with 0.1 mg/kg group in the same day.
*** $p < 0.05$ compared with 0.6 mg/kg group in the same day.
[a] Data presented as mean ± standard deviation (µg/mL).
[b] Data presented as mean ± standard deviation (µg/g).

Sprague-Dawley rats were administered PFOS in 0.05% Tween (in deionized water) once daily by gavage from GD 1 to GD 21 at 0 mg/kg/day, 0.1 mg/kg/day, or 2.0 mg/kg/day. There was a postnatal decline in the serum and brain PFOS levels between PND 0 and PND 21. PFOS concentrations were higher in the serum when compared with the lung in offspring on both PND 0 and PND 21 (Chen et al., 2012b) (Table B-15).

Table B-15. Serum and Lung PFOS Concentration of Sprague-Dawley Rat Pups (Chen et al., 2012b)

Age	Dose (mg/kg/day)	Serum[a]	Lung[b]
PND 0	0.0	ND	ND
	0.1	1.7 ± 0.35*	0.92 ± 0.04*
	2.0	47.52 ± 3.72*	22.4 ± 1.03*
PND 21	0.0	ND	ND
	0.1	0.41 ± 0.11*	0.21 ± 0.04*
	2.0	4.46 ± 1.82**	3.16 ± 0.11**

Notes: ND = not detected; PND = postnatal day.
*$p < 0.05$ compared with control.
** $p < 0.01$ compared with control.
[a] Data presented as mean ± standard deviation (µg/mL).
[b] Data presented as mean ± standard deviation (µg/g).

B.2.3.2.2 Mice

Borg et al. (2010) administered a single dose of 12.5 mg/kg 35S-PFOS by intravenous injection (n = 1) or gavage (n = 5) on GD 16 to C57Bl/6 dams. Using whole-body autoradiography and liquid scintillation, counting distribution of PFOS was determined for the dams/fetuses (GD 18 and GD 20) and neonates (PND 1). Distribution of PFOS in the dams was similar regardless of the route of exposure, with the highest levels in the liver and lungs at all timepoints (liver and lung PFOS levels approximately 4 times and 2 times that of blood, respectively). The distribution of PFOS in the kidneys was similar to blood and the amount in the brain was lower than that of the blood. In the fetuses, the highest concentrations of PFOS were found in the kidneys and liver. In the kidneys, the highest concentration of PFOS was observed in the fetuses on GD 18 (3 times higher than maternal levels). In the fetuses on GD 18, values in the lungs were similar to the maternal lungs, and this value Increased by GD 20.

Accumulation in fetal liver was also observed C57BL/6 mice (Lai et al., 2017b). In the offspring at all timepoints, PFOS was homogeneously distributed in the liver at a level 2.5 times higher than maternal blood and 1.7 times lower than maternal liver. In pups on PND 1, PFOS was

mostly concentrated in the lungs and liver. Pups on PND 1 had PFOS levels that were 3 times higher in the lungs compared with maternal blood with a heterogeneous distribution. In the kidneys, the levels in pups on PND 1 were similar to their respective dams despite being higher in fetuses on GD 18. Levels in the brain were similar at all timepoints in the offspring and higher than in the maternal brain, likely due to an immature brain-blood barrier. Select data are provided in Table B-16.

Table B-16. Concentration Ratios of ^{35}S-PFOS Maternal Serum to Various Organs of C57BL/6 Mouse Dams, Fetuses, and Pups (Lai et al., 2017b)

Group	$[^{35}S\text{-PFOS}]_{organ}/[^{35}S\text{-PFOS}]_{maternal\ blood}$				
	Liver[a] (n = 6–8)	Lungs[a] (n = 5–6)	Kidneys[a] (n = 3–6)	Brain[a] (n = 6–9)	Blood[b] (n = 1–6)
Dams	4.2** ± 0.7	2.0* ± 0.4	0.9 ± 0.1	0.2** ± 0.05	1.0
Fetuses on GD 18	2.6** ± 0.8	2.1* ± 0.6	2.8** ± 0.3	1.2 ± 0.3	2.3
Fetuses on GD 20	2.4** ± 0.5	2.5** ± 0.4	1.4 ± 0.2	0.9 ± 0.1	1.1 ± 0.04
Pups on PND 1	2.4* ± 0.4	3.0** ± 0.5	1.0 ± 0.5	0.9 ± 0.2	1.7** ± 0.3

Notes: ^{35}S-PFOS = ^{35}S-radioisotope perfluorooctance sulfonic acid; GD = gestation day; PND = postnatal day.
*Statistically significant ($p \leq 0.01$) in comparison to maternal blood.
**Statistically significant ($p \leq 0.001$) in comparison to maternal blood.
[a] Data presented as mean ± standard deviation (μg/g).
[b] Data presented as mean ± standard deviation (μg/mL).

Male and female KM mice were administered PFOS by subcutaneous injection one time on PND 7, PND 14, PND 21, PND 28, or PND 35 at concentrations of 0 mg/kg or 50 mg/kg (Liu et al., 2009). Animals were killed 24 hours after treatment and the PFOS concentration levels obtained. The percent distribution found in the blood, brain, and liver are provided in Table B-17. The distribution shows that, beyond PND 14, the levels in the liver are approximately 2–4 times greater than those found on PND 7.

Table B-17. Percent Distribution of PFOS in Male and Female KM Mice After 50 mg/kg Subcutaneous Injection (Liu et al., 2009)

PND	Males			Females		
	Blood[a]	Brain[b]	Liver[b]	Blood[a]	Brain[b]	Liver[b]
7	11.78 ± 2.88	5.04 ± 1.49	14.84 ± 4.01	10.77 ± 1.16	4.17 ± 1.17	16.23 ± 4.84
14	13.78 ± 1.52	1.61 ± 0.80**	26.50 ± 7.36	12.31 ± 2.24	3.26 ± 0.58	26.30 ± 4.54
21	9.85 ± 2.74	2.40 ± 0.60**	51.35 ± 11.06**	12.37 ± 3.80	2.14 ± 0.38**	51.48 ± 3.44**
28	9.89 ± 2.94	0.85 ± 0.19**	63.39 ± 19.78**	12.16 ± 2.32	2.10 ± 0.73**	51.05 ± 10.59**
35	13.33 ± 0.89	1.02 ± 0.28**	73.68 ± 6.86**	11.54 ± 1.28	0.90 ± 0.23**	69.92 ± 18.52**

Notes: PND = postnatal day
**Statistically significant from PND 7 ($p < 0.01$).
[a] Data presented as mean percentage ± standard deviation (μg/mL).
[b] Data presented as mean percentage ± standard deviation (μg/g).

B.2.4 Volume of Distribution

B.2.4.1 Human Studies

None of the available studies provide data for calibration of the volume of distribution (V_d) of PFOS in humans. However, several researchers have attempted to characterize PFOS exposure and intake in humans (Egeghy and Lorber, 2011; Thompson et al., 2010) through pharmacokinetic modeling. In the models discussed below, V_d was defined as the total amount of PFOS in the body divided by the blood or serum concentration.

Both research groups defined a V_d for humans using a simple, first-order, one-compartment pharmacokinetic model (Egeghy and Lorber, 2011; Thompson et al., 2010). The models developed were designed to estimate intakes of PFOS by young children and adults (Egeghy and Lorber, 2011) and the general population of urban areas on the east coast of Australia (Thompson et al., 2010). In both models, the V_d was calibrated using human serum concentration and exposure data from NHANES, and it was assumed that most PFOS intake was from contaminated drinking water. Thus, the value for V_d was calibrated so that model prediction of elevated blood levels of PFOS matched those seen in the study population.

Thompson et al. (2010) adjusted the V_d for PFOS (230 mL/kg) based on the calibrated PFOA data by 35% in accordance with the differences in PFOA and PFOS volumes of distribution calculated by Andersen et al. (2006). The original Andersen et al. (2006) model was developed from oral data in monkeys and optimized a V_d of 220 mL/kg for PFOS and 140 mL/kg for PFOA. Thus, the V_d in monkeys for PFOS was approximately 35% greater than that for PFOA in the optimized models. Therefore, Thompson et al. (2010) used a V_d of 230 mL/kg for humans in their model.

Egeghy and Lorber (2011) used high and low bounding estimates of 3,000 mL/kg and 200 mL/kg for V_d since data in humans were not available. The two separate estimates of V_d were used in a first-order, one-compartment model to estimate a range of intakes of PFOA. They concluded that the V_d was likely closer to the lower value based on a comparison of predicted modeled intake with estimates of intakes based on exposure pathway analyses. Use of the lower value gave a modeled intake prediction similar to that obtained by a forward-modeled median intake based on an exposure assessment. The authors concluded that the lower value of 200 mL/kg was appropriate for their analysis.

Both of the models described above used a V_d calibrated from actual human data on serum measurements and intake estimates. A calibration parameter obtained from human studies, where constant intake was assumed and blood levels were measured, is considered a more robust estimate for V_d than that optimized within a model developed from animal data.

The application of V_d values used in several modeling studies are shown in Table B-18. A single value of 239 mL/Kg has been uniformly applied for most PFOS studies. Gomis et al. (2017) used a V_d of 235 mL/kg by averaging V_d values estimated for both humans and animals. V_d values may be influenced by differences in distribution between males and females, between pregnant and nonpregnant females, and across serum, plasma, and whole blood.

Table B-18. Summary of PFOS Volume of Distribution Values Assigned in Human Studies

Study	Population	Sex	Compartment	V_d	AUC or Mean/Median Concentration Measured in Compartment (ng/mL)	Notes and Considerations; Was Steady State Achieved?
Zhang et al. (2015b)	Adult	Males and females	Whole blood	230 mL/kg	Mean: 12.8; GM: 8.62	Steady state assumed.
	Pregnant, adult	Females	Whole blood	230 mL/kg	Mean: 14.7; GM: 13.4	Steady state not assumed due to variable PFAS levels during pregnancy.
Worley et al. (2017)	>12 yr	Males and Females	Blood (2016)	230 mL/kg bodyweight	Mean: 23.4 (18.5, 28.4)	–
	>12 yr	Males and Females	Blood (2010)	230 mL/kg bodyweight	Mean: 39.8 (30.9, 48.9)	–
Fu et al. (2016)	Adult, occupational	Males and females	Serum	230 mL/kg	Mean: 5,624; median: 1,725	–
Zhang et al. (2013c)	Adults	Males and Females	Serum and whole blood	230 mL/kg	Mean: 31	–
Gomis et al. (2017)	Humans and Animals	Males and Females	Serum	235 mL/kg	Reports an average of human and animal V_d values	Authors note that due to declining values in U.S. and Australian populations, steady state was not achieved.

Notes: AUC = area under the curve; GM = geometric mean; V_d = volume of distribution; U.S. = United States; yr = years.

B.2.4.2 Animal Studies

The Chang et al. (2012) series of pharmacokinetic studies on rats, mice, and monkeys described above, included V_d calculations. Values for all species were calculated following a single oral or IV dose of PFOS. In accordance with these studies, the authors concluded that the V_ds for monkeys, rats, and mice are likely in the range of 200–300 mL/kg.

Two recent studies in rats (Huang et al., 2019a; Kim et al., 2016b) measured toxicokinetic parameters including V_d (Table B-19). In the Kim et al. (2016b) study, V_d values were calculated as Dose × AUMC/$(AUC0\text{-}\infty)^2$, where AUMC is the area under the first moment curve. Rats were dosed with 2 mg/kg PFOS by both oral and IV routes. V_d values were higher after oral administration (382.55 ± 17.59 mL/kg in males and 351.50 ± 19.20 mL/kg in females) compared with the IV administration (279.81 ± 16.71 mL/kg in males and 288.97 ± 15.59 mL/kg in females), but results between the sexes were similar. While organ-specific V_d values were not determined, only the liver exhibited a partition coefficient (P_c) greater than 1, and the liver P_c in males was significantly higher than the P_c in females (2.63 ± 0.04 and 2.04 ± 0.03, respectively). This observation may contribute to the slightly lower V_ds observed after IV administration in

males relative to females. P_cs in other tissues were 1 (kidney, lung) or 2 (heart, spleen), lower that those observed in the liver for both males and females.

Huang et al. (2019a) calculated the apparent volume of central (V_1) and peripheral (V_2) distribution in rats using standard equations (Gabrielsson and Weiner, 2000). In this study, a two-compartment model was the best fit for male rats for both IV and gavage routes of administration and females dosed by the IV route whereas a one-compartment model was the best fit for female rats dosed by oral gavage. As detailed in Table B-19, males and females were administered the same dose (2 mg/kg) used by Kim et al. (2016b). In males, V_d values by the IV route were 417 ± 31 mL/kg and 264 ± 71 mL/kg in the central and peripheral compartments, respectively. Interestingly, it was the V_d in the peripheral compartment that was most similar to that observed by Kim et al. (2016b). V_d values in females after IV administration were lower than that observed in males in both the central and peripheral compartments (297 ± 43 mL/kg, and 124 ± 62 mL/kg, respectively). For the oral route, striking sex differences were noted between the central and peripheral compartments. While V_d values were quite similar in males (244–280 mL/kg) for both compartments, they were notably higher in the central compartment (222 ± 84 mL/kg) compared with the peripheral compartment (93.4 ± 93 mL/kg) in females.

In a third study (Iwabuchi et al., 2017), PFOS was administered to male Wistar rats as a single bolus dose (BD) and V_d was measured as BD/elimination rate constant (ke) × plasma concentration (AUC). V_d values were calculated for whole blood, serum, and several tissues. The V_d of whole blood was much higher than that observed for serum (2.5 kg tissue volume/g bw and 0.96 kg tissue volume/kg bw, respectively). Organ V_d values were highest in the brain (7.9 kg tissue volume/kg bw), heart (4.5 kg tissue volume/kg bw) and spleen (2.8 kg tissue volume/kg bw). V_ds were lower by 1 (kidney) or 2 (liver) orders of magnitude. Interestingly, for this analysis of PFOS, the body organs behaved as an assortment of independent one-compartments, with a longer elimination half-life in liver than serum in the elimination phase.

Table B-19. Summary of PFOS Volume of Distribution in Rats

Study	Method of V_d Calculation	Route	Dose	Species	Age	Sex	V_d	Compartment	AUC or Mean/Median Concentration Measured in Compartment	C_{max}	Steady State Considerations
Kim et al. (2016b)	Dose × AUMC/(AUC0-∞)[2]	IV	2 mg/kg	Sprague-Dawley	8–12 wk	Males	382.55 ± 17.59 mL/kg	Blood Plasma	AUC: 216.47 ± 8.63 µg day/mL	5.23 ± 0.24 µg/mL	NR
						Females	351.50 ± 19.20 mL/kg	Blood Plasma	AUC: 203.60 ± 8.42 µg day/mL	5.69 ± 0.33 µg/mL	NR
		Oral	2 mg/kg	Sprague-Dawley	8–12 wk	Males	279.81 ± 16.71 mL/kg	Blood plasma	AUC: 272.69 ± 20.39 µg day/mL	6.71 ± 0.30 µg/mL	NR
						Females	288.97 ± 15.59 mL/kg	Blood Plasma	AUC: 234.61 ± 10.05 µg day/mL	6.66 ± 0.29 µg/mL	NR
Huang et al. (2019a)	Standard equations (Gabrielsson and Weiner, 2000)	IV	2 mg/kg	Sprague-Dawley	8 wk	Males	417 ± 31 mL/kg	Central	AUC: 7.32 ± 0.42 µM-hr	0.01 ± 0.01 mM	NR
							264 ± 71 mL/kg	Peripheral	AUC: 7.32 ± 0.42 µM-hr	0.01 ± 0.01 mM	NR
						Females	297 ± 43 mL/kg	Central	AUC:10.72 ± 0.78 µM-hr	0.01 ± 0.01 mM	NR
							124 ± 62 mL/kg	Peripheral	AUC:10.72 ± 0.78 µM-hr	0.01 ± 0.01 mM	NR
		Oral	2 mg/kg	Sprague-Dawley	8 wk	Males	280 ± 48 mL/kg	Central	AUC: 9.86 ± 0.74 µM-hr	0.01 ± 0.01 mM	NR
							244 ± 81 mL/kg	Peripheral	AUC: 9.86 ± 0.74 µM-hr	0.01 ± 0.01 mM	NR
						Females	222 ± 84 mL/kg	Central	AUC: 17.74 ± 1.02 µM-hr	0.02 ± 0.01 mM	NR

Study	Method of V_d Calculation	Route	Dose	Species	Age	Sex	V_d	Compartment	AUC or Mean/Median Concentration Measured in Compartment	C_{max}	Steady State Considerations
							93.4 ± 93 mL/kg	Peripheral	AUC: 17.74 ± 1.02 µM-hr	0.02 ± 0.01 mM	NR
			2 mg/kg (×5 d)	Sprague-Dawley	8 wk	Males	176 ± 27 mL/kg	Central	AUC: 58.18 ± 3.00 µM-hr	0.11 ± 0.01 mM	NR
							123 ± 42 mL/kg	Peripheral	AUC: 58.18 ± 3.00 µM-hr	0.11 ± 0.01 mM	NR
						Females	136 ± 25 mL/kg	Central	AUC: 89.18 ± 5.00 µM-hr	0.14 ± 0.02 mM	NR
							86.3 ±37.3 mL/kg	Peripheral	AUC: 89.18 ± 5.00 µM-hr	0.14 ± 0.02 mM	NR
			20 mg/kg	Sprague-Dawley	8 wk	Males	34.6 ± 4.8 mL/kg	Central	AUC: 149.76 ± 10.60 µM-hr	AUC: 0.21± 0.03 µM-hr	NR
							43.9 ± 7.7 mL/kg	Peripheral	AUC: 149.76 ± 10.60 µM-hr	AUC: 0.21± 0.03 µM-hr	NR
						Females	27.9 ± 4.7 mL/kg	Central	AUC: 213.94 ± 16.00 µM-hr	AUC: 0.27 ± 0.03 µM-hr	NR
							27.5 ± 6.5 mL/kg	Peripheral	AUC: 213.94 ± 16.00 µM-hr	AUC: 0.27 ± 0.03 µM-hr	NR
Iwabuchi et al. (2017)	Dose/elimination rate constant (ke) × plasma	Oral	100 µg/kg	Wistar	7–9 wk at start of exposure	Males	7.9 kg tissue volume/kg BW	Brain	180 µg/kg tissue volume – day	9.17 µg/kg tissue volume	NR
							4.5 kg tissue volume/kg BW	Heart	380 µg/kg tissue volume – day	27.7 µg/kg tissue volume	NR

Study	Method of V_d Calculation	Route	Dose	Species	Age	Sex	V_d	Compartment	AUC or Mean/Median Concentration Measured in Compartment	C_{max}	Steady State Considerations
	concentration (AUC).						0.043 kg tissue volume/kg BW	Liver	240,000 µg/kg tissue volume – day	2,730 µg/kg tissue volume	NR
							2.8 kg tissue volume/kg BW	Spleen	650 µg/kg tissue volume – day	46.9 µg/kg tissue volume	NR
							0.85 kg tissue volume/kg BW	Kidney	2,300 µg/kg tissue volume – day	197 µg/kg tissue volume	NR
							2.5 kg tissue volume/kg BW	Whole blood	1,800 µg/kg tissue volume – day	52.6 µg/kg tissue volume	NR
							0.96 kg tissue volume/kg BW	Serum	2,200 µg/kg tissue volume – day	127 µg/kg tissue volume	NR

Notes: AUMC = area under the first moment curve; AUC = area under the curve; BW = body weight; C_{max} = Maximum concentration achieved; IV = intravenous; NR = not reported; V_d = volume of distribution; wk = weeks.

Unlike the sex differences observed in rats, V_d calculations were similar in male and female monkeys as shown in Table B-20 (Chang et al., 2017). Young adult cynomolgus monkeys (Macaca fascicularis) (6 per sex) were sham-dosed with vehicle, a single dose of PFOS (9 mg/kg, low-dose group), or three separate PFOS doses (11–17.2 mg/kg, high-dose group). Blood samples were drawn from all monkeys prior to, during, and after PFOS administration for up to 1 year. Toxicokinetic parameters were determined using a noncompartmental analysis. At the lower dose, a V_d of 127 mL/kg was calculated for both males and females. At the higher dose, the V_d in males was calculated to be 135 mL/kg. V_d was slightly higher in females (141 mL/kg).

Table B-20. Pharmacokinetic Parameters After Acute PFOS Exposure in Cynomolgus Monkeys[a] (Chang et al., 2017)

Parameter	9 mg/kg		14 mg/kg	
	Male	Female	Male	Female
$T_{1/2}$ (day)	124 ± 3.89	102 ± 29.2	117 ± 17.2	102 ± 45.6
K_{e1} (1/day)	0.00559 ± 0.000175	0.00729 ± 0.00223	0.00605 ± 0.000951	0.00757 ± 0.00270
Cl (mL/day/kg)	0.712 ± 0.0812	0.897 ± 0.196	0.816 ± 0.111	1.06 ± 0.510
V_d (mL/kg)	127 ± 10.9	127 ± 18.9	135 ± 6.69	141 ± 38.5
AUC/dose (ng/day/mL/mL/kg)	271,333 ± 21,733	265,200 ± 15,057	249,667 ± 14,468	220,333 ± 9,019

Notes: AUC/dose = area under the curve per dose; Cl = clearance; K_{e1} = elimination rate per day; $T_{1/2}$ = half-life (time); V_d = volume of distribution.
[a] Data presented in mean ± standard deviation.

B.3 Metabolism

A summary of studies that provide information on PFOS metabolism from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD is shown in Figure B-3.

Evidence Stream	Grand Total
Animal	
Human	1
In Vitro	
Grand Total	1

Figure B-3. Summary of PFOS Metabolism Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOS HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

The literature contains no studies on the metabolism of PFOS. It appears that PFOS is not further metabolized once absorbed. Several studies investigating PFOA found no evidence of metabolism (U.S. EPA, 2016d), and it is likely that PFOS is similarly resistant to metabolism in humans, primates, and rodents.

B.4 Excretion

A summary of studies that provide information on PFOS excretion from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD is shown in Figure B-4.

Evidence Stream	Grand Total
Animal	6
Human	29
In Vitro	2
Grand Total	35

Figure B-4. Summary of PFOS Excretion Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOS HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.4.1 Urinary and Fecal Excretion

B.4.1.1 Human Studies

A study in uremic patients illustrates the importance of kidney function in urinary PFOS excretion (Liu et al., 2018c). Uremic patients exhibit higher concentrations of PFOS than the general population, indicating the important role of urinary excretion in PFOS elimination. Interestingly, PFOS can be removed by dialysis, and serum PFOS is negatively correlated with number of hours of dialysis ($p = 0.029$). Three additional studies investigated urinary excretion of PFOS in humans in detail. Zhang et al. (2015b) derived estimates for PFOS's urinary excretion rate using paired urine and blood samples from 54 adults (29 male, 25 female, ages 22–62) in the general population and 27 pregnant females (ages 21–39) in Tainjin, China. Urinary excretion was calculated by multiplying PFOS concentration in first-draw morning urine samples by the predicted urinary volume (1.6 L/day for males and 1.2 L/day for females). PFOS was detected in the blood samples for all participants but only for 48% of the urine samples from the general population (mostly males) and 11% of samples from the pregnant females. Total daily PFOS intake was modeled for the general population with a geometric mean of 89.2 ng/day, resulting in an estimated daily urinary excretion rate of 16% of the estimated total daily intake for PFOS. There was no significant difference in excretion rate between males and females, but a significantly ($p = 0.015$) higher rate among the younger adults. Nonpregnant females aged 21–50 had a higher urine:blood ratio than those age 51–61 (0.0018 and 0.0006, respectively). A lower urine:blood ratio was found in pregnant females compared with nonpregnant females (0.0004 and 0.0013, respectively), suggesting the placenta and cord blood as possible elimination pathways.

Zhang et al. (2013c) measured renal clearance of PFOS in 86 paired blood and morning urine samples from healthy volunteers in Hebei province, China. The calculated median renal clearance rates of 0.044 mL/kg/day in young women and 0.024 mL/kg/day in men and older women for total PFOS. The authors also observed that major branched PFOS isomers were more efficiently excreted than the corresponding linear isomer.

In a later study, Fu et al. (2016) determined renal clearance of PFOS, PFOA and PFHxS in 302 occupational workers (213 male, 89 female) from one of the largest producers of PFOS-related compounds in China. Paired serum and urine samples were collected. Mean and median urine concentrations for PFOS among all workers were 4.4 ng/mL and 1.2 ng/mL, respectively; in serum, the mean and median concentrations PFOS were 5,624 and 1,725 ng/mL, respectively. The correlation coefficient of PFOS concentrations in paired serum and urine samples of 0.72 was found to be highly statistically significant ($p < 0.01$), suggesting that urine concentrations could serve as effective bioindicators for PFOS exposure in occupational settings. Daily renal clearance was calculated for each PFAA as follows:

$$\frac{Urine\ PFAA\ Concentrations\ Daily\ \times\ Daily\ urine\ excretion\ volume}{Serum\ PFAA\ concentrations\ \times\ Body\ weight}$$

Urine excretion volumes were assigned as 1.4 L/day and 1.2 L/day for males and females, respectively), and body weight as reported in questionnaires. The daily renal clearance was the highest for PFOA (GM 0.067 mL/kg/day) and lowest for PFOS (GM 0.010 mL/kg/day). Sex did impact PFOS daily renal clearance values, which were significantly lower in males compared with females ($p < 0.01$).

Fu and colleagues noted their half-life estimates are the shortest values ever, suggesting that the overall elimination potential of PFAAs might have been underestimated. The shorter half-life values presented could suggest that pathways other than renal clearance play important roles in elimination of PFAAs in humans. Another possibility is that the apparent half-lives of PFAAs calculated through annual decline rates could be affected by the high ongoing levels of exposure.

B.4.1.2 Animal Studies

In a study by Chang et al. (2012), three Sprague-Dawley rats/sex/timepoint were administered [^{14}C]PFOS as the potassium salt, one time by oral gavage at a dose of 4.2 mg/kg. Urine and feces were collected after 24 and 48 hours. The amounts recovered in urine and feces were approximately equivalent at each time point: 1.57% and 1.55%, respectively, at 24 hours and 2.52% and 3.24%, respectively, at 48 hours.

Further investigation by Kim and colleagues measured the amounts of unchanged PFOS excreted into the urine and the feces of male and female Sprague-Dawley rats with a single dose of 2 mg/kg by oral or intravenous administration (Kim et al., 2016b). After dosing, urine and feces were measured weekly throughout the 70-day study period. The highest concentrations were found in urine under all conditions. In males, the levels detected in urine (76.13 ± 16.83 μg) and feces (61.65 ± 7.29 μg) were similar after oral administration. After intravenous dosing, urine levels in males (103.04 ± 21.56 μg) were more than 2-fold higher than fecal levels (43.73 ± 5.29 μg). Females also excreted higher levels in urine compared with feces by both dosing routes. After oral administration, urine and fecal levels were 95.42 ± 22.14 μg and 53.29 ± 8.64 μg, respectively. Similar values in urine (88.29 ± 14.91 μg) and feces (48.37 ± 4.98 μg) were measured after intravenous dosing. The similar concentrations in urine and feces translated to similar half-life estimates for PFOS (26.44 and 28.70 days in males and 23.50 and 24.80 days in females by the oral and intravenous routes).

Another study evaluated repeat dosing in 10 male Sprague-Dawley rats (~9 weeks old)/group which were administered 0 mg/kg/day, 5 mg/kg/day, or 20 mg/kg/day PFOS by gavage once daily for 4 weeks (Cui et al., 2010). Urine and feces were collected for 24-hour intervals on the day prior to treatment (day 0), and days 1, 3, 5, 7, 19, 14, 18, 21, 24, and 28. Both dose groups exhibited increased excretion over time, with greater excretion rates in the urine. No notable difference in excretion between the dose groups remained after accounting for decreased food intake and mortality in the high-dose group.

Another study (Gao et al., 2015) compared concentrations in urine and feces of male and female Wistar rats. A mixture of PFOA/PFNA/PFOS were administered to rats by drinking water for 90 days, with each compound at doses of 0 mg/L, 0.05 mg/L, 0.5 mg/L, and 5 mg/L. While the focus of this study was measuring concentrations in the hair of animals (discussed below under Other Routes of Excretion), the authors measured concentrations of each PFAA in urine and feces samples by collecting excreta in standard metabolism cages overnight for 24 h intervals on day 84 (week 12). The intake for each compound was calculated as the drinking volume multiplied by water concentration of 0.05 mg/L, 0.5 mg/L, and 5 mg/L. In contrast to observations by others, there were far higher levels of PFOS in feces compared with urine for both males and females. However, this trend was also observed among female Crl:CD(SD)IGS VAF/Plus rats by Luebker et al. (2005a), in which five groups of 16 dams each were administered 0 mg, 0.1 mg, 0.4 mg, 1.6 mg, or 3.2 mg PFOS/kg bw/day by oral gavage beginning 42 days prior to cohabitation and continuing through GD 14 or GD 20. Urine and feces were collected overnight from dams on the eve of cohabitation day 1 and during GDs 6–7, GDs 14–15, and GDs 20–21. The concentrations in the feces were consistently about 5 times greater than in the urine. It is unclear whether the higher levels of PFOS in feces reflects rat strain or dose differences among the various studies or is driven by differential excretion pathways in rats exposed to a mixture of PFAAs.

In summary, limited evidence supports excretion through the fecal route in both animals and humans. Most studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. There are sex-specific differences in excretion of PFOS through feces. Excretion through the fecal route appears to be more efficient in males compared with females. Also, in male rats, fecal and urinary concentrations were similar after oral but not intravenous dosing. Finally, exposures to mixtures of PFNAs suggests that PFOS in the context of a mixture may be preferentially excreted through the fecal route. The extent to which resorption by hepatic and enteric routes impacts fecal excretion has not been established in either humans or animals.

B.4.2 Physiological and Mechanistic Factors Impacting Excretion

B.4.2.1 Renal Resorption

Urinary excretion is the major route of elimination for PFOS. Excretion through urine is impacted by saturable renal resorption of PFOS from the glomerular filtrate via transporters in the kidney tubules.

Urinary excretion of PFOS in humans is also impacted by the isomeric composition of the mixture present in blood and the sex/age of the individuals. The half-lives of the branched chain

PFOS isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains.

Zhang et al. (2013c) determined half-lives for PFOS isomers based on paired serum samples and early morning urine samples collected from healthy volunteers in two large Chinese cities. Half-lives were determined using a one-compartment model and an assumption of first-order clearance. The mean half-life values for the six branched chain isomers of PFOS were lower than the value for the linear chain with the exception of the 1-methyl heptane sulfonate, suggesting that resorption transporters may favor uptake of the linear chain and 1-methyl branched chain over the other isomers.

B.4.2.2 Enterohepatic Resorption

Early evidence of enterohepatic resorption of PFOS was revealed by Johnson and colleagues (1984), who demonstrated that cholestyramine (CSM) treatment increased mean cumulative carbon-14 elimination in feces by 9.5-fold for male CD rats administered 3.4 mg/kg [^{14}C]PFOS. CSM is a bile acid sequestrant, and its facilitation of PFOS GI clearance suggests enterohepatic circulation.

Evidence of enterohepatic excretion and potential resorption in humans includes Harada et al. (2007), in which serum and bile samples from patients (two male and two female; aged 63–76) undergoing gallstone surgery exhibited higher PFOS levels in the bile than in the serum, suggesting bile as a route of excretion. The biliary resorption rate was 0.97, which could contribute to the long half-life in humans. Method of exposure to PFOS was unknown.

Biliary excretion in humans and the potential for resorption from bile discharged to the GI tract is supported by the Genuis et al. (2010) self-study of the potential for CSM to lower the levels of PFAS in blood. This was a case report and the sole example of excretion analyzed after inhalation PFOS exposure. A 51-year-old exposed through carpet treated with soil/dirt repellants presented with elevated serum levels of perfluorinated compounds including PFOS. After treatment with CSM for 1 week (ingested 4 g/day, 3 times a day), PFOS serum levels decreased from 23 ng/g serum to 14.4 ng/g serum. Additionally, the stool concentration of PFOS was increased from undetectable before treatment (LOD = 0.5 ng/g) to 9.06 ng/g and 7.94 ng/g in the weeks after treatment, suggesting that it may help with removing PFOS that gains access to the GI tract with bile.

Table B-21 summarizes enterohepatic transporters identified in liver hepatocytes and intestinal enterocytes in humans and rats by Zhao and colleagues (2017b; 2015) and suggests that PFOS is a substrate of both sodium-dependent and -independent enterohepatic transporters involved in recirculation of bile acids. For these in vitro studies, the authors used transformed ovary (CHO) and kidney (HEK293) cells stably or transiently transfected with cDNA constructs encoding for the transporters as well as CHO Flp-In cells expressing human OATP2B.Wild-type CHO cells and HEK293 cells transfected with vector only were used as controls. With the exception of rat ASBT, PFOS was demonstrated to be a substrate for all transporters as well as OSTalpha/beta.

Binding efficiency to the enterohepatic transporters was chain-length dependent. Sodium-taurocholate cotransporting polypeptide (NTCP) transported PFSAs with decreasing affinity but increasing capacity as the chain length increased (Zhao et al., 2015). The opposite trend was seen for OATP-mediated uptake (Zhao et al., 2017b). For these five OATPs, PFOS was transported

with the highest affinity compared with transport of PFBS and PFHxS. The authors suggest that transport efficiency generally increased with the increase in chain length, and that this may, at least in part, account for the shorter half-lives of short chained versus long chained perfluoroalkyl sulfonates. While these in vitro studies demonstrate that PFOS is a substrate of enterohepatic transporters found in the livers and intestines of humans and rats, it is unknown whether and to what extent these transporters function in vivo.

Table B-21. Enterohepatic Transporters of PFOS

Type	Human Transporters		Rat Transporters	
	Liver Hepatocyte	Intestinal Enterocyte	Liver Hepatocyte	Intestinal Enterocyte
Sodium-dependent (Zhao et al., 2015)	NTCP	ASBT	NTCP	
Sodium-independent (Zhao et al., 2017b)	OATP1B1[a] OATP1B3[a] OATP2B1[a]	OATP2B1[a]	OATP1A1[a] OATP1B2 OATP2B1	OATP1A5 OATP2B1

Notes: ASBT = human apical sodium-dependent bile salt transporter; NTCP = Na+/taurocholate cotransporting polypeptide; OATP = organic anion transporting polypeptide.
[a] Transporter examined in transfection studies; PFOS also shown to be a substrate of these transporters in HEK293 cells transiently transfected with cDNA constructs encoding these transporters (Zhao et al., 2017b; Zhao et al., 2015).

B.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

PFOS can readily pass from mothers to their fetuses during gestation and through breast milk during lactation. In conjunction with elimination through menstruation discussed in Section B.4.4, females may eliminate PFOS through routes not available to males. The total daily elimination of PFOS in pregnant females was estimated to be 30.1 ng/day, higher than the 11.4 ng/day for PFOA (Zhang and Qin, 2014). The ratio of branched:total PFOS isomers in cord blood was 0.27 and was statistically greater in cord blood compared with maternal blood and placenta. These findings suggest branched PFOS isomers may transfer to the fetus more readily than linear forms.

The distribution of PFOS from maternal serum to the fetus and infants is discussed in detail above (Section B.2.3). A study by Zhang et al. (2013b) exemplifies the routes and amounts of PFOS eliminated by pregnant females. Paired maternal whole blood and cord blood samples were analyzed from 32 females from Tianjin, China. The maternal blood concentration of PFOS was 14.6 ng/mL. The mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.1%, respectively, of those in the mother's blood. Although levels in amniotic fluid correlated to maternal blood for PFOA, the correlation was poor for PFOS. Nevertheless, in addition to cord blood, placenta and amniotic fluids are additional potentially substantial routes of elimination in pregnant females. Blood loss during childbirth could be another source of excretion.

The elimination of PFOS in pregnant women corresponds to an increase in concentrations in the placenta. Mamsen et al. (2019) observed an increase in PFOS accumulation from gestational age 50 to 300 days, with male placentas showing higher levels of than female placentas. The authors estimated a placenta PFOS accumulation rate of 0.13% increase per day during gestation.

Mamsen and colleagues (2017) measured placental samples and fetal organs in relation to maternal plasma levels of 5 PFAS in 39 Danish women who underwent legal termination of pregnancy before gestational week 12 (Mamsen et al., 2017). All PFAS were transferred from mother to fetus with different efficiencies and a significant positive correlation was observed for fetal age (exposure duration) and for fetal:maternal plasma ratios for all PFAS compounds. Fetal organ levels of PFOS were lower than maternal blood. The average concentration of PFOS was 0.6 ng/g in fetal organs compared with 1.3 ng/g in placenta and 8.2 ng/g in maternal plasma. Increasing fetal PFOS levels with fetal age suggests that the rate of elimination of PFOS from mother to fetus may increase through the gestational period.

The same group (Mamsen et al., 2019) measured PFOS accumulation in fetal tissues across the three trimesters from 78 pregnant women who underwent elective pregnancy terminations and from cases of intrauterine fetal death. Fetal tissues (placenta, liver, lung, heart, central nervous system (CNS) and adipose) were collected for 38 first trimester pregnancies, 18 second trimester pregnancies and 22 third trimester pregnancies. PFOS was above LOQ in 100% of maternal serum samples, in 93% of placenta samples and 76% of fetal organs. In general, the concentrations of PFOS in fetal tissue increased from first trimester to third trimester except for liver and heart which showed highest levels in the second trimester compared with the third trimester. Analysis of the placenta:serum ratio of PFOA revealed a higher ratio in male fetuses than in female fetuses, but unlike PFOA, the difference between the sexes did not reach statistical significance. These studies support the placenta and fetus as important routes of PFOS elimination in pregnant women.

Underscoring the importance of pregnancy as a lifestage when excretion is altered, Zhang et al. (2015a) observed that the partitioning ratio of PFOS concentrations between urine and whole blood in pregnant women (0.0004) was significantly lower ($p = 0.025$) than the ratios found in nonpregnant women (0.0013) and may be affected by the increase in blood volume during pregnancy (Pritchard, 1965).

After birth, women can also eliminate PFOS via lactation. Tao and colleagues measured 45 human breast milk samples collected in 2004 from Massachusetts and PFOS (mean 131 ng/L) and PFOA (mean 43.8 ng/L) were the predominant PFAS compounds measured (Tao et al., 2008). Elimination through breast was more recently measured in 293 samples collected from 127 mothers in the Children's Health and Environmental Chemicals in Korea (CHECK) Cohort (Lee et al., 2017). Results were stratified by age, parity, body mass, delivery method, and infant sex. The median PFOS concentrations in breast milk across all samples was 47.4 ng/L (range of 36.4–63.8 ng/L) and the median concentration for all PFAS chemicals measured was 151 ng/L (range of 105–212 ng/L). Pooled breast milk samples were measured to follow the time course of PFOS in breast milk after birth. Concentrations in breast milk measured 30 days after birth were significantly higher than those measured prior to 7 days after birth. These findings are contrast with results of Thomsen et al. (2010) that reported that breast milk levels of PFOA and PFOS decreased by 7% and 3.1%, respectively, during the first month after birth. Demographic factors,

maternal diets, sample sizes, the lactational periods measured may account for these discrepancies.

Lee and colleagues also observed that parity impacts PFOA levels in breastmilk. While primiparous mothers showed higher levels of PFOA in breast milk to mothers giving birth to more than one child ($p < 0.05$), levels of PFOS were not significantly different between these two groups. In contrast, another study of a Slovakian cohort, multivariable models estimated that parous women had 40% lower PFOS (95% CI: −56%, −17%) concentrations in colostrum compared with nulliparous women (Jusko et al., 2016). The geometric mean concentration in was 35.3 ng/L for PFOS and 32.8 ng/L for PFOA. These findings are also consistent with higher PFOS levels ($p < 0.001$) in second trimester maternal serum (18.1 ± 10.9 ng/mL) than maternal serum levels at delivery (16.2 ± 10.4 ng/mL), which were higher than the levels found in cord serum (7.3 ± 5.8 ng/mL; $p < 0.001$) (Monroy et al., 2008). In this study, samples were measured in 101 pregnant women at 24–28 weeks of pregnancy, at delivery, and in umbilical cord blood.

PFOS was also measured in maternal serum, cord serum and breast milk from 102 female volunteers hospitalized between June 2010 and January 2013 for planned cesarean delivery in Tolouse, France (Cariou et al., 2015). Mean PFOS concentrations were 3.67, 1.38 and 0.040 in maternal serum, cord serum and breast milk respectively (compared with 1.22, 0.9191 and 0.041 ng/mL for PFOA). The observed ratios of cord and maternal serum for PFOS was 0.38 in this study, much lower than the ratio of 0.78 for PFOA. However, the ratio between breast milk and maternal serum was 0.038 ± 0.016 (essentially the same as measured for PFOA). Thus, PFOS exhibits a low transfer from maternal blood to cord blood and a 10-fold lower transfer from maternal blood to breast milk.

In summary, partitioning to the fetus and breast milk represent important routes of elimination in humans, and may account for some of the differences observed for blood and urinary levels of PFOS by sex and age.

B.4.4 Other Routes of Elimination

Wong et al. (2014) looked at the role of menstrual blood as an excretory pathway to explain the shorter half-life of PFOS in females than males. They fit a population-based pharmacokinetic model to six cross-sectional National Health and Nutrition Examination Survey (NHANES) datasets (1999–2012) for males and females. They concluded that menstruation could account for about 30% of the elimination half-life difference between females and males. Wong et al. (2014) did not account for other possible loss pathways of PFOS that are unique to women of reproductive age such as the amount of blood loss in child delivery, amniotic fluid, breast feeding. Verner and Longnecker (2015) suggested a need to consider the non-blood portion of the menstrual fluid and its albumin content in the Wong et al. (2014) estimate for the menstrual fluid volume. A yearly estimate for serum loss of 868 mL/year by Verner and Longnecker (2015) compared with the 432 mL/year estimate of Wong et al. (2014) suggests that the menstrual fluid loss can account for >30% of the difference in the elimination half-life between females and males.

Two earlier studies supported an association between increased serum concentrations of PFOA and PFOS and early menopause (Taylor et al., 2014; Knox et al., 2011). However, a re-analysis of this data (Ruark et al., 2017) suggested that this association could be explained by reversed

causality and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Also challenging the assumption that this is due to menstruation, Singer et al. (2018) failed to find evidence of associations between menstrual cycle length and PFAS concentrations.

Furthermore, Lorber et al. (2015) compared individuals who had undergone blood removal treatments for medical reasons to menstruating females. Measurements showed lower PFOA and PFOS concentrations in the groups experiencing regular blood loss. Estimated concentrations based on a one-compartment model were consistent with measured serum concentrations. Overall, this study provides data and modeling that support the initial hypothesis that ongoing blood loss explains lower PFAA concentrations in humans. These authors suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may also help explain the differences in elimination rates between males and females. Curiously, studies providing direct measurements of PFOS in menstrual blood were not identified. However, for PFOS to be selectively retained from the blood lost through menstruation would require a specific mechanism for that process and no such mechanism has been demonstrated or proposed.

Hair has been demonstrated as a route of elimination in animals (Gao et al., 2015). Adult male and female Wistar rats were exposed via drinking water to 0 mg/L, 0.05 mg/L, 0.5 mg/L, and 5 mg/L of PFOS, PFNA, and PFOA for 90 days. At the end of the exposure period, dorsal hair samples were collected, washed twice in Triton buffer to remove external contaminants and alkaline digested to extract PFAS. PFOS was detected in hair samples of all the treatment groups, suggesting a potential route of elimination. Hair from male and female rats contained PFOS concentration ranged from 20.3 ng/g to 2,086 ng/g in 0.05 mg/L and 5 mg/L treatment groups, respectively. Notably, the PFOS concentration in hair was significantly higher than the levels of PFOA (3.31–444 ng/g) and PFNA (14.2–1,604 ng/g) at 0.05 mg/L to 5 mg/L doses. Unlike PFOA and PFNA which showed a sexually dimorphic pattern, where male rats have significantly higher hair concentrations than female rats, hair PFOS levels were lower in males of the 0.05 mg/L group than females of the same dose group and there were no significant differences in hair PFOS concentrations between males and females of the 0.5 mg/L and 5 mg/L dose.

Gao et al. (2015) also measured the composition of the mixture excreted in in urine, feces, and hair after administration of 0.5 mg/L or 0.05 mg/L of a mixture of PFAS (Table B-22). At the lower dose of 0.05 mg/L, PFOS was the dominant constituent in urine of males and made up a smaller proportion of total mixture excreted in hair but not feces. In females however, PFOA was the predominant constituent excreted in urine, but made up the minority constituent excreted in feces and especially in hair. These findings underscore the impact of mixtures and sex on PFOA excretion.

Table B-22. Estimated Percentage of the Sum of PFOS, PFNA, and PFOA in Excreta and Serum of Male and Female Wistar Rats[a] as Reported by Gao et al. (2015)

Sex	PFAA	Serum	Urine	Feces	Hair
Males	PFOS	24.6	89.0	20.8	30.0
	PFNA	59.9	11.0	53.0	45.4
	PFOA	15.6	ND	26.1	24.6

Sex	PFAA	Serum	Urine	Feces	Hair
Females	PFOS	89.0	ND	62.4	78.0
	PFNA	11.0	38.9	21.7	18.0
	PFOA	ND	61.1	16.1	4.2

Notes: ND = not detected; PFAA = perfluoroalkyl acid; PFNA = perfluorononanoic acid.
[a] Data are presented in % total perfluoroalkyl acids administered. Animals exposed to 0.05 mg/L in Gao (2015)

A single case report study (Genuis et al., 2010) examined PFOS excretion through sweat. PFOS was measured in sweat as well as urine and stool from a single male subject exposed to perfluorinated chemicals via inhalation exposure and subjected to treatment with bile sequestrants. With the exception of PFHxS, no other PFAS chemicals, including PFOS, were detected in sweat.

Thus far, no single study has conducted a comparative analysis of elimination of PFOS through all possible routes of excretion. A comprehensive analysis stratified by age and sex would be necessary to advance the understanding PFOS excretion by all possible routes, and to establish factors that influence the proportion of PFOS excreted through urine versus other excreta matrices.

B.4.5 Half-life Data

B.4.5.1 Overview

In general a half-life represents elimination by all routes, which includes metabolism for other chemicals, but because PFOA/PFOS are not metabolized, it can be interpreted for excretion (after correction for body weight (BW) changes). The calculated values of PFOS half-lives reported in the literature vary considerably, which poses challenges in predicting both the routes and rates of excretion. Several interrelated physiological and mechanistic factors impacting excretion are summarized here:

1. The capacity of PFOS to be reabsorbed via renal and enterohepatic routes of excretion and binding affinities to relevant transporters including organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), MRPs, and sodium-dependent transporters involved in bile acid transport including NTCP and the apical sodium-dependent bile acid transporter (ASBT). Exposure to high levels of PFOS under acute conditions (e.g., contaminated drinking water) or in occupational settings may result in saturation of resorption transporters and increased excretion.

2. Binding affinity to serum proteins limiting the concentration of the unbound fraction available for resorption through renal or enterohepatic transporters. Moreover, binding to serum proteins may limit passive diffusion of perfluorinated chemicals across the placental barrier.

3. Phospholipid lipid binding affinity (phospholipidphilicity), which can further reduce the unbound fraction of PFOS as well as uptake into cells. As reported by Sanchez Garcia and colleagues, phospholipophilicity shows the highest correlation to cellular accumulation data compared with other measures of lipophilicity. Also, phospholipid binding affinity could distinguish between high and low accumulating compounds as well

as half-life measures (Sanchez Garcia et al., 2018).

4. Chain length and branching. The half-lives of the branched chain PFOS isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains. Interactions with transporters also vary by chain length.

5. Exposure to mixtures of perfluorinated compounds with differential binding affinities to transporters, serum binding proteins and phospholipids could impact both the rate and route of PFOS excretion.

6. Sex and species can influence both the rate and route excretion. First, several elimination pathways are specific to females including menstruation, pregnancy, and lactation. Second, sex-specific hormones can impact expression of transporters involved in resorption. Furthermore, elimination half-lives vary dramatically by species, with much longer half-lives calculated in humans compared with animals.

Differences between species were observed in studies determining the elimination half-life ($T_{1/2}$) of PFOS in rats, mice, monkeys, and humans. Sex differences in rats do not appear to be as dramatic for PFOS as they are for PFOA (Loccisano et al., 2012b, a).

B.4.5.2 Human Studies

Blood sampling was performed on retirees from the 3M plant in Decatur, Alabama where PFOS was produced. These samples were taken approximately every 6 months over a 5-year period to predict the half-life of PFOS. Results ranged from approximately 4 years to 8.67 years (3M, 2002, 2000). Both of these studies exhibited some deficiencies in sample collection and methods.

More recently, Olsen et al. (2007) obtained samples from 26 retired fluorochemical production workers (24 males and 2 females) from the 3M plant in Decatur, Alabama to determine the half-life of PFOS. Periodic serum samples (total of 7–8 samples per person) were collected over a period of 5 years, stored at −80 °C, and at the end of the study, High-performance liquid chromatography/mass spectrometry was used to analyze the samples. The study took place from 1998 to 2004. The mean number of years worked at the plant was 31 years (range: 20–36 years), the mean age of the participants at the initial blood sampling was 61 years (range: 55–75 years), and the average number of years retired was 2.6 years (range: 0.4–11.5 years). The initial arithmetic mean serum concentration of PFOS was 0.799 µg/mL (range: 0.145–3.490 µg/mL), and when samples were taken at the end of the study the mean serum concentration was 0.403 µg/mL (range: 0.037–1.740 µg/mL). Semi-log graphs of concentration versus time for each of the 26 individuals were created, and individual serum elimination half-lives were determined using first-order elimination. The arithmetic and geometric mean serum elimination half-lives of PFOS were 5.4 years (95% confidence interval (CI): 3.9, 6.9 years) and 4.8 years (95% CI: 4.1, 5.4 years), respectively.

The rate of serum PFOS decline was measured in residents of two communities exposed to contaminated municipal drinking water contaminated in Bleking County, Sweden in 2013 (Li et al., 2018). A biomonitoring program ensued between 2014 and 2016 for residents exposed to contaminated water and an unexposed community. A subset of residents (age range of 15–50 years) were included in a panel study to estimate PFOS half-lives. Drinking water PFOS levels were 8,000 ng/L prior to closure of the waterworks facility and 27 ng/L in the unexposed

community. The mean serum levels among the 106 participants 6 months after the end of exposure was 387 ± 259 ng/mL. The average decrease in PFOA was 20% of its previous value each year. The excretion rate constant after the end of exposure was 0.20 (95% CI: 0.19, 0.22) and was significantly higher in females (0.22) than males (0.15). The mean half-life was 3.4 years and was also significantly shorter in females (3.1 years) than in males (4.6 years). There was a high level of inter-individual variation in half-lives.

Fu et al. (2016) determined the half-life of PFOS in 302 occupational workers (213 male and 89 female) from one of the largest producers of PFOS-related compounds in China. The half-lives of PFAAs in workers were estimated by daily clearance rates and annual decline rates of PFAAs in serum by a first-order model based on fasting blood and urine samples collected over a period of five years. Mean and median serum concentrations for PFOS among all workers were 5,624 ng/mL and 1,725 ng/mL, respectively, whereas in urine, mean and median PFOS were 4.4 and 1.2 ng/mL. Fu et al. calculated that the renal clearance rate for PFOS ranged from 5.0×10^{-5} mL/kg/day to 0.54 mL/kg/day (Geometric mean of 0.010 mL/kg/day).

Half-lives were calculated by Ln2/k using two approaches. In the first approach, k was defined as Cl_{total}/V_d, where V_d stands for the volume of distribution of PFAAs in the human body and Cl_{total} represents the total daily PFAAs clearance in the human body. Cl_{total} was defined as renal clearance for men and women older than 50, and as the sum of menstrual and renal clearance in young women. V_d was set to 230 mL/kg for PFOS. In the second approach, k was defined as the average annual decline rates of PFAAs in workers who participated in this study.

The half-life of PFOS estimated using daily clearance rate of all workers had a geometric mean and median value of 32.6 and 21.6 years, respectively. However, when measured by annual decline rate, the half-life of PFOS was estimated to be 1.9 years. The GM values of the half-life of PFOS for men here was 60.9 years and 8.0 years for women. The authors suggest that half-lives estimated by the limited clearance route information could be considered as the upper limits for PFAAs and that the unrealistically long half-lives determined using urine clearance values may indicate that other clearance play important roles in elimination of PFAAs in humans including fecal elimination. Another possibility is that the apparent half-lives of PFAAs calculated through annual decline rates could be affected by the high ongoing levels of exposure.

Calculated half-lives of PFOS were much longer than for PFOA. The authors postulate differential accumulation kinetics of the pollutants and suggest that PFOS reaches a steady state much faster than PFHxS and PFOA in humans. The longer half-life estimates for PFOS compared with PFOA may also reflect its stronger affinity for serum albumin as reported previously (Salvalaglio et al., 2010). Other factors impacting half-lives could include higher enterohepatic and renal reabsorption rates of PFOS relative to PFOA. The authors conclude that the shorter half-lives of PFHxS and PFOS estimated by annual decline compared with those estimated by daily clearance rates suggest that other important elimination pathways operate to remove PFOS and might have been underestimated.

Worley and colleagues (2017) calculated PFOS half-lives in community members (age 12-years old or older) living near a PFAS manufacturer in Alabama that had discharged waste into a local wastewater treatment plant. Sewage sludge from this plant was applied to local agricultural fields. In 2010, ATSDR collected blood samples from subjects and followed up with blood and urine measurements in 2016. Biological half-lives were estimated for PFOA and PFOS using a

one-compartment pharmacokinetic model. Geometric mean serum PFOS concentrations were significantly higher in subjects in both 2010 (39.8 μg/L) and 2016 (23.4 μg/L) relative to national averages reported by NHANES (9.32 μg/L in 2009–2010 and 4.99 μg/L in 2013–2014).

Half-lives for PFOA and PFOS were estimated to be 3.9 and 3.3 years, respectively. When V_d and intake values were altered by ± 20%, half-life values varied by several months (half-life estimates for PFOA and PFOS ranged from 3.5 to 4.1, and 3.0 to 3.6 years). The authors suggest these parameters have a significant impact on half-life estimates.

Xu et al. (2020c) estimated the half-life of PFAS by sampling urine (4 times) and blood (5 times) from 26 airport employees between 2 weeks and 5 months after the end of a 2-month exposure to PFAS-contaminated drinking water. The levels of PFOS in the airport's contaminated water were 62 ng/L (0.062 ng/mL) for linear PFOS and 64 ng/L (0.064 ng/mL) for branched PFOS. Specific gravity adjusted urine concentrations for PFOS were generally below detectable limits for linear and branched forms of PFOS with respective ranges of <LOD–0.084 ng/mL and <LOD–1.6 ng/mL (determined from the second to the fifth sampling periods).

Serum levels of PFOS in the first serum sample taken from all 26 employees was 9.5 and 6.4 ng/mL for linear and branched PFOS, respectively. The serum/water ratio was reported as 153 for linear PFOS and 100 for branched PFOS. PFOS median concentrations measured in serum obtained from the second to the fifth sampling were reported as 10 ng/mL and 2.1 ng/mL for linear and branched PFOS, respectively, with an average urine/serum ratio of 0.00092 (linear) and 0.0051 (branched) in paired serum and urine samples. The significant difference between the serum/water ratio and the urine/serum ratio is suggestive of the influence of the clearance rate on the overall serum levels (lower the clearance rate and higher serum levels correlate to longer the half-lives). PFOS half-lives were reported as 2.91 years for linear PFOS and ranged from 1.04 to 1.27 years for branched forms.

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOS half-lives along with measured intake and serum and urine PFOS concentrations (Xu et al., 2020c; Worley et al., 2017; Fu et al., 2016; Zhang et al., 2013d) (Table B-23). PFOS half-life values among these 4 studies varied from 1.04 years in Xu et al. (2020c) to 60.9 years in Fu et al. (2016). These comparisons support principles suggested by the broader literature. First, sex-related differences with males exhibiting somewhat longer half-lives compared with (especially females of reproductive age) may relate, at least in part, to menstruation as routes of elimination (Zhang et al., 2013c). Second, blood and urine concentrations varied by several orders of magnitude across these 4 studies. This variability in serum and urine concentrations may reflect the role of non-urinary routes of PFOS excretion; the variability in concentrations may also reflect the difficulty in measuring renal resorption. Finally, only two studies estimated PFOS intake in subjects (Xu et al., 2020c; Worley et al., 2017). The multiple routes of exposure to PFOS and the need to understand historical exposure levels to estimate PFOS intake is an ongoing challenge for many studies that examine PFOS elimination. These factors, as well as age and health status of subjects, likely contribute to the reported variability in PFOS half-life estimates in humans.

Table B-23. Summary of PFOS Concentration in Blood and Urine in Relation to Half-life Values in Humans

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half-Life (y)	Considerations
Xu et al. (2020c)	26 19 Males 7 Females	22–62 yr	Oral	Drinking water at airport 62 ng/µL (linear) 64 ng/µL (branched) 130 ng/µL Total	Linear PFOS: Median: 10 ng/mL (4.1–24 ng/mL) 2/6m-PFOS: Median: 2.1 ng/mL (0.57–8.1 ng/mL)	Linear PFOS: mean <LOD–0.084 ng/mL Median: <LOD 2/6m-PFOS mean: <LOD–1.6 ng/mL, Median: <LOD (not creatinine adjusted)	Linear PFOS: 2.91, 1m-PFOS: 1.27 3/4/5m-PFOS: 1.09 2/6m-PFOS: 1.04	• 1 woman was previously pregnant 2018 during sampling year • PFOS also measured in the private well of one airport employee living near the airport (PFOS concentration in well was lower than the airport at 1.9 ng/µL linear and 0.24 ng/µL branched)
Worley et al. (2017)	153 (2010) 63 Males 90 Females 45 (2016) 22 Males 23 Females	2010: Mean 52.0 2016: Mean 62.6	Oral	Drinking water	2010 Geometric mean 39.8 ng/mL (30.9–48.9, 95% CI) 2016 Geometric mean 23.4 (18.5–28.4, 95% CI)	Not determined due to high proportion of <LOD samples (creatinine adjusted)	3.9 (2010) 3.3 (2016)	• PFOS was detected in 45.7% of samples. LOD was 0.02 µg/L • Estimate intake rate for PFOS was 6 ng/h , based on PFOS drinking water concentration of 0.12 µg/L, Volume of distribution of PFOS was reported as 230 mL/kg body weight

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half-Life (y)	Considerations
								• Clearance rate was not reported
Fu et al. (2016)	302 213 Males 89 Females	Males: 19–65, median 41 Females: 19–50, Median 37	Occupational (assuming oral and inhalation but not directly addressed in study)	NR	Mean 5,624 ng/mL Median 1,725 ng/mL (50.3–118,000 ng/mL)	Mean: 4.4 ng/mL, Median 1.2 ng/mL (not creatinine adjusted)	Male (n = 136): GM 60.9 Females (n = 71): GM 8.0 Overall (n = 207): GM 32.6	• Urinary samples were only taken from 274 participants while there were serum samples for every participant • For half -life calculation for females, menstrual clearance was added to renal clearance • PFOS clearance rate 0.017 mL/kg-day
Zhang et al. (2013c)	86 47 Males 37 Females	22–68	Unspecified (Oral likely, Shijazhuang is a capital city and Handan is an industrial city)	NR	Mean 21 ng/mL Median 19 ng/mL (1.4–180 ng/mL) Branched	Mean 47 ng/g creatinine Median 28 ng/g creatinine (range 2.8–232 ng/g creatinine)	Young females: 6.2 Males and older females: 27	• All participants had paired (whole blood/serum and urine) • For young females, menstrual clearance was estimated and added to renal clearance. • Renal clearance rate for total PFOS: mean 0.050 mg/kg/day (young females), 0.037 mg/kg/day

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half-Life (y)	Considerations
								(males and older females)

Notes: CI = confidence interval; GM = geometric mean; LOD = limit of detection; NR = not reported; yr = years.

All human PFOS half-life values identified in the literature review are provided in Table B-24. A prominent feature of this data includes a very wide range of values ranging from less than 1 year in a single male child of 16 years of age (Genuis et al., 2014) to up to 60.9 years for males occupationally exposed in a plant in China (Fu et al., 2016). Second, with one exception (Genuis et al., 2014), half-lives estimated for males are longer than those estimated for females. Third, studies that stratified by ages show an age-related increase in half-life values (Genuis et al., 2014; Zhang et al., 2013d). Fourth, linear isomers exhibit longer half-lives than branched isomers (Zhang et al., 2013c).

Gomis et al. (2017) estimated half-lives in the general populations in the U.S. and in Australia and reported a range of 3.3 to 5.4 years. Olsen et al. (2012b) estimated a similar value in blood samples from Red Cross volunteer donors of 4.3 years. Interestingly, these values were also in line with the half-life (2.3 y) estimated for subjects likely exposed to contaminated drinking water in West Virginia and Ohio (Bartell et al., 2010). Other studies of subjects exposed to contaminated drinking water in Sweden (Li et al., 2018) estimated half-lives of 3.1 (for females) to 4.6 years (for males). Among the highest values are those for occupationally exposed workers that ranged from 8.67 years (retired workers from a PFOS production plant in Decatur, Alabama) to 60.9 years for workers in Hubei province, China.

While most studies were conducted in adults and/or adolescents, at least one study examined PFOS half-lives in newborns (Spliethoff et al., 2008). Whole blood was collected as dried spots on filter paper from almost all infants born in the United States. One hundred and ten of the Newborn Screening Programs (NSPs) collected in the state of New York from infants born between 1997 and 2007 were analyzed for PFOS. The analytical methods were validated by using freshly drawn blood from healthy adult volunteers. The mean whole blood concentration for PFOS ranged from 0.00081 μg/mL to 0.00241 μg/mL. The study grouped the blood spots by two different time points; those collected in 1999–2000 and in 2003–2004, which corresponded to the intervals reported by NHANES. The PFOS concentrations decreased with a mean value of 0.00243 μg/mL reported in 1999–2000 and 0.00174 μg/mL in 2003–2004. The study authors determined the half-life of PFOS using the regression slopes for natural log blood concentrations versus the year 2000 and after. The calculated half-life for PFOS was 4.1 years.

Table B-24. Summary of Human PFOS Half-Life Values

Study	Number of Subjects	Age Range	Estimated Half-Life (y)	Subjects
3M (2002)	9 7 males 2 females	61 (55–64)	8.67 ± 6.12 (range: 2.29–21.3)	Retirees from the 3M plant in Decatur, Alabama where PFOS was produced. Derived from 4 measurements over 18-month time period from November of 1998 to May of 2000.
Bartell et al. (2010)	200 100 males 100 females	54.5 ± 15	2.3	Subjects were a subcohort of the C8 Health Project, conducted in 2005–2006, who had lived in at least one of six affected water districts near the DuPont Washington Works plant.
Fu et al. (2016)	302 213 males 89 females	Males: 19–65, median 41 Females: 19–50, median 37	Based on daily clearance rate Male (n = 136): GM 60.9 Females (n = 71): GM 8.0 Overall (n = 207): GM 32.6 Based on annual decline rate Overall (n = 207): GM 1.9	Occupationally exposed subjects working in one of the largest fluorochemical plants (Henxin Chemical Plant) in Yingcheng, Hubei province, China
Genuis et al. (2014)	53 father 47 mother 22 first male child 19 second female child 17 third male child 16 fourth male child	16–53	Father: 1.14 Mother: 1.93 First male child: 0.65 Second female child: 1.03 Third male child: 0.78 Fourth male child: 0.61	A family (6 patients) identified to have elevated serum concentrations of PFAAs, likely through repeated commercial spraying of their home carpets with stain-repellants. Patients were treated by intermittent phlebotomy over a 4- to 5-yr period.
Glynn et al. (2012)	413 women	19–41	8.2	Primiparous women 3 wk after delivery in Uppsala County, Sweden 1996–2010 (the POPUP study; Persistent Organic Pollutants in Uppsala Primiparas)
Gomis et al. (2017)	Australia: A total of 24–84 pools per survey containing between 30 and 100 individual samples. USA: 2,000 individuals were sampled throughout the USA	12+ (USA) <16–>60 (Australia)	Australian men: 4.9 American men: 3.8 Australian women: 5 American women: 3.3	Population-based model using Australian biomonitoring studies from Toms et al. (2014, 2009) and NHANES from the U.S. A total of 24–84 pools per survey were obtained, with each pool containing between 30 (2007) and up to 100 individual samples (2003, 2009 and 2011) Study reports intrinsic elimination half-lives.

Study	Number of Subjects	Age Range	Estimated Half-Life (y)	Subjects
Li et al. (2018)	50 Males: 20 Females 30	15–50	Males: 4.6 Females: 3.1	Subjects in Ronneby, Sweden, exposed to contaminated water through a municipal water source.
Olsen et al. (2007)	26 24 males 2 females	55–75	5.4	Retirees from the 3M plant in Decatur, Alabama where PFOS was produced.
Olsen et al. (2012b)	600 Males: 300 Females: 300	5 age groups (20–29, 30–39, 40–49, 50–59, 60–69)	4.3	Six American Red Cross adult blood donor centers each provided 100 plasma samples for analysis of 11 PFAA concentrations in 2010: 10 samples per every 10-yr age interval (20–29, 30–39, 40–49, 50–59, and 60–69) for each sex. The six American Red Cross blood donor centers represented the following areas: Boston, Massachusetts; Charlotte, North Carolina; Hagerstown, Maryland; Los Angeles, California; Minneapolis-St. Paul, Minnesota; and Portland, Oregon
Splitehoff et al. (2008)	240	Newborn infant (1–2 d)	4.1	New York State newborn screening program blood spot specimens from newborn infants
Wong et al. (2014)	Approx. 2,000 per dataset (6 datasets) Males and females analyzed separately	Eight age groups (age 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80+)	Males: 4.7 Females: 3.7 Females (accounting for rate of menstrual blood loss): 4.0	Population-based pharmacokinetic model (Ritter) to six cross-sectional datasets from 1999 to 2012 from U.S. NHANES. Data from age-stratified biomonitoring data for PFOS extracted from U.S. NHANES from the years 1999–2000, 2003–2004, 2005–2006, 2007–2008, 2009–2010, and 2011–2012
Worley et al. (2017)	153 (2010) 63 males 90 females 45 (2016) 22 males 23 females	2010: mean 52.0 2016: mean 62.6	3.9 (2010) 3.3 (2016)	Residentially exposed population from Lawrence, Morgan and Limestone Counties, Alabama recruited by ATSDR
Xu et al. (2020d)	26 19 males 7 females	22–62 yr	Linear PFOS: 2.91 1m-PFOS: 1.27 3/4/5m-PFOS: 1.09 2/6m-PFOS: 1.04	Subjects in Arvidsjaur, Sweden exposed to contaminated drinking water occupationally (working at the airport) and through residential drinking water

Study	Number of Subjects	Age Range	Estimated Half-Life (y)	Subjects
Yeung et al. (2013)	420 Munster: 270 Halle: 150	20–29	Munster: 4.3 Halle: 4.8	Residents of Munster and Halle, Germany; samples collected between 1982 and 2009 in Munster and between 1995 and 2009 in Halle.
Zhang et al. (2013c)	86 47 males 37 females	22–68	∑PFOS Young females: 6.2 males and older females: 27 n-PFOS Young females: 6.7 males and older females: 34 iso-PFOS Young females: 5.9 males and older females: 24 1m-PFOS Young females: 10 males and older females: 90 4m-PFOS Young females: 5.8 y males and older females: 27 3 + 5m-PFOS Young females: 5 y males and older females: 21 ∑m2-PFOS Young females: 5.1 males and older females: 14	Healthy volunteers in Shijiazhuang and Handan, Hebei province, China, in April–May 2010

Notes: ATSDR = Agency for Toxic Substances and Disease Registry; C8HP = C8 Health Project; GM = geometric mean; NHANES = National Health and Nutrition Examination Survey; PFAA = perfluoroalkyl acid; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; U.S. = United States; USA = United States of America; yr = year.

B.4.5.3 Animal Studies

B.4.5.3.1 Nonhuman Primates

In the study by Chang et al. (2012), three male and three female monkeys were administered a single IV dose of PFOS of 2 mg/kg and followed for 161 days. All monkeys were observed twice daily for clinical signs, and body weights were obtained weekly. Urine and serum samples were taken throughout the study. There was no indication that elimination was different from males versus females. Serum elimination half-lives ranged 122–146 days in male monkeys and 88–138 days in females. Mean values are shown in Table B-25. The V_d values suggest that distribution was predominately extracellular.

In a second primate study, Seacat et al. (2002) administered 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS orally in a capsule by intragastric intubation to six young-adult to adult cynomolgus monkeys/sex/group, except for the 0.03 mg/kg/day group, which had 4/sex, daily for 26 weeks (182 days) in a GLP study. Two monkeys/sex/group in the control, 0.15, and 0.75 mg/kg/day groups were monitored for 1 year after the end of the treatment period for reversible or delayed toxicity effects. The elimination half-life for potassium PFOS in monkeys was estimated from the elimination curves as approximately 200 days. This value is consistent with that reported by Chang et al. (2012) above.

B.4.5.3.2 Rats and Mice

Half-lives rodents are very short relative to those observed in humans and primates (Table B-25). In mice, Chang et al. (2012) measured slightly higher half-lives in males (36–43 days) compared with females (30–38 days). Ranges in mice were similar to those observed in rats.

Two recent studies evaluated toxicokinetic parameters informing half-lives in rats (Huang et al., 2019a; Kim et al., 2016b). In the Kim study, Sprague-Dawley rats were administered 2 mg/kg PFOS by either the IV or oral route. Urine and feces were collected weekly, and blood was collected at 10 time points over the first day and then up to 70 days after exposure. Half-lives in females and males were similar. In females, half-lives of 23.50 ± 1.75 and 24.80 ± 1.52 days were estimated after oral and IV dosing, respectively. In males, values were slightly longer (26.44 ± 2.77 and 28.70 ± 1.85 after oral and IV dosing, respectively).

In a similar study (Huang et al., 2019a), male and female Sprague-Dawley rats were administered a single dose of 2 mg/kg by IV injection or a single dose of 2 mg/kg or 20 mg/kg by oral gavage and observed from 5 minutes to 20 weeks after dosing. After IV administration of 2 mg/kg, the overall half-life was 22 and 23 days in males and females, respectively days. Similar values were obtained after a single gavage administration of the same dose (19.9 days in males and 28.4 days in females) and after repeated dosing by oral gavage (19.0 in males and 21.1 in females). Half-lives in females administered the higher dose of 20 mg/kg were slightly longer (18 days) than in males (14.5 days) and were slightly longer after repeated administration (19.0 and 21.1. days in males and females, respectively). Half-life values in the terminal elimination phase were much longer than those measured in the initial elimination phase.

Table B-25. Summary of Animal PFOS Half-Life Values Identified in the Literature Review

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex/Half-Life Approach	Dose	Estimated Half Life[a]
Chang et al. (2012)	Cynomolgus Monkey	IV	NR	Male	2 mg/kg and followed for 161 d	132 ± 7
				Female	2 mg/kg and followed for 161 d	110 ± 15
		Oral	4–6 yr	Male	9 mg/kg	124 ± 3.89
					14 mg/kg	117 ± 17.2
				Female	9 mg/kg	102 ± 29.2
					14 mg/kg	102 ± 45.6
Seacat et al. (2002)	Cynomolgus Monkey	Oral	Young-adult to adult	Male	0.15 mg/kg	~200
				Female	0.75 mg/kg	~200
Chang et al. (2012)	Mice, CD-1	Oral	8–10 wk	Male	1 mg/kg, followed for 20 wk	42.81
					20 mg/kg, followed for 20 wk	36.42
				Female	1 mg/kg, followed for 20 wk	37.80
					20 mg/kg, followed for 20 wk	30.45
Benskin et al. (2012)	Rat, Sprague-Dawley	Oral	Adult (429 g)	male	0.4 mg/kg PFOS (0.27 mg/kg n-PFOS)	n-PFOS: 33.7 iso-PFOS: 23.4 5m-PFOS: 24.4 4m-PFOS: 23.1 3m-PFOS: 33.8 1m-PFOS: 102 tb-PFOS: 19.6 B7-PFOS: 15.4 B8-PFOS: 11.3 B9-PFOS11.1
Chang et al. (2012)	Rat, Sprague-Dawley	IV	8–10 wk	Male	2 mg/kg, followed for 24 hr	7.99 ± 4.94
				Female (1 rat)	2 mg/kg, followed for 24 hr	5.62
		Oral	8–10 wk	Male	4.2 mg/kg, followed for 144 hr	8.23 ± 1.53
					2 mg/kg, followed for 10 wk	38.31 ± 2.32
					15 mg/kg, followed for 10 wk	41.19 ± 2.01
				Male (1 rat)	2 mg/kg, followed for 24 hr	3.1
				Female	2 mg/kg, followed for 24 hr	1.94 ± 0.13
					2 mg/kg, followed for 10 wk	62.30 ± 2.09

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex/Half-Life Approach	Dose	Estimated Half Life[a]
					15 mg/kg, followed for 10 wk	71.13 ± 11.25
Huang et al. (2019a)	Rat, Sprague-Dawley	IV	8 wk	Male – overall elimination half-life	2 mg/kg	22.0 ± 2.1
				Male – initial phase	2 mg/kg	4.6 ± 2.7
				Male – terminal phase	2 mg/kg	39.7 ± 4.4
				Female – overall elimination half-life	2 mg/kg	23.0 ± 3.7
				Female – initial phase	2 mg/kg	0.3 ± 0.3
				Female – terminal phase	2 mg/kg	32.8 ± 3.7
		Oral	8 wk	Male – overall elimination half-life	2 mg/kg	19.9 ± 3.8
					2 (×5) mg/kg	19.0 ± 3.2
					20 mg/kg	14.5 ± 2.1
				Male – initial phase	2 mg/kg	3.1 ± 2.4
					2 (×5) mg/kg	0.3 ± 0.1
					20 mg/kg	4.0 ± 2.9
				Male – terminal phase	2 mg/kg	40.5 ± 5.5
					2 (×5) mg/kg	33.4 ± 4.2
					20 mg/kg	35.8 ± 4.2
				Female – overall elimination half-life	2 mg/kg	28.4 ± 11.0
					2 (×5) mg/kg	21.1 ± 4.3
					20 mg/kg	18.0 ± 3.1
				Female – initial phase	2 mg/kg	0.8 ± 2.1
					2 (×5) mg/kg	0.3 ± 0.2
					20 mg/kg	2.2 ± 3.0
				Female – terminal phase	2 mg/kg	40.7 ± 3.5
					2 (×5) mg/kg	40.0 ± 2.5
					20 mg/kg	36.0 ± 4.0
Kim et al. (2016b)	Rat, Sprague-Dawley	IV	8–12 wk	Male	2 mg/kg	28.70 ± 1.85
				Female	2 mg/kg	24.80 ± 1.52

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex/Half-Life Approach	Dose	Estimated Half Life[a]
		Oral	8–12 wk	Male	2 mg/kg	26.44 ± 2.77
				Female	2 mg/kg	23.50 ± 1.75

Notes: d = days; IV = intravenous; NR = not reported; wk = weeks; yr = years.
[a] Data reported in mean days ± standard deviation.

Appendix C. Nonpriority Health Systems Evidence Synthesis and Integration

C.1 Reproductive

The U.S. Environmental Protection Agency (EPA) identified 60 epidemiological and 22 animal studies that investigated the association between perfluorooctane sulfonic acid (PFOS) and reproductive effects. Of the epidemiological studies addressing male reproductive endpoints, 2 were classified as *high* confidence, 15 as *medium* confidence, 6 as *low* confidence, and 1 was considered *uninformative* (Section C.1.1). Of the epidemiological studies addressing female reproductive endpoints, 5 were classified as *high* confidence, 24 as *medium* confidence, 17 as *low* confidence, and 2 were considered *uninformative* (Section C.1.1). Of the animal studies, 2 were classified as *high* confidence, 15 as *medium* confidence, 4 as *low* confidence, and 1 was considered *mixed* (*medium/low*) (Section C.1.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.1.1 Human Evidence Study Quality Evaluation and Synthesis

C.1.1.1 Male

C.1.1.1.1 Introduction

The 2016 Health Advisory (U.S. EPA, 2016b) and Health Effects Support Document (HESD) (U.S. EPA, 2016c) reports identified limited evidence of effects of PFOS on reproductive effects in men and boys. Analyses of male children in the C8 Health Project (Lopez-Espinosa et al., 2011) suggested an association between increasing PFOS exposure and delayed onset of puberty, defined by measured testosterone levels (>50 ng/dL testosterone and >5 pg/mL free testosterone). The effects of PFOS on semen quality parameters were mixed. In healthy, young Danish males Joensen et al. (2014) observed significantly inverse associations with testosterone, calculated free testosterone, free androgen index (FAI), and ratios of testosterone/luteinizing hormone (LH), free testosterone/LH, and FAI/LH. Significant associations for semen quality parameters were not observed among these young men. Regarding other studies examining semen quality parameters, three studies (Buck Louis et al., 2015; Toft et al., 2012; Joensen et al., 2009) out of nine observed associations with morphologically abnormal sperm. In a cross-sectional sample of military recruits (n = 105), Joensen et al. (2009) observed significantly lower sperm counts in men with higher combined perfluorooctane sulfonic acid/perfluorooctanoic acid (PFOS/PFOA) exposure. A Texas- and Michigan-based cohort (n = 462), the Longitudinal Investigation of Fertility and the Environment (LIFE) study (Buck Louis et al., 2015), observed limited evidence of the effects of PFOS. Only one significant association was observed for a morphological parameter, namely decreased percentage of sperm with coiled tails.

For this updated review, 23 studies[5] (24 publications) report on the association between PFOS and endocrine effects since the 2016 document. Eleven of the studies were in children and adolescents (Jensen et al., 2020b; Liu et al., 2020b; Di Nisio et al., 2019; Ernst et al., 2019;

[5] Zhou, 2016, 3856472 and Zhou, 2017, 3858488 analyze participants from the same population using the same outcome.

Wang et al., 2019a; Goudarzi et al., 2017a; Lind et al., 2017a; Zhou et al., 2017c; Itoh et al., 2016; Lopez-Espinosa et al., 2016; Zhou et al., 2016), one study was in pregnant women (Anand-Ivell et al., 2018) and the remainder of the publications were in the general population. Different study designs were utilized, including four cohort studies (Jensen et al., 2020b; Ernst et al., 2019; Goudarzi et al., 2017a; Itoh et al., 2016), one case-control study (Anand-Ivell et al., 2018) with the remainder of the studies following a cross-sectional design. All observational studies measured PFOS in blood components (i.e., blood, plasma, or serum), however, PFOS was additionally measured in semen for four studies (Cui et al., 2020; Di Nisio et al., 2019; Pan et al., 2019; Song et al., 2018) and amniotic fluid in one study (Anand-Ivell et al., 2018). The studies were conducted in different study populations including populations from Australia, China, Denmark, the Faroe Islands, Greenland, Italy, Japan, the Netherlands, Poland, Taiwan, Ukraine, and the United States. There were several pairs of studies investigating the same population, including the Biopersistent Organochlorines in Diet and Human Fertility (INUENDO) cohort (Leter et al., 2014; Kvist et al., 2012), the Odense Child Cohort (OCC) (Jensen et al., 2020b; Lind et al., 2017a), the Genetic and Biomarkers study for Childhood Asthma (GBCA) (Zhou et al., 2017c; Zhou et al., 2016), and a cross-sectional sample of men from an infertility clinic in Nanjing, China (Cui et al., 2020; Pan et al., 2019). Two studies assessed populations from related cohorts belonging to the Hokkaido Study on the Environment and Children's Health (Goudarzi et al., 2017a; Itoh et al., 2016).

C.1.1.1.2 Study Quality

There are 24 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and male reproductive effects. Study quality evaluations for these 24 publications are shown in Figure C-1.

Of the 24 studies identified since the 2016 assessment, two studies were classified as *high* confidence, 15 studies as *medium* confidence, six studies as *low* confidence, and one study (Song et al., 2018) was determined to be *uninformative*. Anand-Ivell, 2018, 4728675 was considered *low* confidence for cryptorchidism and *uninformative* for amniotic fluid hormones. Publications from the GBCA (Zhou et al., 2017c; Zhou et al., 2016) were rated *low* confidence because of concerns of selection bias and confounding. Cases and controls in Zhou, 2017, 3858488 were drawn from separate sources resulting in some concern for selection bias by recruiting individuals from different catchment areas. One *low* confidence study (Di Nisio et al., 2019) adjusted results only for age, resulting in concerns about potential for residual confounding by socioeconomic status (SES). One National Health and Nutrition Examination Survey (NHANES) study (Lewis et al., 2015) did not adjust for the participant sampling design in the analysis which contributed to a *low* confidence rating. Song, 2018, 4220306 only reported bivariate correlations between exposure levels and semen parameters with no accounting for potential confounders which contributed to the study being classified as *uninformative*.

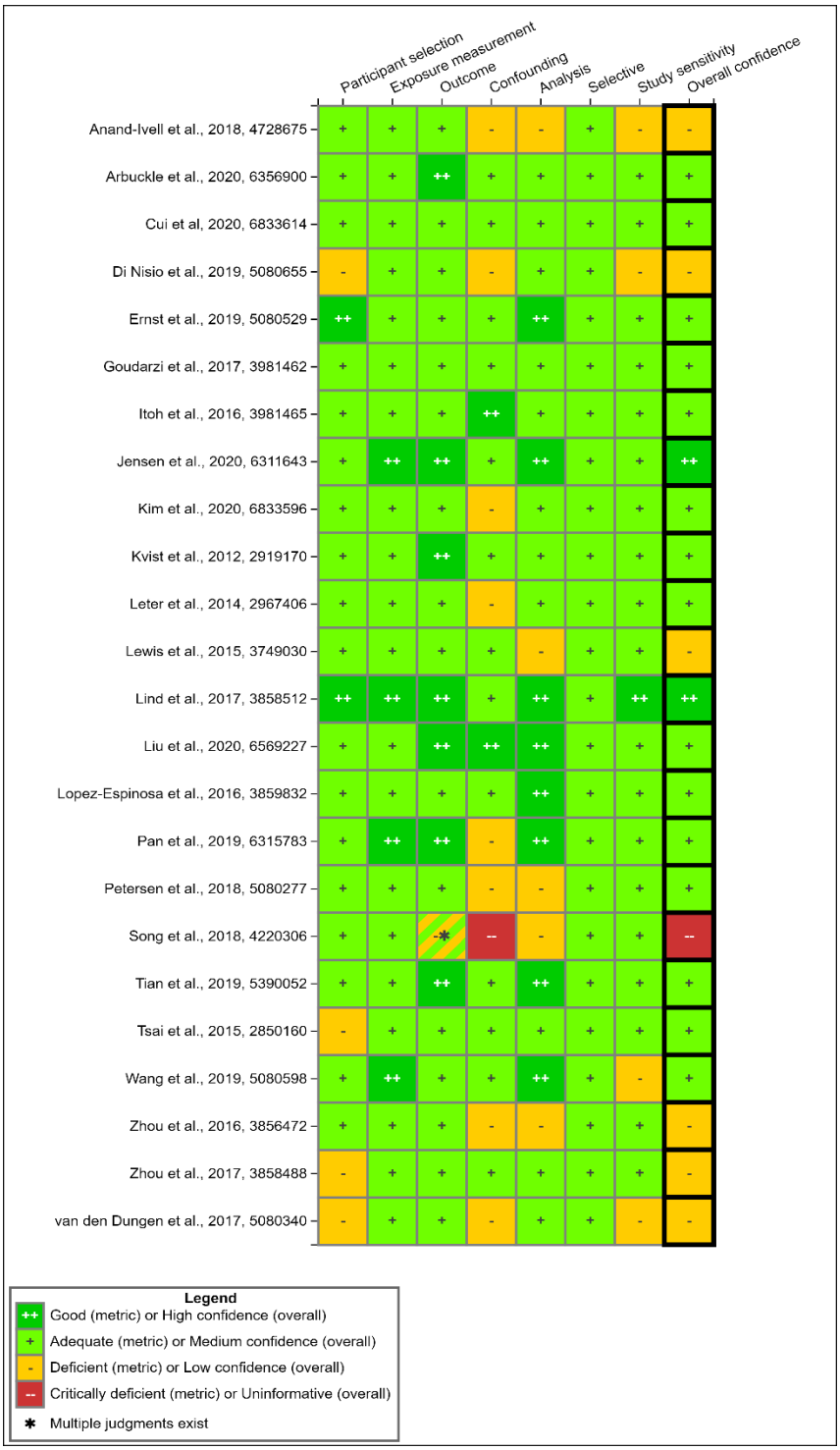


Figure C-1. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Male Reproductive Effects

Interactive figure and additional study details available on HAWC.

C.1.1.1.3 Findings From Children and Adolescents

Sex hormone levels and related steroid hormone levels were examined in nine studies (Jensen et al., 2020b; Liu et al., 2020b; Di Nisio et al., 2019; Wang et al., 2019a; Goudarzi et al., 2017a; Zhou et al., 2017c; Itoh et al., 2016; Lopez-Espinosa et al., 2016; Zhou et al., 2016) and three observed significant effects (Appendix D). A *high* confidence prospective study on the Odense cohort (Jensen et al., 2020b; Lind et al., 2017a) did not find evidence of effects on steroid hormones in the sex hormone metabolic pathway (e.g., dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17-OHP)) in four-month-old male infants. Similarly, a prospective cohort study (Goudarzi et al., 2017a) in boys from the Hokkaido Study on the Environment and Children's Health reported no significant results with steroid hormones in cord blood. However, a *medium* confidence study (Itoh et al., 2016) from a related cohort within the Hokkaido Study observed a significant positive association ($p = 0.033$) for estradiol (E2). Increases in E2 potentially contributed to a significant decrease ($p = 0.002$) in the testosterone-E2 ratio in male infants. Inverse associations were also observed for progesterone ($p = 0.043$) and inhibin B ($p < 0.001$), and quartile analyses supported significant trends for E2 (p-trend = 0.027), T/E2 (p-trend = 0.015), and inhibin B (p-trend < 0.001) but did not support a significant trend for progesterone (p-trend = 0.231). A *medium* confidence cross-sectional study (Lopez-Espinosa et al., 2016) observed inverse associations for E2 and total testosterone in children 6–9 years of age. Analyses by quartile of exposure supported this trend for decreasing testosterone. A cross-sectional analysis in a *medium* confidence study (Wang et al., 2019a) from China observed a positive association ($p < 0.001$) for estriol (E3) in cord blood but did not find an association for E2.

Decreases in testosterone were seen in *low* confidence cross-sectional analyses (Zhou et al., 2017c; Zhou et al., 2016) in children and adolescents (10–15 years of age) from the GBCA in Taiwan. In boys, testosterone was observed to have a significant inverse association, and a decreasing trend. No effects on E2 in boys were observed. A follow-up study (Zhou et al., 2017c) observed significant decreases in testosterone among children with asthma but not in children without asthma. Sex-stratified analyses for reproductive hormones were not conducted in this study.

A cross-sectional study (Di Nisio et al., 2019) in Italian high school students examined associations between PFOS levels and possible risk factors for diseases of the male reproductive system and observed significantly higher serum PFOS levels and testosterone ($p < 0.001$) in exposed individuals compared with unexposed controls.

Pubertal development and semen parameters were examined in two studies (Di Nisio et al., 2019; Ernst et al., 2019) and effects were seen in one (Appendix D). One *medium* confidence study (Ernst et al., 2019) observed no associations between prenatal PFOS exposure from first-trimester maternal serum samples and pubertal stages (i.e., Tanner stages) and pubertal landmarks (e.g., acne, voice break, or first ejaculation). Comparisons of semen analysis in Italian high school students (Di Nisio et al., 2019) observed a reduced number of sperm with normal morphology ($p < 0.001$) and a slight increase in semen pH ($p = 0.005$).

Anthropometric measurements of male reproductive organs were examined in four studies (Arbuckle et al., 2020; Di Nisio et al., 2019; Tian et al., 2019b; Lind et al., 2017a) and three observed effects (Appendix D). A *high* confidence Danish study (Lind et al., 2017a) in children

from the Odense cohort observed a significant positive association with anoscrotal distance (AGDas) in the highest prenatal PFOS exposure group. Positive non-significant associations were observed for anopenile distance (ADGap). Children from the Shanghai-Minhang Birth Cohort Study (Tian et al., 2019b) were evaluated at birth, six months, 12 months of age for changes in anogenital distance (AGD). At birth, significant decreases in AGDas ($p = 0.043$) were observed in continuous analyses, and in the highest quartile of exposure. Results were similar at six months of age. In contrast, associations were positive and largely not significant at 12 months of age. However, a significant increase in ADGap was observed among boys in the third quartile of exposure at 12 months. Results from a *medium* confidence study (Arbuckle et al., 2020) in children from the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort were inconsistent regarding the relationship between prenatal PFOS exposure and AGD. Di Nisio et al. (2019) reported smaller AGD in exposed compared with unexposed adolescents ($p = 0.019$). Significant differences ($p < 0.001$) were also observed for penile and testicular measurements among adolescents, including smaller testicular volume, shorter penis length, and smaller penis circumference. A smaller borderline significant pubis-to-floor distance was also observed ($p = 0.064$).

C.1.1.1.4 Findings From the General Adult Population

Serum sex hormones were examined in four studies (Cui et al., 2020; Petersen et al., 2018; Lewis et al., 2015; Tsai et al., 2015) and two observed effects (Appendix D). A *medium* confidence study (Cui et al., 2020) evaluated serum hormone concentrations in men with fecundity issues and men from couples with female factor infertility. Serum and semen PFOS were significantly correlated (Spearman's $r = 0.793$, $p < 0.01$). Total testosterone and sex hormone binding globulin (SHBG) were inversely associated ($p < 0.05$) with serum and semen PFOS. The total testosterone-LH ratio was negatively associated ($p < 0.05$) with semen PFOS, and borderline significant with serum PFOS ($p = 0.058$). Results for total testosterone remained among those 30 years old or younger after stratifying by age but were no longer observed in men over 30 years of age. The pattern was similar for SHBG, but the association with serum PFOS did not reach significance ($p = 0.069$). Analyses by quartile showed agreement with the continuous regression analyses, indicating significant trends for total testosterone and SHBG with serum and semen levels of PFOS. A *medium* confidence cross-sectional study (Petersen et al., 2018) on Faroese men observed a significant increase ($p = 0.04$) in luteinizing hormone with increasing serum PFOS levels.

Semen characteristics and genomic effects in sperm were examined in five studies (Pan et al., 2019; Petersen et al., 2018; Song et al., 2018; Leter et al., 2014; Kvist et al., 2012) and three observed effects (Appendix D). One *medium* confidence study (Kvist et al., 2012) evaluating men from the INUENDO cohort from Greenland, Poland, or Ukraine observed a significant positive association ($p = 0.026$) with the Y:X-chromosome ratio in sperm when pooling data across countries. This association was also observed in trend analyses for the Greenland subset of the cohort but not in other country-specific analyses. Chromosomal changes were further characterized in another INUENDO study (Leter et al., 2014) using a sperm DNA global methylation assay. Methylation of the Satα repeats, a non-transposonic repetitive satellite DNA sequence generally found in or adjacent to every centromere, was significantly increased ($p < 0.05$) in men from Ukraine, but no effect was observed in other INUENDO communities or in the pooled analysis. Another method of analysis of sperm DNA methylation utilized flow-cytometry to measure cell-by-cell methylated cytosines (% 5-mCs) by immunodetection. A

significant inverse relationship was observed among Polish men but was not seen in other populations or the entire cohort. These results indicate hyper- and hypomethylated states, respectively. Differences in results may be related to differences in each method's approach.

A *medium* confidence cross-sectional study (Pan et al., 2019) on a sample of men from Nanjing, China, described above (Cui et al., 2020), investigated the effects of PFOS on semen characteristics. Two separate analyses were conducted, each using either serum or semen as the biomonitoring matrix for PFOS exposure determination. In linear regression analyses using semen PFOS exposure levels, significant positive associations ($p < 0.05$) were observed for the sperm DNA fragmentation index (DFI)—a measure of the percentage of sperm with damaged DNA. Significant inverse associations were observed for progressive motility, and sperm straight-line velocity, suggesting an overall deleterious effect on sperm motility. No significant associations were observed in analyses using serum PFOS levels.

C.1.1.2 Female

C.1.1.2.1 Introduction

Reproductive health outcomes of interest in females vary with biological maturity over the life course and by pregnancy status. Of interest across the life stages, reproductive hormone levels, such as prolactin, follicle stimulating hormone (FSH), LH, testosterone, and E2, are commonly examined as indicators of reproductive health. Additional reproductive health outcomes of interest include timing of pubertal milestones among children and adolescents; fertility indicators, impacts to menstruation, and occurrence of menopause among non-pregnant adult females; and preeclampsia, gestational hypertension, pregnancy loss, and breastfeeding duration among pregnant females.

The 2016 *Health Assessment and Health Effects Support Document for PFOS* (U.S. EPA, 2016c) concluded that there was suggestive evidence of an association with risk of gestational hypertension or preeclampsia (Zhang et al., 2015a; Darrow et al., 2013; Stein et al., 2009). There was generally consistent evidence of associations between serum PFOS and reduced female fertility and fecundity (Bach et al., 2015; Vélez et al., 2015; Jørgensen et al., 2014; Fei et al., 2009). There were concerns over the possibility of reverse causality explaining observed associations between PFOS exposure and various female reproductive outcomes due to menstruation being a route of PFOS excretion (Whitworth et al., 2012b).

There are 48 studies (50 publications) that have investigated relationships between PFOS exposure and female reproductive outcomes since the 2016 document (U.S. EPA, 2016c). Among the 50 publications available for review, there were 20 cohort studies, 17 cross-sectional studies, and 13 case-control studies. 19 studies were conducted in adults, 6 were conducted in children and adolescents, 13 were conducted in both adults and children, and 12 were conducted in pregnant women. Most studies used blood PFOS measures to assess exposure while others used amniotic fluid and follicular fluid.

C.1.1.2.2 Study Quality

There are 48 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and female reproductive effects. Study quality evaluations for these 48 studies are shown in Figure C-2 and Figure C-3.

Among the 48 publications available for review, 5 were classified as *high* confidence, 24 as *medium* confidence, 16 as *low* confidence, and three were considered *uninformative*. Because menstruation is a primary route of PFOS excretion for people who menstruate, reverse causality was a specific concern for cross-sectional studies that measured blood PFOS and certain reproductive hormones with known menstrual fluctuations without reporting sample collection timing (Heffernan et al., 2018; Zhang et al., 2018b). Several *low* confidence studies lacked an appropriate strategy for identifying potential confounders (Mccoy et al., 2017; Zhou et al., 2017a) or failed to adjust for key confounders, such as age and SES (Heffernan et al., 2018; Zhou et al., 2016). *Low* confidence studies had deficiencies in participant selection (Bach et al., 2018; Heffernan et al., 2018; Zhang et al., 2018b), exposure measurement methods (Campbell et al., 2016), reliance on self-reporting for exposure, outcome, or covariate information (Campbell et al., 2016), and small sample size (Heffernan et al., 2018; Mccoy et al., 2017). Maekawa, 2017, 4238291 was considered *uninformative* due to lack of information on participant selection, lack of adjustment in analyses for key confounders. Lee, 2013, 3859850 was also considered *uninformative* due to lack of consideration of key confounders in analyses. Arbuckle, 2013, 2152344 was considered *uninformative* because PFOS was evaluated as the outcome and reproductive measures were considered as predictors.

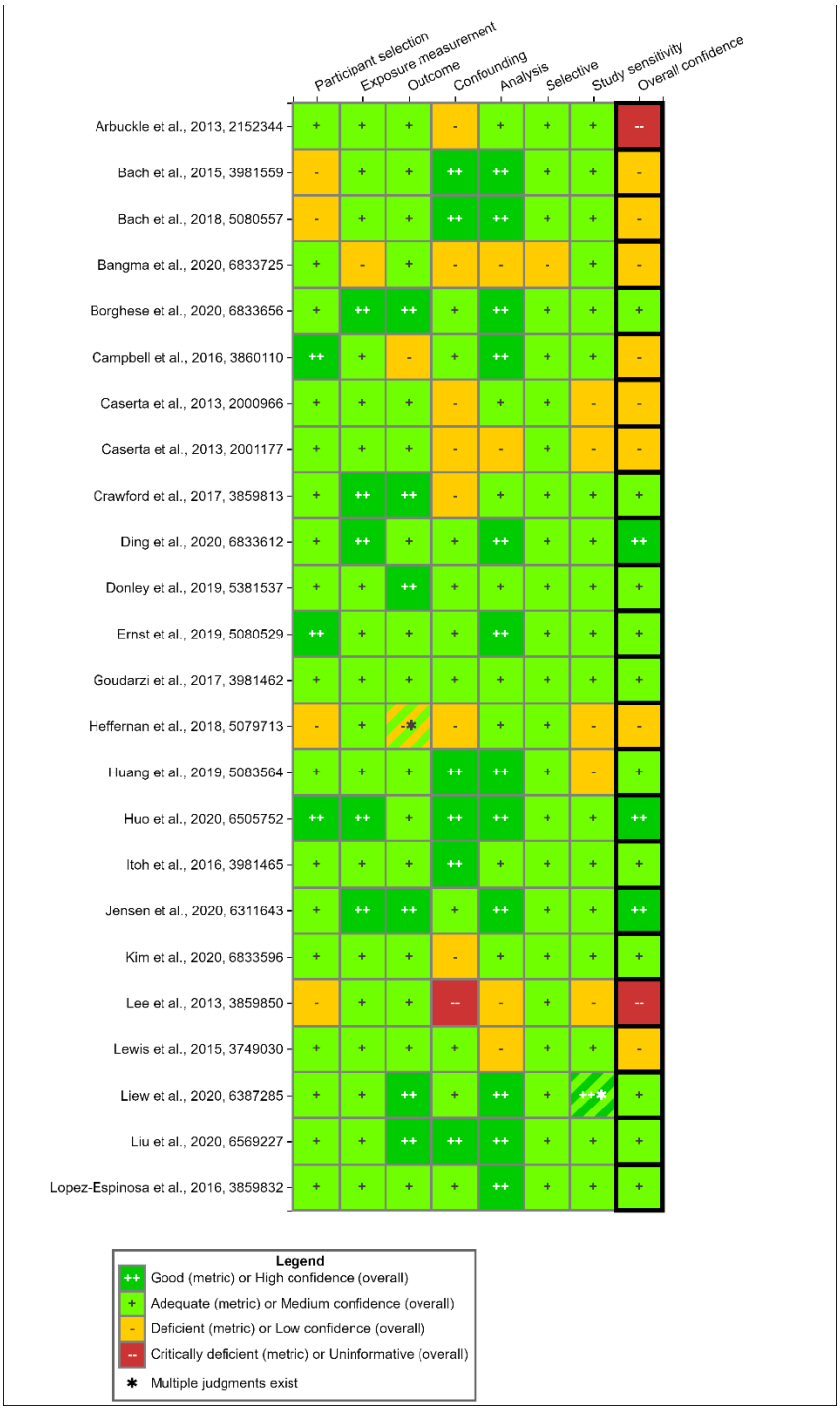


Figure C-2. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Female Reproductive Effects

Interactive figure and additional study details available on HAWC.

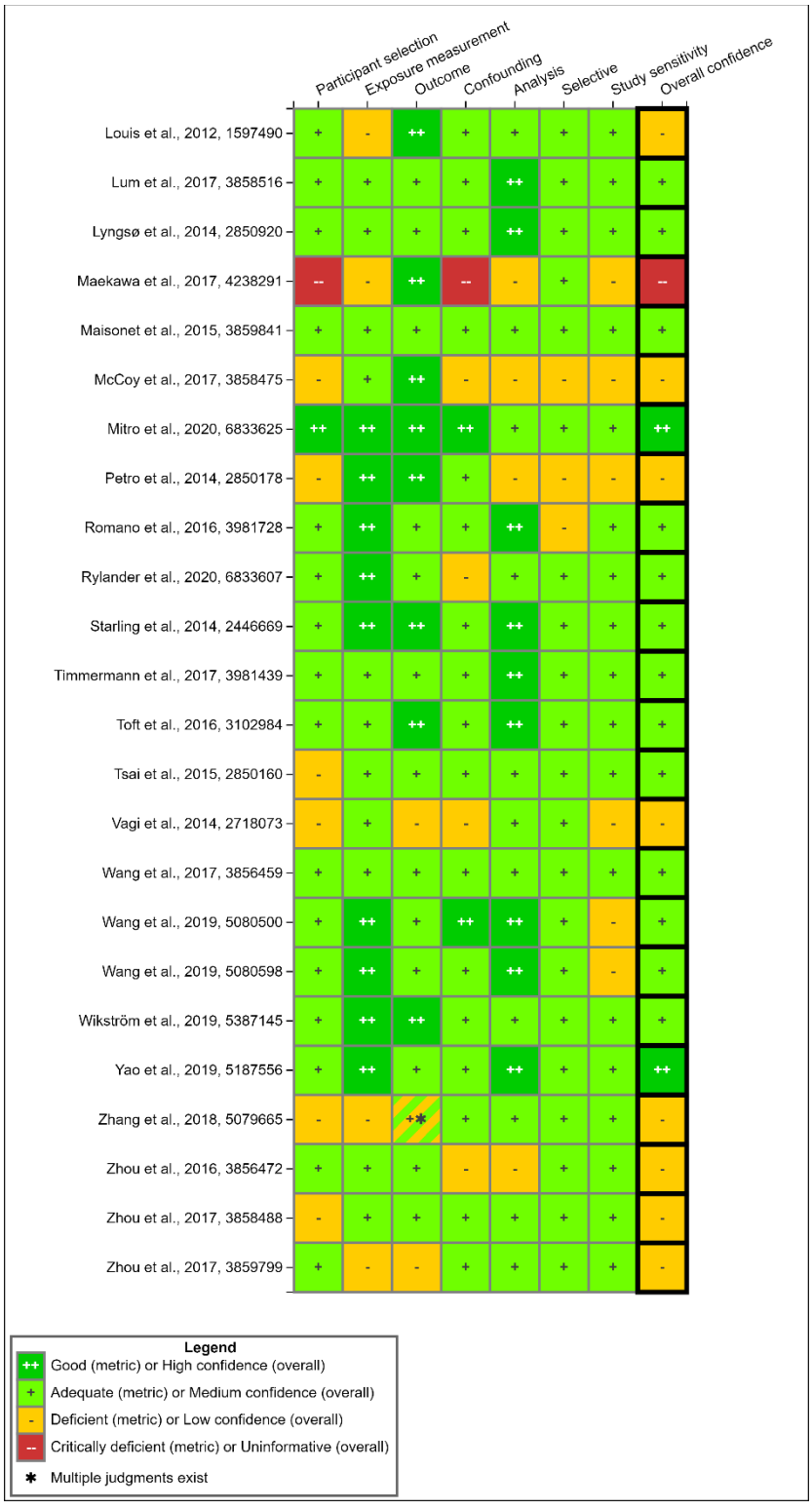


Figure C-3. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Female Reproductive Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.1.1.2.3 Findings From Children and Adolescents

Two *high* confidence, eight *medium* confidence, and three *low* confidence studies assessed relationships between PFOS exposure and female reproductive outcomes in children and adolescents (Appendix D). Studies in infants primarily focused on reproductive hormone levels, while studies in adolescents focused on reproductive hormone levels as well as pubertal milestones.

Two *high* confidence (Jensen et al., 2020b; Yao et al., 2019) and four *medium* confidence studies (Liu et al., 2020b; Wang et al., 2019a; Goudarzi et al., 2017a; Itoh et al., 2016) examined the effects of PFOS exposure on reproductive hormone levels in female infants, reporting mixed results. Itoh, 2016, 3981465, a study of the Hokkaido birth cohort, observed a significant negative association between maternal serum PFOS and progesterone in cord blood (regression coefficient per unit change in PFOS (log10-ng/mL) = −0.6; 95% CI: −0.9, −0.2) as well as prolactin in cord blood (regression coefficient per unit change in PFOS (log10-ng/mL) = −0.5; 95% CI: −0.8, −0.2). A significant positive association was observed between cord blood PFOS and E3 (regression coefficient per unit increase in cord blood PFOS (log10-ng/mL) = 0.5; 95% CI: 0.3, 0.7) in another *medium* confidence study (Wang et al., 2019a). The two *high* confidence studies and four *medium* confidence studies found no significant associations between maternal serum or cord blood PFOS and reproductive hormones such as testosterone, the testosterone-to-estradiol ratio (Yao et al., 2019); E2, testosterone, SHBG, the testosterone-to-SHBG ratio (Itoh et al., 2016); 17-OHP, androstenedione, FSH, LH, DHEA, dehydroepiandrosterone sulfate (DHEAS) (Jensen et al., 2020b); 17-OHP, progesterone (Liu et al., 2020b); androstenedione, DHEA (Goudarzi et al., 2017a); β-E2, and estrone (Wang et al., 2019a).

Three *medium* confidence (Lopez-Espinosa et al., 2016; Maisonet et al., 2015a; Tsai et al., 2015) and three *low* confidence (Zhou et al., 2017c; Zhou et al., 2016; Lewis et al., 2015) studies assessed the relationship between PFOS and reproductive hormone levels in adolescent females. As part of the C8 Health Project, Lopez-Espinosa, 2016, 3859832 observed negative associations for total testosterone across serum PFOS quartiles and per unit increase in serum PFOS among females 6–9 years old with high exposure (percent difference for quartile 2 vs. quartile 1 = −1.1; 95% CI: −8.6, 7.1; percent difference for quartile 3 vs. quartile 1: −7.8%; 95% CI: −15, −0.1; percent difference for quartile 4 vs. quartile 1: −11.1%; 95% CI:−18.2, −3.5; percent difference per unit increase in serum PFOS (ln-ng/mL) = −6.6%; 95% CI: −10.1, −2.8). Maisonet, 2015, 3859841 found significantly increased serum testosterone among 15-year-old females in the highest tertile of maternal serum PFOS during pregnancy (beta: 0.18, 95% CI: 0.01, 0.35). No significant associations were observed for E2 (Zhou et al., 2017c; Lopez-Espinosa et al., 2016; Zhou et al., 2016), testosterone (Zhou et al., 2016; Lewis et al., 2015), SHBG (Maisonet et al., 2015a; Tsai et al., 2015), or FSH (Tsai et al., 2015).

One *medium* confidence study drew data from the Danish National Birth Cohort (DNBC) to examine the effects of prenatal PFOS exposure on pubertal milestones in female adolescents, such as breast development (age at attainment of Tanner stages 2–5), pubic hair development (age at attainment of Tanner stages 2–5), axillary hair development, and age at menarche in adolescent girls (Ernst et al., 2019). Average age at attainment for all pubertal indicators was significantly reduced across PFOS tertiles), while no other significant associations were observed for breast development, age at menarche, axillary hair development, or pubic hair development.

C.1.1.2.4 Findings From Pregnant Women

One *high* confidence, five *medium* confidence studies, and one *low* confidence study examined the relationship between PFOS exposure and preeclampsia (Appendix D). One *medium* confidence study (Wikström et al., 2019) reported significant positive associations between serum PFOS and odds of preeclampsia in both continuous and quartile analyses (OR = 1.53; 95% CI: 1.07, 2.2; OR for PFOS highest vs. lowest quartile = 2.68; 95% CI: 1.17, 6.12). The remaining five studies reported mixed non-significant associations (Borghese et al., 2020; Huo et al., 2020; Rylander et al., 2020; Huang et al., 2019b; Starling et al., 2014a). Huo, 2020, 6505752, a *high* confidence cohort study of 3,220 pregnant women study observed a non-significant reduction in odds of preeclampsia for women above the 80th percentile for plasma PFOS compared with women in or below the 80th percentile and observed a non-significant increase in odds of preeclampsia. In two *medium* confidence cohort studies, non-significant positive associations were observed (Borghese et al., 2020; Starling et al., 2014a). Non-significant negative associations were observed in *medium* confidence case-control (Rylander et al., 2020) and cross-sectional (Huang et al., 2019b) studies. A *low* confidence study found no association between median PFOS levels and hypertensive disorders of pregnancy (Bangma et al., 2020).

One *high* confidence and two *medium* confidence studies examined the relationship between PFOS exposure and gestational hypertension reporting non-significant mixed associations for gestational hypertension and significant positive associations for blood pressure. Huo, 2020, 6505752, a *high* confidence cohort study of 3,220 pregnant women, observed a non-significant negative association between plasma PFOS and odds of gestational hypertension. Borghese, 2020, 6833656, a *medium* confidence prospective cohort study, followed 1,708 women from early pregnancy to delivery for gestational hypertension, preeclampsia, and changes in blood pressure, measuring plasma PFOS once per trimester and again at delivery. Borghese, 2020, 6833656 observed a non-significant positive association between plasma PFOS and odds of gestational hypertension. A significant positive association was reported for systolic blood pressure (SBP) mmHg) per $\log_2$-µg/L increase PFOS at delivery (beta: 1.19, 95% CI: 0.28, 2.1). Significant positive associations were also observed in each trimester for diastolic blood pressure (DBP) (mmHg) (beta for trimester 3: 0.66, 95 % CI 0.18, 1.14) but not at delivery. No association between plasma PFOS levels and gestational hypertension was observed by Huang, 2019, 5083564.

Two *medium* confidence studies (Liew et al., 2020; Buck Louis et al., 2016) and one *low* confidence study (Jensen et al., 2015) investigated the effect of PFOS exposure on pregnancy loss and reported non-significant mixed results. In a cohort study of 501 couples, Louis, 2016, 3858527 reported a non-significant, negative association between serum PFOS levels and pregnancy loss during the first seven weeks of pregnancy. A case-control study nested within the DNBC comparing 222 pregnancies ending in miscarriage to 218 pregnancies resulting in live births observed non-significant positive associations across maternal plasma PFOS levels for odds of miscarriage in both continuous and quartile analyses. Jensen, 2015, 2850253 also reported non-significant positive associations for odds of miscarriage in both continuous and tertile analysis.

Two *medium* confidence studies assessed the relationship between serum PFOS levels in pregnancy and breastfeeding duration, with both reporting significant, inverse associations between the two (Timmermann et al., 2017b; Romano et al., 2016). Using data from two Faroese

birth cohorts (n = 1,130), Timmermann, 2017, 3981439 observed a significant reduction in total breastfeeding duration per doubling of maternal serum PFOS (regression coefficient per doubling of serum PFOS (ng/mL) = −1.4; 95% CI: −2.1, −0.6) and a non-significant reduction in exclusive breastfeeding duration per doubling of maternal serum PFOS (regression coefficient per doubling of serum PFOS (ng/mL) = −0.3; 95% CI: −0.6, 0.1). These observations were supported by a prospective birth cohort study of 336 women investigating the relationship between serum PFOS levels during pregnancy and relative risk of breastfeeding termination at three and six months postpartum (Romano et al., 2016). This study observed a positive trend for relative risk of breastfeeding termination across maternal serum PFOS quartiles for both time points. Relative risk for stopping breastfeeding by 3 months increased in maternal serum PFOS quartiles 2, 3, and 4 compared with quartile 1, with a significant increase observed for quartile 3 (relative risk for PFOS quartile 2 vs. 1 = 1.32; 95% CI: 0.97, 1.79; relative risk for PFOS quartile 3 vs. quartile 1 = 1.39; 95% CI: 1.04, 1.88; relative risk for PFOS quartile 4 vs. quartile 1 = 1.08; 95% CI: 0.79, 1.46). Relative risk for stopping breastfeeding by 6 months was non-significantly increased in maternal serum PFOS quartiles 2, 3, and 4 compared with quartile 1 as well.

One *high* confidence study and one *medium* confidence study examined relationships between PFOS exposure and female reproductive hormone levels in pregnant women. In a *medium* confidence case-control study of 545 mother-infant pairs, Toft, 2016, 3102984 observed a significant, positive association between PFOS in amniotic fluid and 17-OHP, with a significant percent difference in the continuous analysis and a significant increase for tertile 3 compared with tertile 1 (percent difference in median 17-OHP level per unit increase in amniotic fluid PFOS (ln-ng/mL) = 0.15; 95% CI: 0.11, 0.2; percent difference in median 17-OHP for women in amniotic fluid PFOS tertile 3 vs. tertile 1 = 18%; 95% CI: 11, 26). A significant, positive association was also observed between amniotic fluid PFOS and androstenedione in the continuous analysis and for tertile 3 compared with tertile 1 (percent difference in median androstenedione level per unit increase in amniotic fluid PFOS (ln-ng/mL) = 0.15; 95% CI: 0.1, 0.21; percent difference in median androstenedione for women in amniotic fluid PFOS tertile 3 vs. tertile 1 = 17; 95% CI: 8, 25). Significant, positive associations across tertiles of PFOS were observed for progesterone (percent difference per 1% increase in PFOS (ln-ng/mL) = 0.21; 95% CI: 0.14, 0.29; percent difference for PFOS tertile 2 vs. 1 = 11%; 95% CI: 0, 23; percent difference for PFOS tertile 3 vs. 1 = 22; 95% CI: 11, 34) and testosterone (percent difference per 1% increase in PFOS (ln-ng/mL) = 0.16; 95% CI: 0.09, 0.23; percent difference for PFOS tertile 2 vs. tertile 1 = 9%; 95% CI: −2, 20; percent difference for PFOS tertile 3 vs. tertile 1 = 18%; 95% CI: 7, 29), but no association was observed for DHEAS. In a *high* confidence study, Mitro, 2020, 6833625, no significant association was observed between plasma PFOS during pregnancy and SHBG levels 3 years postpartum.

One *medium* confidence study (Lyngsø et al., 2014) examined the effects of serum PFOS levels on pre-pregnancy menstruation. While evidence of increased odds of menstrual cycle irregularity was reported, the association was not significant.

C.1.1.2.5 Findings From the General Adult Population

Five *medium* confidence (Kim et al., 2020b; Donley et al., 2019; Crawford et al., 2017; Lum et al., 2017; Wang et al., 2017), three *low* confidence studies (Bach et al., 2018; Zhang et al., 2018b; Mccoy et al., 2017) and one *uninformative* study (Arbuckle et al., 2013) examined implications of PFOS exposure on female fertility, reporting mixed results (Appendix D).

Significant positive associations were reported in *low* confidence studies, including for odds of premature ovarian insufficiency (POI) across plasma PFOS quartiles (Zhang et al., 2018b) and for the fecundity ratio for parous women in plasma PFOS quartiles (Bach et al., 2018). Non-significant positive associations were observed for day-specific probability of pregnancy (Lum et al., 2017) and cycle and day-specific time to pregnancy (Crawford et al., 2017). Associations with indicators of ovarian function were largely non-significant, including no association observed between serum PFOS and anti-Mullerian hormone (AMH) (Crawford et al., 2017). Associations between maternal serum PFOS during pregnancy and female adolescent AMH levels were also not observed (Donley et al., 2019). No significant associations were reported for infertility measures including endometriosis-related infertility (Wang et al., 2017), and fertilization rate (Kim et al., 2020b). Additionally, McCoy, 2017, 3858475 reported non-significant negative correlations between PFOS in follicular fluid and blast conversion rate, fertilization rate, and follicle count. No associations were observed for other outcomes related to menstrual cycles and gynecologic pathologies, including menstrual cycle length (Lum et al., 2017), endometriosis, polycystic ovary syndrome (PCOS), genital tract infections, and idiopathic infertility (Kim et al., 2020b).

One *high* confidence study examined the relationship between PFOS exposure and age at natural menopause: the Study of Women's Health Across the Nation (SWAN), a prospective cohort of 1,120 premenopausal women aged 45–56 (Ding et al., 2020). Significant, positive associations were reported between serum Sm-PFOS and risk of natural menopause for women in Sm-PFOS tertile 3 versus tertile 1 (HR = 1.27; 95% CI: 1.01, 1.59) and between serum n-PFOS and risk of natural menopause for women in n-PFOS tertile 3 versus tertile 1 (HR = 1.26; 95% CI: 1.02, 1.57). Non-significant positive associations were observed for both Sm-PFOS and n-PFOS when analyzed as a continuous variable and for women in tertile 2 versus tertile 1.

One *medium* confidence (Tsai et al., 2015) and five *low* confidence studies (Heffernan et al., 2018; Zhang et al., 2018b; Mccoy et al., 2017; Lewis et al., 2015; Petro et al., 2014) reported associations between PFOS and female reproductive hormone levels in non-pregnant adult women. Three *low* confidence studies reported significant mixed effects. In women with and without PCOS, Heffernan, 2018, 5079713 observed significant negative associations with FAI only in controls. McCoy, 2017, 3858475 observed a negative correlation with plasma E2. In women with and without POI, Zhang, 2018, 5079665 observed significant negative associations for E2 in both cases and controls and positive associations for FSH and prolactin in cases only. No significant associations were observed for testosterone (Lewis et al., 2015); mean FSH and SHBG in young women (ages 12–30 years) (Tsai et al., 2015); testosterone, E2, and SHBG (Heffernan et al., 2018); E2 (Petro et al., 2014); or for LH and testosterone (Zhang et al., 2018b).

C.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and 16 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and reproductive effects. Study quality evaluations for these 22 studies are shown in Figure C-4.

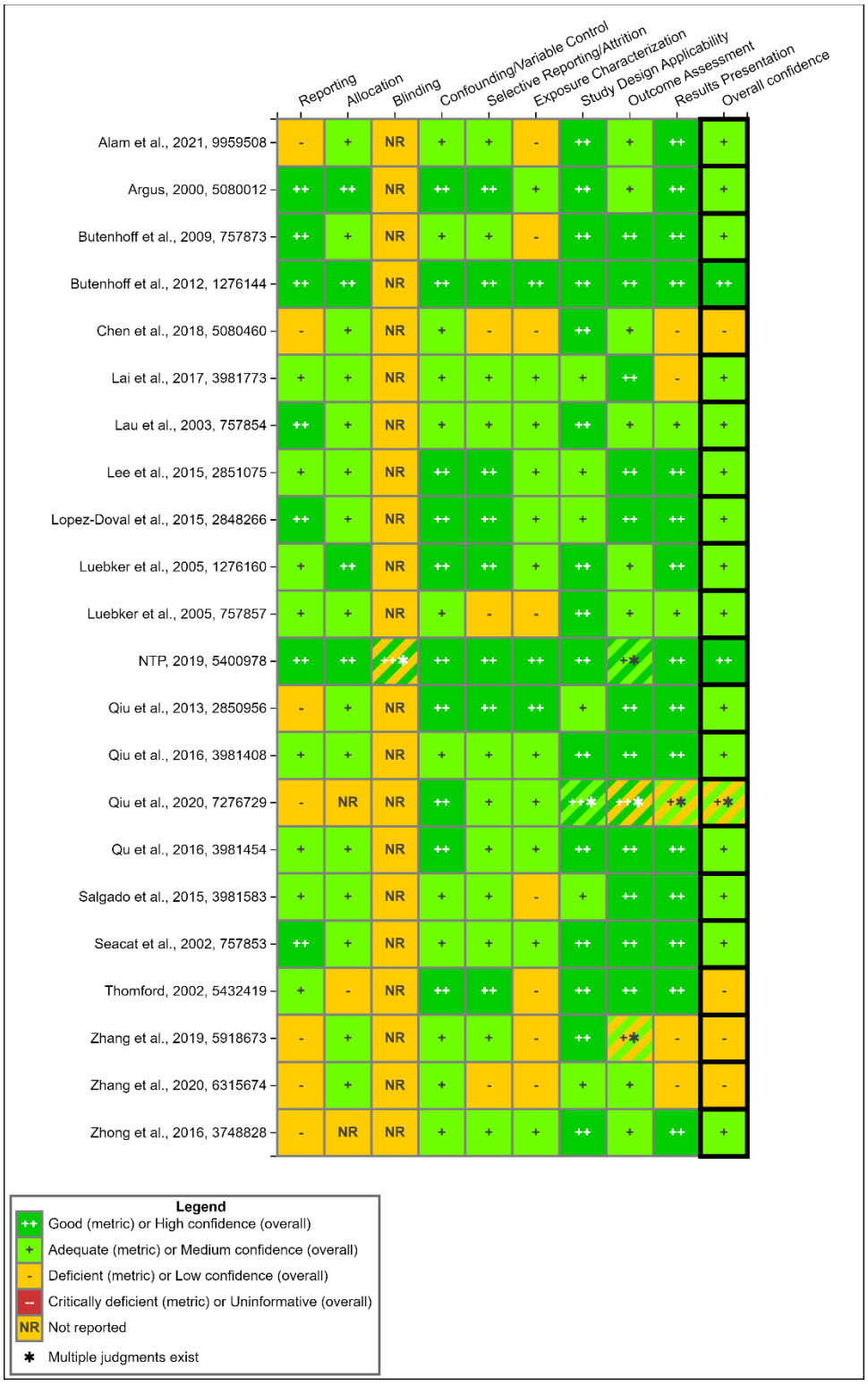


Figure C-4. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Reproductive Effects

Interactive figure and additional study details available on HAWC.

Short-term, subchronic, chronic, and reproductive/developmental animal studies suggest that oral exposure to PFOS can adversely affect the male and female reproductive systems. However, it is not often clear whether the observed alterations reflect specific toxicity to the reproductive system or if they result from concurrent systemic toxicity (i.e., reductions in body weight). Effects observed in male rodents included alterations to hormone levels (prolactin, luteinizing hormone, FSH, E2, and testosterone), as well as decreased testis weights, and decreased sperm count. In female mice exposed to PFOS, effects on prolactin-family hormones were observed. Although effects were predominately seen in rodent species there were inconsistencies among rats and mice. In cynomolgus monkeys no effects were noted in reproductive organ weights and histopathology, although a decrease in male E2 levels was observed (Seacat et al., 2002).

C.1.2.1 Male and Female Fertility Parameters and Pregnancy Outcomes

Male and female fertility parameters and pregnancy outcomes were evaluated in rodent and rabbit species. Mating and fertility parameters, such as number of pregnancies per number of rats that mated, number of days to inseminate, and number of matings during the first week of cohabitation were unaffected by PFOS doses as high as 3.2 mg/kg/day in a two-generation reproduction study in rats(Butenhoff et al., 2009; Luebker et al., 2005a). Gestation and fertility indices were unaffected in one- and two-generation rat reproduction studies (Luebker et al., 2005b; Luebker et al., 2005a); however, gestation length was significantly decreased in a dose-dependent manner in dams exposed to ≥ 0.8 mg/kg/day in the one-generation study (Luebker et al., 2005b) and in P_0 dams exposed to 3.2 mg/kg/day in the two-generation study(Luebker et al., 2005a) (Figure C-5). Decreases in maternal bodyweight change were noted in both studies (Luebker et al., 2005b; Luebker et al., 2005a)(see Toxicity Assessment, (U.S. EPA, 2024)). In contrast, Butenhoff et al. (2009) reported no significant differences in gestation length for rats treated with up to 1 mg/kg/day PFOS from GD 0 to PND 20. That study also found no significant differences in the number of litters delivered or live litter size at birth (Butenhoff et al., 2009).

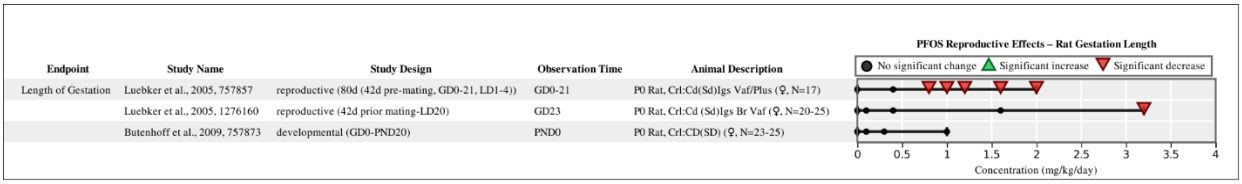


Figure C-5. Gestation Length in Rats Following Exposure to PFOS

LD = lactation day; GD = gestation day; P_0 = parental generation.
Interactive figure and additional study details available on HAWC.

In mice, reproductive outcomes were examined in pregnant CD1 mice treated at 1.5, 3, and 6 mg/kg/day from GD 6–GD 18. Body weight and body weight change were significantly reduced in dams given PFOS at 6 mg/kg/day in comparison to the controls (Fuentes et al., 2006). The number of live and dead fetuses per litter and number of implantation sites were not statistically significant even though high fetal mortality was observed in dams exposed to PFOS at 6 mg/kg. Lastly, there was no observed effect on gravid uterine weight in pregnant CD1 mice on GD 18.

In a single study in New Zealand white rabbits, dams were administered 0 mg/kg/day, 0.1 mg/kg/day, 1.0 mg/kg/day, 2.5 mg/kg/day, or 3.75 mg/kg/day PFOS via intubation from GD 7 to GD 20 (Argus Research Laboratories, 2000). The number of rabbits pregnant at the time of sacrifice (GD 29) decreased with increasing dose due to an increased incidence of abortion with higher PFOS doses (see Toxicity Assessment, (U.S. EPA, 2024)). Only 12/21 (57%) of dams that became pregnant in the study from the 3.75 mg/kg/day dose group were pregnant on GD 29 compared with 100% pregnancy maintained in the 0 mg/kg/day, 0.1 mg/kg/day, and 1.0 mg/kg/day groups and 94% pregnancy maintained in the 2.5 mg/kg/day group. Each individual doe that aborted exhibited weight loss and severely reduced feed consumption. Overall, maternal body weight gains were significantly reduced in the 1.0 mg/kg/day, 2.5 mg/kg/day, and 3.75 mg/kg/day groups (Argus Research Laboratories, 2000).

C.1.2.2 Male Sperm Parameters

Sperm parameters were evaluated in studies of male rats and mice, with conflicting results (Figure C-6). In a 28-day study conducted by NTP in which Sprague-Dawley rats, exposed to PFOS for 28 days had no effect on spermatid headcount in the testis, sperm count in the epididymis and cauda epididymis, or epididymal sperm motility in animals treated with 1.25 mg/kg/day to 5.0 mg/kg/day (NTP, 2019). In contrast, a general reduction in epidydimal sperm count was observed in mice among studies of varying durations including two 4-week studies in ICR mice exposed to 2.5 mg/kg/day or 5 mg/kg/day, a 4-week study in ICR mice exposed to 5 mg/kg/day and 10 mg/kg, a 5-week study in C57 mice exposed to 10 mg/kg/day, and CD-1 pups on PND 63 exposed to 3 mg/kg/day during gestation (Qiu et al., 2020; Lai et al., 2017a; Qiu et al., 2016; Qu et al., 2016; Qiu et al., 2013). Qiu et al. (2016) did not observe alterations in epididymis weight that may have influenced epididymal sperm counts.

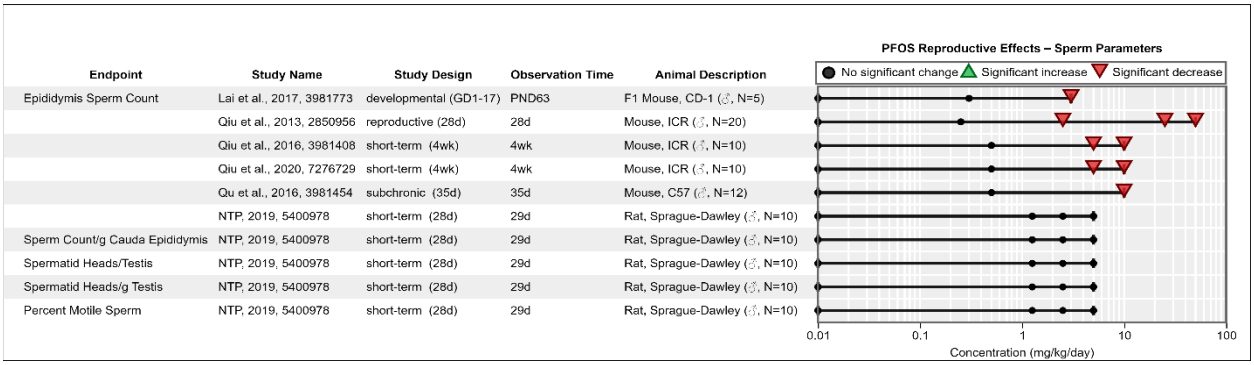


Figure C-6. Sperm Parameters in Male Rodents Following Exposure to PFOS

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; F_1 = first generation; d = day; wk = week.

C.1.2.3 Reproductive Hormones

C.1.2.3.1 Males

Alterations in testosterone levels in males were inconsistent across studies and species (Figure C-7). Lopez-Doval et al. (2015; 2014) observed decreases of 40, 39, 32, and 37% at 0.5 mg/kg/day, 1 mg/kg/day, 3 mg/kg/day, and 5 mg/kg/day, respectively, in male rats treated by gavage for 28 days. Conversely, in a subchronic study, Alam et al. (2021) observed significantly

increased serum testosterone and progesterone levels in comparison to the controls at 0.015 and 0.15 mg/kg via oral gavage for 60 days in Wistar rats. However, in a 28-day study conducted by NTP (2019), no effects on testosterone levels were noted in male rats treated with up to 5 mg/kg/day. A 46% decrease relative to controls was also noted in mice treated with 10 mg/kg/day for five weeks (Qu et al., 2016). Developmental studies in mice showed a 31% decrease in testosterone at PND 63 in CD-1 mice exposed to 3 mg/kg/day throughout gestation (Lai et al., 2017a). C57BL/6 mouse pups treated with 1 and 5 mg/kg/day showed 35% and 52% decreases, respectively, at postnatal week 4 (PNW 4) after maternal oral exposure from GD 1 to GD 17 (significantly different in the 5 mg/kg/day group) (Zhong et al., 2016). In the same study, 38% and 34% decreases were observed in the 1 and 5 mg/kg/day groups, respectively, at PNW 8, though only the response in the 1 mg/kg/day group was statistically different from controls. Similarly, Qiu et al. (2020) observed a significant decrease in serum testosterone levels at 5, and 10 mg/kg/day in comparison to the controls for four weeks in ICR mice. Cynomolgus monkeys treated up to 0.75 mg/kg/day for 182 days showed no statistically significant effects on testosterone levels (Seacat et al., 2002).

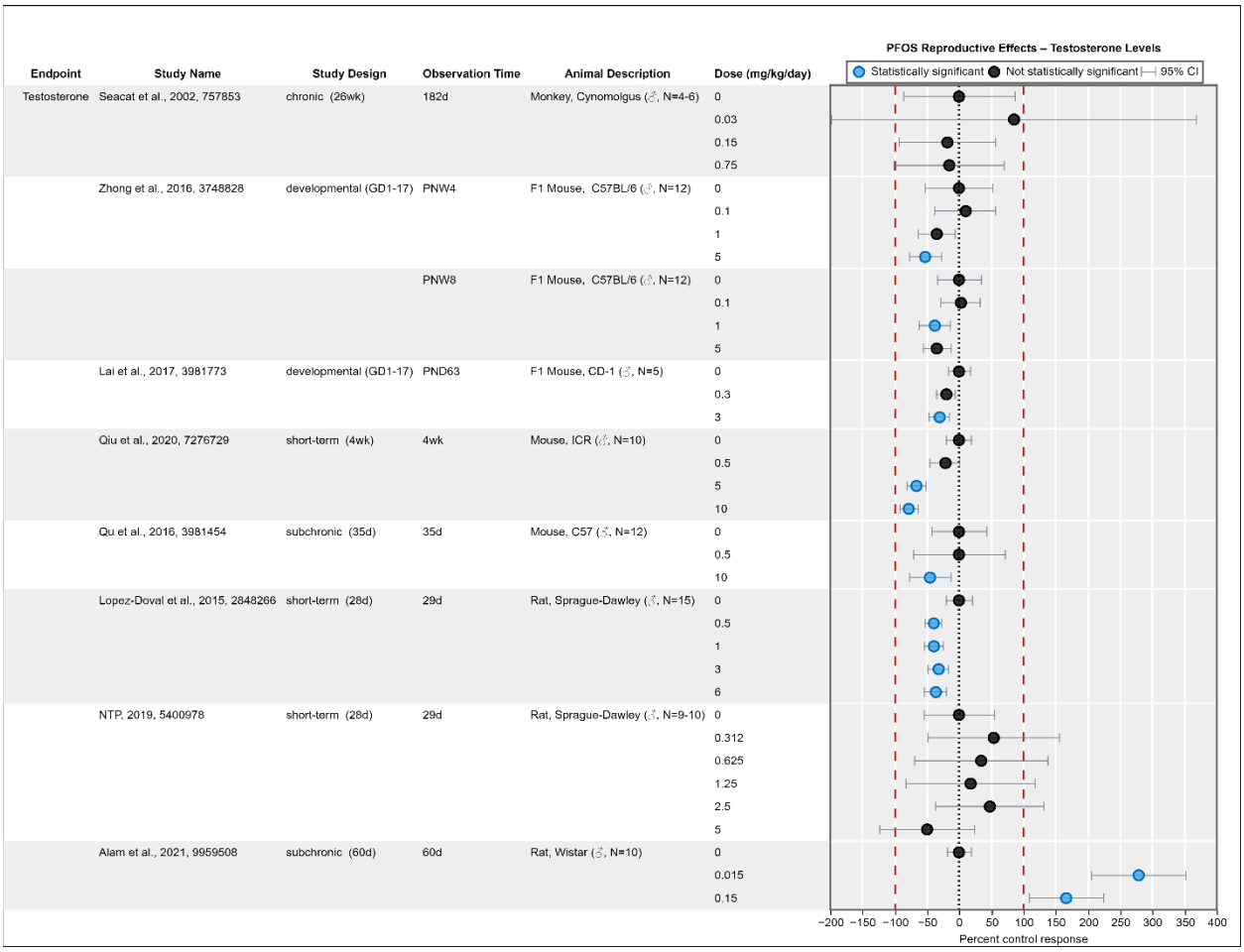


Figure C-7 Percent Change in Testosterone Levels Relative to Controls in Male Rodents and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
The red dashed lines indicate a 100% increase or 100% decrease from the control response.

GD = gestation day; PND = postnatal day; PNW = postnatal week; F_1 = first generation

Changes in E2 levels in males were noted in rats, mice, and cynomolgus monkeys across studies of varying durations (Figure C-8); however, the direction of the change was not consistent across the studies. In two studies from the same laboratory, following a 28-day exposure, Salgado et al. (2015) and Lopez-Doval et al. (2015) noted decreases in E2 ranging from 13% to 19% in rats treated with 3.0 and 6.0 mg/kg/day and ≥ 1.0 mg/kg/day, respectively. Decreases were similar across dose groups and were not dose dependent. In mice, subchronic exposure to PFOS (35 days) at doses of 0.5 mg/kg/day and 10 mg/kg/day showed no statistically significant effect on E2 levels, but there was a general increasing trend with increasing dose (5% and 10% increase, respectively) (Qu et al., 2016). Male mouse pups exposed to 5.0 mg/kg/day from GD 1 to GD 17 exhibited a 42% increase in serum E2 levels at PNW 4 (Zhong et al., 2016). By PNW 8 the increase was no longer statistically significant but remained 28% higher than the control group (Zhong et al., 2016). There was an apparent dose-dependent increase in serum E2 at both PNW 4 and PNW 8. Conversely, no significant change or trend in serum E2 levels was observed in adult ICR male mice exposed to 0 mg/kg/day, 0.5 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day for four weeks (Qiu et al., 2020). Seacat et al. (2002) observed a 97% decrease in serum E2 in male cynomolgus monkeys treated at 0.75 mg/kg/day for 182 days (Seacat et al., 2002).

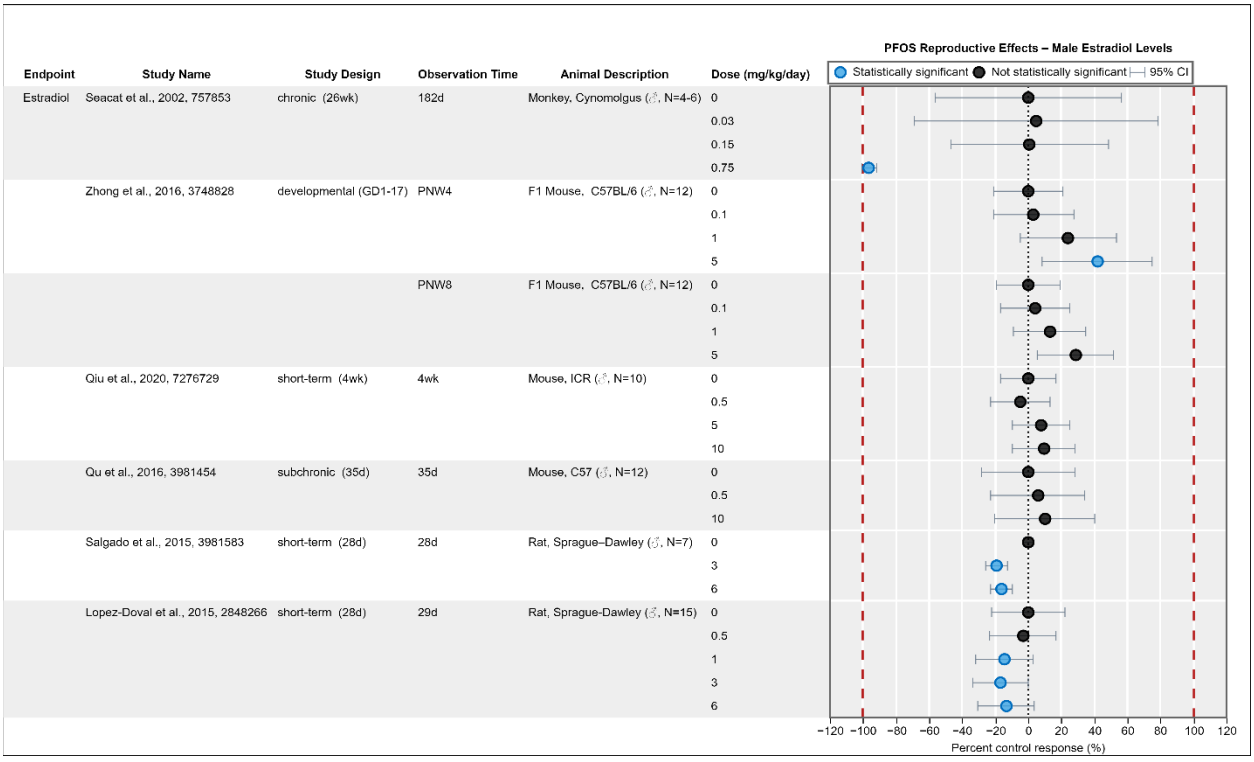


Figure C-8. Percent Change in Estradiol Levels Relative to Controls in Male Rodent and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; PNW = postnatal week; F_1 = first generation

Short-term exposure studies examining the effect of PFOS exposure on LH, FSH, and prolactin levels in male rats were available (Figure C-9). Groups treated for 28 days with

doses ≥ 0.5 mg/kg/day as well as 3.0 mg/kg/day and 6.0 mg/kg/day exhibited decreases in LH (15%–30%) and prolactin (54%–78%), respectively (Lopez-Doval et al., 2015; Salgado et al., 2015; López-Doval et al., 2014). Additionally, increases ranging from 88% to 133% in serum FSH levels were observed in all treated groups (0.5 mg/kg/day–6 mg/kg/day) when compared with controls (López-Doval et al., 2014). However, in a study by Qiu et al. (2020), PFOS exposure did not significantly alter serum FSH and LH levels.

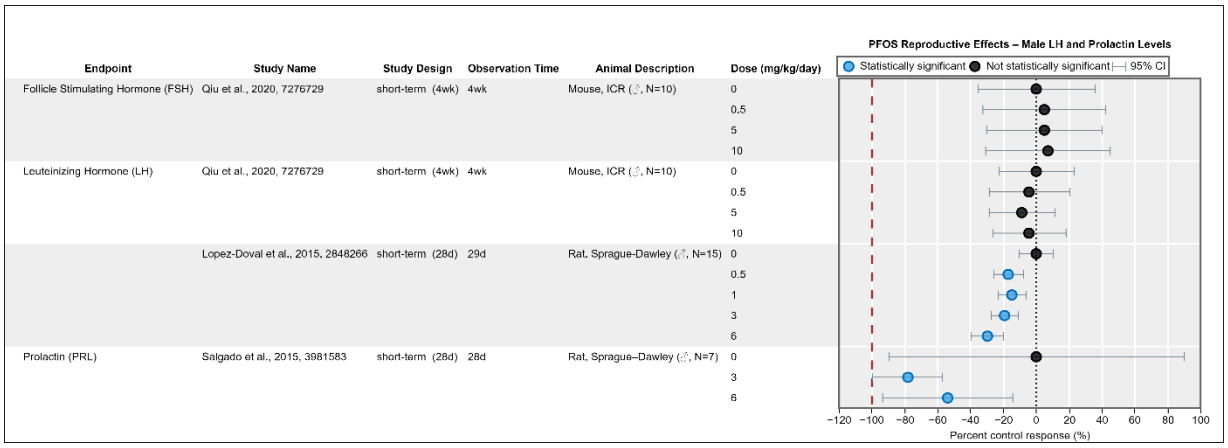


Figure C-9. Percent Change in LH and Prolactin Levels Relative to Controls in Male Rats Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
The red dashed line indicates a 100% decrease from the control response.

C.1.2.3.2 Females

Evidence that oral exposure to PFOS results in changes to levels of prolactin-family hormones in female mice was noted in an investigation by Lee et al. (2015) (Figure C-10). In this study, the authors reported dose-dependent reductions in prolactin-family hormones, including mouse placental lactogen (mPL-II) (46%–71%), mouse prolactin-like protein (mPLP)-Cα (20%–53%), and mPLP-K (30%–57%), in pregnant CD-1 mice exposed to 0.5 mg/kg/day, 2.0 mg/kg/day, and 8 mg/kg/day PFOS from GD 11 to GD 16. Concurrent dose-dependent decreases in bodyweight of 2%, 6%, and 21%, respectively, were also observed in these mice (Lee et al., 2015).

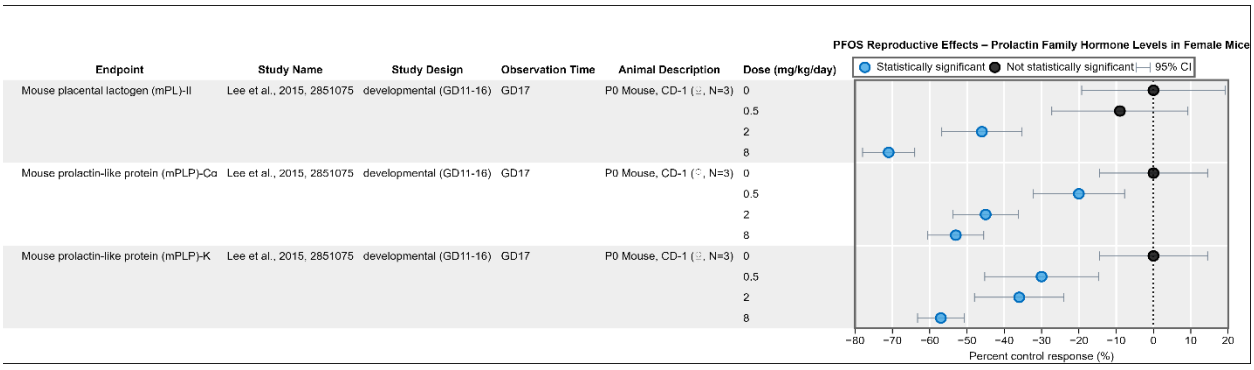


Figure C-10. Percent Change in Prolactin-Family Hormone Levels Relative to Controls in Female Mice Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation.

In female cynomolgus monkeys treated with PFOS for 182 days, E2 levels decreased in a dose-dependent manner relative to controls (decreases of 16%, 52%, and 73% in the 0.03 mg/kg/day, 0.15 mg/kg/day, and 0.75 mg/kg/day dose groups, respectively) (Seacat et al., 2002) (Figure C-11). In the same study, testosterone levels were not affected in females in a dose-dependent or statistically significant manner, though a decrease of 72% was observed in the 0.15 mg/kg/day dose group (Seacat et al., 2002). In contrast to female monkeys, evaluation of F_1 female mouse pups treated with 0.1 mg/kg/day, 1.0 mg/kg/day, or 5.0 mg/kg/day from GD 1 to GD 17 showed increases in E2 levels relative to the control at PNW4 (increases of 10%, 17%, and 8%, respectively) and PNW8 (increases of 11%, 19%, and 12%, respectively), although statistical significance was not achieved (Zhong et al., 2016). A dose-dependent decrease in testosterone levels when compared with controls was noted at PNW4 in females (decreases of 18%, 26%, and 30% in the 0.1 mg/kg/day, 1 mg/kg/day, and 5 mg/kg/day groups, respectively), but was not statistically significant (Zhong et al., 2016). In female rats exposed to PFOS for 28 days, testosterone levels were significantly increased with 1.25 mg/kg/day and 2.5 mg/kg/day PFOS (increases of approximately 37% in both groups) but not in the 5 mg/kg/day dose group (NTP, 2019).

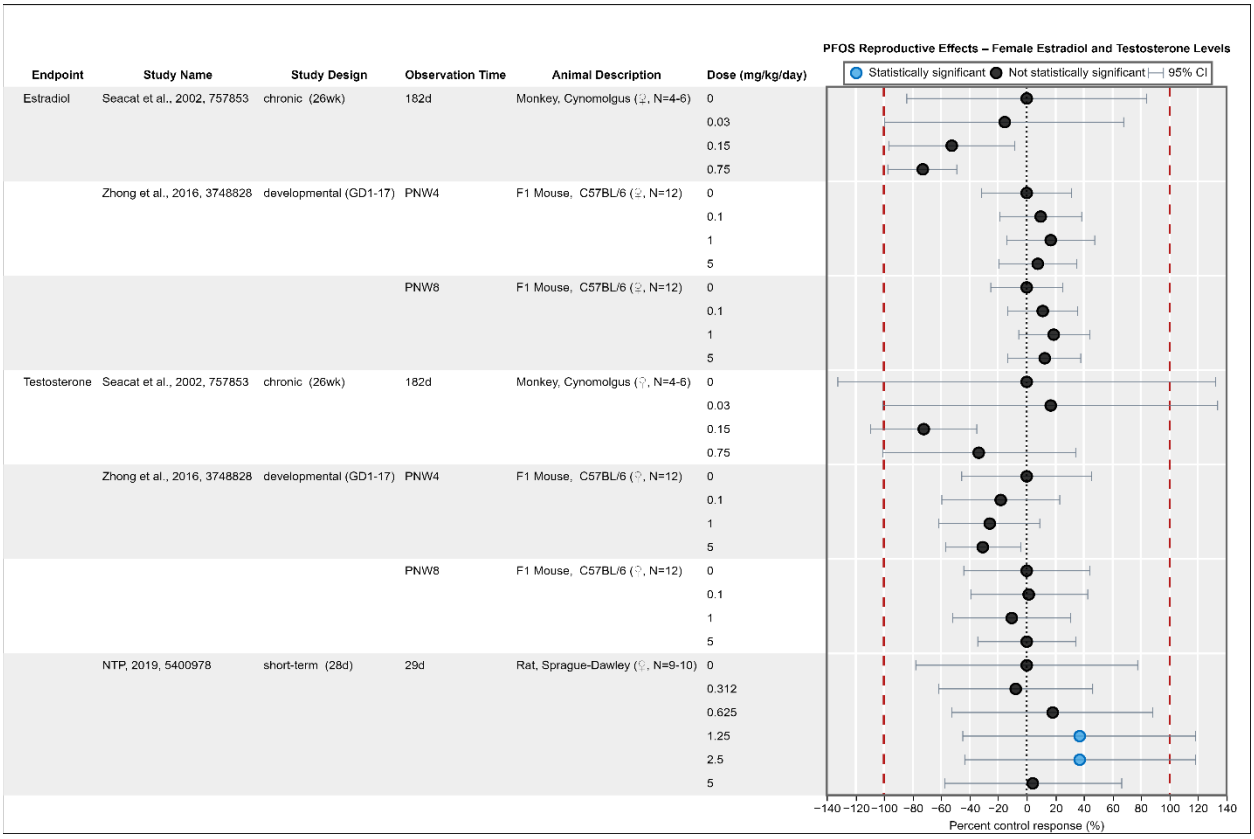


Figure C-11. Percent Change in Estradiol and Testosterone Levels Relative to Controls in Female Rodents and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; F_1 = first generation; PNW = postnatal week; d = day; wk = week.

The red dashed lines indicate a 100% increase or 100% decrease from the control response.

C.1.2.4 Estrous Cyclicity and Ovarian Function (Female) and Reproductive System Development, Including Markers of Sexual Maturation (Female and Male)

In females, a dose-dependent increase in estrous cycle length was observed in rats treated with 0.625 mg/kg/day to 2.5 mg/kg/day over the course of 28-days (increased length of 0.4 days in the 2.5 mg/kg/day group compared with controls); however, this finding was not statistically significant (NTP, 2019). Summary statistics indicated that the proportion of time spent in each phase was unaffected, although Markov analysis indicated that females in all assessed groups had an increased likelihood of transitioning to prolonged diestrus when compared with controls. In the same study, the number of cycles was considered unaffected by treatment (NTP, 2019). In a two-generation reproduction study in rats, no significant effects were observed on the number of estrous cycles of P_0 females treated with up to 3.2 mg/kg/day for 6 weeks prior to mating (Luebker et al., 2005a).

No significant changes in the number or distribution of corpora lutea were noted in P_0 rats exposed prior to mating and during gestation in the one- and two-generation reproductive toxicity studies (Luebker et al., 2005b; Luebker et al., 2005a). Likewise, no changes in the number of corpora lutea were seen in P_0 female rabbits exposed during gestation (Argus Research Laboratories, 2000). Reproductive and developmental studies additionally reported no impact of gestational PFOS exposure on the timing of preputial separation or vaginal opening in rats (Butenhoff et al., 2009; Luebker et al., 2005a; Lau et al., 2003).

C.1.2.5 Reproductive Organ Weights and Histopathology

C.1.2.5.1 Male

Several studies investigated the effect of PFOS exposure on male reproductive organ weights. No effects were noted in the absolute or relative epididymal and testes weights in rats treated up to 5.0 mg/kg/day for 28 days (NTP, 2019) or in absolute or relative testis weight in rats exposed to 20 ppm in the diet for 53 weeks (equivalent to 0.984 mg/kg/day) (Butenhoff et al., 2012). In a subchronic study, no significant changes were observed in relative or absolute testis weight upon exposure to PFOS at doses of 0.015 mg/kg/day and 0.5 mg/kg/day for a duration of 60 days in Wistar rats (Alam et al., 2021). Effects in mice exposed to PFOS were observed in a subchronic study in which significant decreases in absolute and relative testis weights were noted in mice exposed to 10 mg/kg/day for 35 days (Qu et al., 2016). No effects were seen in relative epididymis or testis weights of mice treated up to 10 mg/kg/day for four weeks (Qiu et al., 2016), nor were any effects noted in the relative testes weight of mouse pups treated from GD 1 to GD 17 (Lai et al., 2017a). Similarly, no significant changes in relative epididymis or testis weight were observed for ICR mice treated up to 10 mg/kg/day for four weeks (Qiu et al., 2020). Male cynomolgus monkeys treated with up to 0.75 mg/kg/day for 182 days showed no changes in absolute or relative epididymis or testis weights (Seacat et al., 2002).

Histopathological examination of rats following 28 days or 2 years of exposure revealed no treatment-related changes in the testes, epididymis, seminal vesicle, or prostate (NTP, 2019; Butenhoff et al., 2012). However, Lopez-Doval et al. (2014) noted edema around seminiferous

tubules and malformed spermatids in male rats treated at ≥ 1 mg/kg/day with marked edema and loss and degeneration of the spermatozoids observed at 6 mg/kg/day following PFOS exposure up to 6 mg/kg/day for 28 days. The specific incidences of histopathological findings were not reported in this study, and statistical analysis was not conducted. In another study, subchronic exposure in rats revealed lesions including vacuolations in spermatogonia, spermatocytes, and Leydig cells, as well as exaggerated intracellular space and disturbed germ cells in rats treated at 10 mg/kg/day; however, incidences of specific findings were not reported, and statistical analyses were not conducted (Qu et al., 2016).

Relevant histopathological findings in a 28-day study in mice included Sertoli cell vacuolization and derangement of the cell layers at 2.5 mg/kg/day, 25 mg/kg/day, and 50 mg/kg/day and dislocated immature germ cells in seminiferous tubules at 50 mg/kg/day (Qiu et al., 2013); however, incidences of specific findings were not reported, and methods used for statistical analysis are unclear. These findings were confirmed by observing the ultrastructure of seminiferous epithelia by electron microscopy. In addition, PFOS was observed to disrupt the blood-testis barrier in vitro and in vivo in two studies, suggesting that Sertoli cells in the testes are a target for PFOS toxicity (Qiu et al., 2016; Qiu et al., 2013). Along with observations of reduced epididymal sperm count in these studies, these results collectively suggest the potential for PFOS exposure to induce deterioration of the testis and impair spermatogenesis in mice.

In a single study in cynomolgus monkeys, histopathology of the testes, prostate, and seminal vesicles and cell proliferation in the testes were examined following exposure to PFOS for 182 days, however no differences were noted when compared with controls (Seacat et al., 2002).

C.1.2.5.2 Females

Female organ weight and histopathological data in rats were only available from the 28-day NTP study (NTP, 2019). In females, relative and absolute uterus with cervix and vagina weights in Sprague-Dawley rats were not affected following a 4-week exposure to PFOS at doses up to 5 mg/kg/day. In addition, no treatment-related histopathological changes were observed in the uterus or ovary (NTP, 2019). A chronic study in rats (Butenhoff et al., 2012) measured the weight of the uterus with cervix at the 53-week interim evaluation and evaluated histopathology of the ovaries, uterus, vagina, and cervix after two years of exposure to concentrations up to 20 ppm in the diet (equivalent to 1.251 mg/kg/day) and reported no significant findings for those organs. Similarly, Seacat et al. (2002) did not report alterations in ovary weight or uterine or vaginal histopathology in female cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 182 days. Effects on placental characteristics such as weight and capacity, as well as histopathological effects were noted in rats and mice exposed to PFOS during gestation (see Toxicity Assessment, (U.S. EPA, 2024)).

C.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse reproductive outcomes is discussed in Sections 3.2.5, 3.3.4, and 3.4.1.2 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are 57 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to reproductive effects. A summary of these studies is shown in Figure C-12. Additional

mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS may cause respiratory effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	1	2
Atherogenesis And Clot Formation				
Big Data, Non-Targeted Analysis	4	1	5	9
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	23	29
Cell Signaling Or Signal Transduction	9	1	18	27
Extracellular Matrix Or Molecules	2	0	2	4
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	5	1	1	6
Hormone Function	10	1	13	23
Inflammation And Immune Response				
Oxidative Stress	2	0	5	7
Renal Dysfunction				
Xenobiotic Metabolism	2	0	4	6
Other	2	0	1	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	21	3	38	57

Figure C-12. Summary of Mechanistic Studies of PFOS and Reproductive Effects

Interactive figure and additional study details available on HAWC.

C.1.4 Evidence Integration

C.1.4.1 Reproductive Effects in Males

There is *slight* evidence for an association between PFOS exposure and male reproductive effects based on inverse associations with testosterone in male children. Inverse associations with testosterone were observed in two *medium* confidence studies in children, and one study reported an inverse association for E2. Among *low* confidence studies, there was mixed evidence for an association between PFOS exposure and testosterone in cross-sectional studies (Di Nisio et al., 2019; Zhou et al., 2017c; Zhou et al., 2016) in children and adolescents. However, these mixed associations were observed in populations at different stages of pubertal development. Results showed decreasing testosterone with increasing serum PFOS in children, but increased

testosterone with higher PFOS exposure levels in adolescents. In adolescents, there were no effects on pubertal development, but associations were observed for penile measurements, testicular measurements, and sperm parameters (Di Nisio et al., 2019). Evidence was also inconsistent for AGD in infants. In adults, there was evidence in one study (Cui et al., 2020) of an inverse association between serum PFOS and testosterone, and these associations were also observed using semen PFOS. Inverse associations were also seen for E2, SHBG, and the total T/LH ratio. Regarding semen and sperm characteristics in adults, associations were observed for several parameters in analyses of semen PFOS, including increased sperm DNA fragmentation and decreased measures of sperm motility. Other results for markers of genotoxic effects (e.g., sperm Y:X-chromosome ratio, sperm DNA methylation) in sperm were inconsistent.

The animal evidence for an association between PFOS exposure and reproductive toxicity in males is *slight* based on several *high* or *medium* confidence studies of varying durations showing that oral exposure to PFOS can affect the male reproductive system. However, many of the observed reproductive effects (e.g., decreased E2 levels in male monkeys) occurred at doses that also resulted in reduced body weight which can be confounding effects for reproductive endpoints. Additionally, several of the observed effects were not consistent across species (e.g., sperm parameters, testis weight, E2 levels in males) which increases uncertainty about the relevance of these effects to humans or potential differences in the MOA between species.

Several studies reported effects of PFOS exposure on male mouse and rat reproductive organ histopathology (Qiu et al., 2016; Qu et al., 2016; Lopez-Doval et al., 2015; López-Doval et al., 2014; Qiu et al., 2013). However, these studies did not report incidence data which hinders further quantification or conclusions about these results. In male mice, these histopathological alterations were accompanied by a reduction in epididymal sperm count, though this effect was not observed in male rats. Although reductions in epididymal sperm counts across mouse studies ranged from 25% to 70% at the highest doses tested (Lai et al., 2017a; Qiu et al., 2016; Qu et al., 2016; Qiu et al., 2013) and are consistent with effects seen in humans, fertility may be normal in male rodents even with sperm reductions as great as 90% (Gray et al., 1988). Without further evidence of reduced fertility or quantitative evidence of histopathological changes in the testes or epididymis, it is unclear whether reductions in sperm counts can be considered adverse.

Similar uncertainties arise when linking the observed hormonal alterations with functional reproductive consequences. Changes in LH, FSH, and prolactin were observed in male rats, however, lack of histopathological and sperm parameter effects (specific to rats), as well as inconsistent effects on testosterone levels, make it difficult to assess the relevance of these changes. It is difficult to ascertain the magnitude of change in hormone levels that can be considered adverse without concurrent supporting evidence of functional or histopathological reproductive consequences.

C.1.4.2 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause reproductive effects in males under relevant exposure circumstances (Table C-1). This conclusion is based primarily on effects on inverse associations with testosterone in male children and adults, and decreased AGD in children observed in studies in humans exposed to median PFOS ranging from 1.4 to 34.8 ng/mL. Although there is some evidence of negative effects of PFOS exposure on semen and sperm characteristics in adults, there is considerable uncertainty in the results due to

inconsistency across studies and limited number of studies. For male reproductive toxicity, the conclusion is based primarily on observed changes in hormonal parameters in adult rodents following exposure to doses as low as 0.5 mg/kg/day PFOS. However, findings from animal studies are similarly inconsistent as in epidemiological studies. In animal studies, there are uncertainties in the adversity of the observed effects, a lack of quantifiable histopathological evidence in reproductive organs, and inconsistencies in responses observed across studies and species.

Table C-1. Evidence Profile Table for PFOS Reproductive Effects in Males

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.1.1)					⊕⊙⊙ ***Evidence Suggests***
Male reproductive hormones 1 *High* confidence study 8 *Medium* confidence studies 6 *Low* confidence studies	In children and adolescents, inverse associations for total testosterone were observed in two studies (2/8), including a *medium* confidence study reporting a significant inverse trend. One study reported higher total testosterone levels among highly exposed adolescents but was of *low* confidence. Findings for estradiol in male children were generally less precise, however, one *medium* confidence study (1/6) observed a significant, dose-dependent increase in estradiol, which was accompanied by a significant decrease in the testosterone/estradiol ratio. Findings for LH and FSH were mixed, but significantly increased LH was observed in one *low* confidence study, and significantly decreased FSH was	• *High* and *medium* confidence studies • *Consistent direction* of effects for testosterone levels	• *Low* confidence studies • *Imprecision* of most findings • Potential for *residual confounding* by SES and smoking status	⊕⊙⊙ *Slight* Evidence for male reproductive effects is based on several studies reporting consistent and coherent changes to sex hormones. Effects on sex hormones were supported by adverse effects observed for other outcomes such as sperm quality (i.e., sperm DFI and HDS) and anthropometric measures. Uncertainties remain regarding mixed results in adults and imprecise results in some *medium* confidence studies. There were also a limited number of studies evaluating certain endpoints such as semen parameters and pubertal development.	*Primary basis*: Human evidence indicted effects on inverse associations with testosterone in male children and adults, and decreased AGD in children observed in studies in humans exposed to median PFOS. Although there is some evidence of negative effects of PFOS exposure on semen and sperm characteristics in adults, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. Animal evidence indicated changes in hormonal parameters in adult rodents following exposure to PFOS. However, findings from animal studies are similarly inconsistent as in epidemiological studies. In animal studies, there are uncertainties in the

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	observed in a *medium* confidence study. In adults, one study (1/3) observed significant decreases in total testosterone and the testosterone/estradiol ratio. Another *medium* confidence study reported a non-significant increase in total testosterone, but other results for testosterone were imprecise. One study reported a non-significant decrease in estradiol, and one study reported a significant increase in LH. Findings for SHBG were mixed.				adversity of the observed effects, a lack of quantifiable histopathological evidence in reproductive organs, and inconsistencies in responses observed across studies and species. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Semen parameters 4 *Medium* confidence studies 1 *Low* confidence studies	One study examined semen parameters in high school students and observed significant increases in semen pH and increased deficits in sperm morphology. Semen parameter findings in adults were generally consistent between endpoints but did not always indicate adverse effects. Sperm count was non-significantly increased in	• *Medium* confidence studies • *Consistent direction* of effects for most findings	• *Low* confidence study • *Imprecision* of most findings • *Incoherence* of direction of effect for adult semen parameters • Potential for *residual confounding* by SES and smoking status		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	two studies (2/3), non-significant positive associations were observed for sperm concentration in three studies (3/4), and semen volume was reported to be non-significantly increased in two studies (2/4). Adverse effects were also observed, including decreased normal morphology (1/2), increased sperm HDS, and significantly increased sperm DFI. Sperm HDS and DFI are measures of sperm chromatin integrity and sperm DNA damage, respectively.				
Anthropometric measurements of male reproductive organs 1 *High* confidence study 2 *Medium* confidence studies 1 *Low* confidence study	Three studies examined measurements in male infants. Non-significant increases in AGD were observed in two studies (2/3), but findings were not consistent across timepoints. One study examined anthropometric measurements in male	• *High* and *medium* confidence studies • *Consistent direction* of effects • *Coherence* of findings	• *Low* confidence study • *Imprecision* of some findings • Potential for *residual confounding* by SES and smoking status		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	high school students. Adverse effects were observed in adolescents with higher exposure levels, including smaller testicular volume, shorter penis length, and smaller penis circumference.				
Male pubertal development 1 *Medium* confidence study	Findings for changes in timing of pubertal development were largely non-significant. Study authors reported earlier onset of individual Tanner stages (G2 and G5) and earlier onset of voice break, but none were significant.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.1.2)					
Male mating and fertility 1 *Medium* confidence study	No effects on male mating or fertility parameters were observed in a two-generation reproduction study in rats with exposure beginning six weeks prior to mating (1/1).	• Medium confidence study	• Limited number of studies examining outcome	⊕⊙⊙ *Slight* Evidence was based on 15 *high* and *medium* confidence studies. There were no observed effects on mating or fertility in the only available two-generation reproduction study; however, other studies observed effects on hormone levels, sperm count, and testis weight and histopathology. Some of the reproductive	
Male reproductive hormones 1 *High* confidence study 8 *Medium* confidence studies	Alterations in testosterone levels in male rats (3/8), mice (4/8), and monkeys (1/8) were inconsistent. Reports of decreases (5/8), increases (1/8), and	• *High and medium* confidence studies	• *Inconsistent direction* of effects across species • Changes in body weight may limit ability to interpret these responses		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	no change (2/8) in serum testosterone were reported following developmental, short-term, and subchronic exposure. Mixed effects on serum estradiol included decreased levels in rats and monkeys (3/6), increases (1/6) in mice, or no effects (2/6). Short-term studies in male rodents reported no effect on FSH (1/1), decreases in LH (1/2), and decreases in prolactin (1/1).			effects observed (e.g., decreased testosterone and estradiol levels) may be secondary effects because they occurred at doses that also resulted in reduced body weight. Additionally, several of the observed effects were not consistent across species (e.g., sperm parameters, testosterone and estradiol levels) which increases uncertainty about the relevance of these effects to humans or potential differences in the mode of action between species. Studies reporting alterations in testis histopathology did not report incidence data which hinders conclusions about these results. In male mice, these histopathological alterations were accompanied by a reduction in epididymal sperm count. Without further evidence of reduced fertility or quantitative evidence of	
Sperm parameters 1 *High* confidence study 5 *Medium* confidence studies	In mice, five short-term, subchronic, or developmental studies observed dose-dependent reductions in epididymal sperm count (5/5). However, in rats, no effects on epididymal or testicular sperm counts or epididymal sperm motility were reported (1/1).	• *High and medium* confidence studies • *Consistent direction* of effects within species	• *Inconsistent direction* of effects across species		
Male pubertal development 3 *Medium* confidence studies	No effects on age at preputial separation were observed in reproductive and developmental studies in male rats (3/3).	• *Medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Organ weights 2 *High* confidence studies 7 *Medium* confidence studies	Most studies in rats, mice, or monkeys found no effects on absolute or relative testis weight (8/9). One subchronic study in mice observed decreases in absolute and relative testis weight (1/9) only at the highest dose tested. No effects on absolute or relative epididymis weight were observed (4/4).	• *High and medium* confidence studies	No factors noted	histopathological changes in the testis or epididymis, it is unclear whether reductions in sperm counts can be considered adverse. Changes in LH, FSH, and prolactin were observed in male rats; however, the lack of histopathological and sperm parameter effects (specific to rats), as well as inconsistent effects on testosterone levels, make it difficult to assess the relevance of these changes.	
Histopathology 2 *High* confidence studies 4 *Medium* confidence studies	Two *high* confidence studies in rats and one *medium* confidence study in monkeys found no histopathological changes in the testes, prostate, epididymides, or seminal vesicles following short-term or chronic exposure (3/6). Three studies in mice observed histopathological changes in the testes following 4–5 wk of exposure (3/6). These changes included vacuolations in spermatogonia, spermatocytes, Leydig cells, and Sertoli cells, and disturbed germ cell layers; however, results	• *High and medium* confidence studies	• *Inconsistent direction* of effects across species		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	were all reported qualitatively only.				

Notes: LH = luteinizing hormone; FSH = follicle stimulating hormones; SHBG = sex hormone binding globulin; SES = socioeconomic status; DFI = DNA fragmentation index; HDS = high DNA stainability; DNA = deoxynucleic acid; AGD = anogenital distance; G2 = genital stage 2; G5 = genital stage 5; wk = weeks.

C.1.4.3 Reproductive Effects in Females

There is *slight* evidence for an association between PFOS exposure and female reproductive effects in humans based on observed increases in preeclampsia and gestational hypertension, with most studies observing positive non-significant associations, in populations with high exposure levels and at levels typical in the general population.

Epidemiological evidence of a relationship between PFOS exposure and female fertility is mixed. Since the 2016 Health Assessment, nine studies have investigated associations between PFOS exposure and fertility. While some studies reported more frequent or intense female fertility problems with increasing PFOS exposure (Zhang et al., 2018b; Crawford et al., 2017; Mccoy et al., 2017), others found PFOS to be positively associated with female fertility indicators (Kim et al., 2020b; Bach et al., 2018; Lum et al., 2017), and some did not observe any clear trends (Wang et al., 2017). Kim et al. (2020b) also observed some non-significant, positive associations between follicular fluid PFOS and fertility etiology factors for other gynecologic pathologies, including endometriosis, PCOS, genital tract infections, and idiopathic infertility.

There is limited, consistent epidemiological evidence of an inverse association between serum PFOS levels in pregnancy and breastfeeding duration. Timmermann et al. (2017b) observed negative associations between PFOS exposure and exclusive and total breastfeeding duration, while Romano et al. (2016) observed increased relative risk of breastfeeding termination with increasing PFOS exposure.

Human epidemiological evidence of a relationship between PFOS exposure and the female reproductive milestones of age at menarche and menopause is mixed. In the 2016 Health Assessment, Christensen et al. (2011) observed a non-significant decreased adjusted OR for earlier age at menarche for continuous prenatal PFOS exposure. Since the 2016 Health Assessment, Ernst et al. (2019) observed a significant inverse association between age at attainment for overall puberty indicators and a non-significant inverse association for continuous prenatal PFOS exposure and age at menarche. In the 2016 Health Assessment, Knox et al. (2011) observed significant increased odds of natural menopause across PFOS quintiles for women ages 51–65 years in the C8 Health Project. Since the 2016 Health Assessment, Ding et al. (2020) observed significant, positive associations for serum Sm-PFOS and n-PFOS and risk of natural menopause in women aged 45–56. However, findings from studies concurrently assessing menstruation events and PFOS levels in blood must be interpreted with caution due to potential reverse causality, as menstruation is a primary route of PFOS excretion for people who menstruate.

Since the 2016 Health Assessment, 20 studies have assessed relationships between PFOS exposure and various female reproductive hormones. 12 of these studies were conducted in female infants and adolescents. Commonly assessed female reproductive hormones were 17-OHP, DHEA, E2, FSH, SHBG, and testosterone. While most studies did not report significant associations or consistent trends between PFOS exposure and these outcomes, Itoh et al. (2016) observed significant negative associations for maternal serum PFOS and cord blood prolactin and progesterone levels and Wang et al. (2019a) observed significant positive associations for cord blood PFOS and cord blood estrone and E3. In pregnant women, Yao et al. (2019) observed significant, positive associations for cord blood PFOS and testosterone and testosterone to E2

ratio and Toft et al. (2016) observed significant, positive trends in 17-OHP, androstenedione, progesterone, and testosterone across amniotic fluid PFOS tertiles.

The recent epidemiological evidence is also suggestive of an association between PFOS and preeclampsia and gestational hypertension, though there is conflicting evidence on altered puberty onset and limited data suggesting reduced fertility and fecundity. The association are inconsistent across reproductive hormone parameters, and it is difficult to assess the adversity of these alterations.

The animal evidence for an association between PFOS exposure and female reproductive toxicity is *slight* based on several *high* or *medium* confidence studies of varying durations showing that oral exposure to PFOS can affect the female reproductive system. However, many of the observed reproductive effects (e.g., decreased gestation length in female rats, decreased prolactin levels in female mice) occurred at doses that also resulted in decreased gestational body weight which can be confounding effects for reproductive endpoints.

Uncertainties arise when linking the observed hormonal alterations with functional reproductive consequences. NTP (2019) reported modest increases in testosterone concentrations (37% increase) in female rats with PFOS doses of 1.25 mg/kg/day and 2.5 mg/kg/day, but not the highest dose of 5 mg/kg/day. The response in the highest dose was confounded by decreased body weight. The alterations in testosterone were accompanied by dose-dependent increases in estrous cycle length, though this increase was not statistically significant and alterations in the estrous cycle were not observed in a second study in female rats (Luebker et al., 2005a). It is difficult to ascertain the magnitude of change in hormone levels that can be considered adverse without concurrent supporting evidence of functional or histopathological reproductive consequences.

C.1.4.4 Evidence Integration Judgment

Overall, evidence ***suggests*** that PFOS exposure has the potential to cause reproductive effects in females under relevant exposure circumstances (Table C-2). This conclusion is based primarily on effects on preeclampsia and gestational hypertension, female reproductive milestones, and female reproductive hormonal outcomes observed in studies in humans exposed to median PFOS ranging from 1.4 ng/mL to 34.8 ng/mL. There is considerable uncertainty in the results due to inconsistency across studies and the limited number of studies. For female reproductive toxicity, the conclusion is based primarily on observed changes in hormonal parameters in adult rodents following exposure to doses as low as 1.25 mg/kg/day PFOS. However, findings from animal studies are similarly inconsistent as in epidemiological studies. In animal studies, there are uncertainties in the adversity of the observed effects, a lack of quantifiable histopathological evidence in reproductive organs, and inconsistencies in responses observed across studies and species.

Table C-2. Evidence Profile Table for PFOS Reproductive Effects in Females

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.1.1)					⊕⊙⊙ ***Evidence Suggests***
Female reproductive hormones 3 *High* confidence studies 10 *Medium* confidence studies 7 *Low* confidence studies	Results from assessment of female reproductive hormones were mixed. In 13 studies of female children and adolescents, 7 studies reported significant associations. One *medium* confidence study reported increased E1 and E3 and an inverse association with E2 (1/7). Two other studies reported increased E2 (2/7), and one also reported increased FSH (1/2). Three studies, one *high,* one *medium,* and one *low* confidence, reported increases in testosterone (3/7). One *medium* confidence study observed inverse associations with progesterone and prolactin (1/7). Eight studies examined adult women, though many were *low* confidence (5/8). Four studies reported significant effects (4/8). Two *low* confidence studies observed inverse	• *High* and *medium* confidence studies • *Coherence* of findings for testosterone	• *Low* confidence studies • *Inconsistent direction* of effects • *Imprecision* of most findings • Potential for *selection bias* and *residual confounding* by age and SES	⊕⊙⊙ *Slight* Evidence for female reproductive effects is based on several studies reporting effects on sex hormones and increased odds of preeclampsia. There was also evidence for changes in age at natural menopause. Uncertainties remain regarding mixed findings in studies of sex hormones, and a limited number of studies examining outcomes such as female reproductive milestones and anthropometric measurements.	*Primary basis*: Human evidence indicted effects on preeclampsia and gestational hypertension, female reproductive milestones, and female reproductive hormonal outcomes observed in studies in humans exposed to median PFOS. There is considerable uncertainty in the results due to inconsistency across studies and the limited number of studies. Animal evidence indicated changes in hormonal parameters in adult rodents following exposure to PFOS. However, findings from animal studies are similarly inconsistent as in epidemiological studies. In animal studies, there are uncertainties in the adversity of the observed effects, a lack of quantifiable histopathological evidence in reproductive organs,

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	associations with E2 (2/4), one *medium* study observed increased progesterone, testosterone, and 17-OHP (1/4) and one observed an inverse association with free androgen index (1/4).				and inconsistencies in responses observed across studies and species. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Preeclampsia and gestational hypertension 1 *High* confidence study 5 *Medium* confidence studies	Six studies examined preeclampsia in pregnant women. One study reported significant positive results, while four studies of *medium* and *high* confidence reported non-significant positive associations with preeclampsia. Three studies reported inverse associations (3/6). Of the three studies examining gestational hypertension (3/6), two reported inverse associations but neither reached significance (2/3). After observing non-significant increased odds of gestational hypertension, one *medium* confidence study reported significantly increased DBP.	• *High* and *medium* confidence studies	• *Imprecision* of all findings • *Inconsistent direction* of effects • Potential for *reverse causality*		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Female reproductive milestones 1 *High* confidence study 2 *Medium* confidence studies 1 *Low* confidence study	Three studies examined reproductive milestones related to menstruation, two in adolescent populations (2/3) and one in an adult population (1/3). Two studies, one *low* confidence study in adolescents (1/2) and one *medium* confidence study in adults (1/1), reported non-significant increases in long menstrual cycles. A significant inverse association was observed among adolescents for average age at attainment for all pubertal indicators (1/2). One *high* confidence study reported significant positive associations with age at natural menopause.	• *High* and *medium* confidence studies • *Consistent direction* of effects	• *Low* confidence study • Potential for *residual confounding* by not identifying confounders • *Limited number* of studies examining specific outcomes		
Fertility indicators 6 *Medium* confidence studies 6 *Low* confidence studies	Examinations of fertility indicators include fecundability, fertilization rate, and measures of ovarian health, such as AMH levels or endometriosis. Twelve studies evaluated fertility indicators in non-pregnant women with mixed results. One *medium* confidence study	• *Medium* confidence studies	• *Low* confidence studies • *Imprecision* of most findings • Potential for *residual confounding* by not identifying confounders • *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	reported significant inverse associations with endometriosis-driven infertility. In contrast, *low* confidence studies observed significantly increased odds of endometriosis (1/3) and ovarian syndromes (2/3). Other studies reported non-significant positive associations with endometriosis (2/12). Results from remaining studies were inconsistent and did not reach significance.				
Breastfeeding 2 *Medium* confidence studies	Two *medium* confidence cohort studies reported significant inverse associations with breastfeeding duration (2/2).	• *Medium* confidence studies • *Consistent direction* of effects • *Precision* of findings	• *Limited number* of studies examining outcome		
Anogenital distance 1 *High* confidence study 1 *Medium* confidence study	Two studies examined measures of AGD, including anoclitoris and anofourchette distances, in female infants. A *high* confidence study reported significant inverse associations with anoclitoris distance for the highest exposure group and in continuous analysis. Results for	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent* direction of effects		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	anofourchette distances were inverse but not significant. A *medium* confidence study observed non-significant mixed results for both measures.				
Evidence From In Vivo Animal Studies (Section C.1.2)					
Female mating and fertility 2 *Medium* confidence studies	No effects on female mating or fertility parameters were observed in one- and two-generation reproduction studies in rats with PFOS exposure beginning six weeks prior to mating (2/2).	• *Medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome	⊕⊙⊙ *Slight* Evidence is based on 10 *high* and *medium* confidence studies. There were no observed effects on mating or fertility in the only available two-generation reproduction study; however, other studies observed effects on length of gestation, hormone levels, and estrous cyclicity. Some of the observed reproductive effects (e.g., decreased gestation length in female rats, decreased prolactin-family hormones in female mice) may be secondary effects because they occurred at doses that also resulted in decreased gestational body weight. One study reported modest increases	
Female gestation length 3 *Medium* confidence studies	Duration of gestation was slightly decreased in a one-generation rat reproduction study and in a two-generation rat study, both with exposure beginning six weeks prior to mating (2/3). No effect on gestation length was observed in another rat study with exposure beginning on the first day of gestation.	• *Medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome • *Small magnitude* of effect		
Female reproductive hormones 1 *High* confidence study 3 *Medium* confidence studies	Significant alterations in female testosterone levels were found (1/3). No significant changes in serum E2 were found in	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	female monkeys exposed for 26 wk or in female mice exposed in utero from GD 1–17. One mouse study measured maternal serum concentrations of prolactin-family hormones (i.e., mPL-II, mPLP-Cα, mPLP-K) during pregnancy and found dose-dependent decreases (1/1).		• Changes in body weight may limit ability to interpret these responses	in testosterone concentrations in females, but the response in the highest dose was affected by decreased body weight. The increases in testosterone were accompanied by dose-dependent increases in estrous cycle length, though this increase was not statistically significant and alterations in the estrous cycle were not observed in a second study in female rats.	
Estrous cyclicity and ovarian function 1 *High* confidence study 3 *Medium* confidence studies	No significant effect on estrous cyclicity were found in two rat studies (2/2). However, a *high* confidence study in rats observed a dose-dependent, but not significant, increase in estrous cycle length and prolonged diestrus (1/1) compared with controls. No effects on the number and distribution of corpora lutea in the ovaries were observed in pregnant rats and rabbits (3/3).	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *Small magnitude* of effect		
Female pubertal development 3 *Medium* confidence studies	No effects on age at vaginal opening were observed in reproduction and developmental studies in rats (3/3).	• *Medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Organ weights 2 *High* confidence studies 1 *Medium* confidence study	No effects were observed on absolute or relative weights of the uterus (2/2) or ovaries (1/1).	• *High* and *medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome		
Histopathology 2 *High* confidence studies 1 *Medium* confidence study	No exposure-related histopathological findings were reported for the ovaries (2/2), uterus (3/3), vagina (2/2), or cervix (1/1).	• *High* and *medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome		

Notes: E1 = estrone; E3 = estriol; E2 = estradiol; FSH = follicle stimulating hormones; 17-OHP = 17-hydroxyprogesterone; SES = socioeconomic status; DBP = diastolic blood pressure; AMH = anti-Mullerian hormone; AGD = anogenital distance; wk = weeks; GD = gestation day; mPL-II = mouse placental lactogen II; mPLP-Cα = mouse prolactin-like protein-Cα; mPLP-K = mouse prolactin-like protein-K.

C.2 Endocrine

EPA identified 35 epidemiological and 14 animal studies that investigated the association between PFOS and endocrine effects. Of the epidemiological studies, 4 were classified as *high* confidence, 15 as *medium* confidence, 9 as *low* confidence, 4 as *mixed* (1 *high/medium*, 1 *medium/low*, 1 *medium/uninformative*, and 1 *low/uninformative*) confidence, and 3 were considered *uninformative* (Section C.2.1). Of the animal studies, 1 was classified as *high* confidence, 10 as *medium* confidence, 2 as *low* confidence, and 1 was *mixed* (*medium/low*) (Section C.2.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.2.1 Human Evidence Study Quality Evaluation and Synthesis

C.2.1.1 Introduction

Thyroid disease is more common in females than in males and encompasses conditions such as hypothyroidism and hyperthyroidism. Hypothyroidism is characterized by elevated thyroid stimulating hormone (TSH) and concurrently low thyroxine (T4) concentrations, while subclinical hypothyroidism is characterized by elevated TSH in conjunction with normal T4 and triiodothyronine (T3) levels. Hyperthyroidism is characterized by elevated T4 and low TSH, and subclinical hyperthyroidism is characterized by low levels of TSH with normal T4 and T3 levels.

The 2016 Health Advisory (U.S. EPA, 2016a) and HESD (U.S. EPA, 2016c) reports identified evidence of endocrine effects of PFOS for thyroid disease, hypothyroidism, and hypothyroxinemia. Occupational studies examining the relationship between PFOS exposure and endocrine outcomes did not find any significant associations. Studies on NHANES populations (Wen et al., 2013; Melzer et al., 2010) reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease. One study (Melzer et al., 2010) reported associations with thyroid disease in men, and another study (Wen et al., 2013) saw associations with subclinical hypothyroidism in men and women. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (i.e., TSH, T3 or T4) show mixed effects across cohorts. In cross-sectional studies where thyroid hormones were measured in association with serum PFOS, increased TSH was associated with PFOS exposure in most cases (Berg et al., 2015; Webster et al., 2014; Wang et al., 2013). Increasing PFOS was associated with increased T4 in children aged 1 to 17 years from the C8 cohort (Lopez-Espinosa et al., 2012); however, PFOS was not associated with hypothyroidism. A small South Korean study examining correlations between maternal PFAS during pregnancy and fetal thyroid hormones in cord blood (Kim et al., 2011) found an association for PFOS and increased fetal TSH, as well as with decreased fetal T3. TSH was the outcome most frequently associated with PFOS in studies of pregnant women. In studies of pregnant women, PFOS was associated with increased TSH levels (Berg et al., 2015; Wang et al., 2013). Pregnant women testing positive for the anti-thyroid peroxidase (TPO) biomarker for autoimmune thyroid disease showed a positive association with PFOS and TSH (Webster et al., 2014). A case-control study of hypothyroxinemia (normal TSH and low free T4) in pregnant women (Chan et al., 2011), did not show associations of hypothyroxinemia with PFOS exposure.

For this updated review, 34 studies (35 publications)[6] report on the association between PFOS exposure and endocrine effects. Seven of the publications were studies in pregnant women (Aimuzi et al., 2020; Dreyer et al., 2020; Inoue et al., 2019; Itoh et al., 2019; Reardon et al., 2019; Berg et al., 2017; Shah-Kulkarni et al., 2016), and the remainder of the publications were on the general population. Different study designs were utilized, including seven cohort studies (Kim et al., 2020a; Lebeaux et al., 2020; Reardon et al., 2019; Liu et al., 2018a; Berg et al., 2017 Blake, 2018, 5080657; Crawford et al., 2017), seven cohort and cross-sectional studies (Dreyer et al., 2020; Itoh et al., 2019; Xiao et al., 2019; Preston et al., 2018; Kato et al., 2016; Wang et al., 2014), one case-control study (Predieri et al., 2015), one case-control and cross-sectional study (Zhang et al., 2018b), and 19 cross-sectional studies (Abraham et al., 2020; Aimuzi et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Inoue et al., 2019; Jain and Ducatman, 2019b; Byrne et al., 2018; Dufour et al., 2018; Heffernan et al., 2018; Kang et al., 2018; Khalil et al., 2018; Seo et al., 2018; Li et al., 2017; Tsai et al., 2017; van den Dungen et al., 2017; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Lewis et al., 2015; Audet-Delage et al., 2013; Jain, 2013). All observational studies measured PFOS in blood components (i.e., blood, plasma, or serum). Six studies measured PFOS in cord blood (Liu et al., 2020b; Aimuzi et al., 2019; Dufour et al., 2018; Tsai et al., 2017; Shah-Kulkarni et al., 2016; Yang et al., 2016a) and eight studies measured PFOS in maternal blood or serum during pregnancy (Dreyer et al., 2020; Lebeaux et al., 2020; Reardon et al., 2019; Xiao et al., 2019; Preston et al., 2018; Kato et al., 2016; Yang et al., 2016a; Wang et al., 2014). The studies were conducted in different study populations including populations from Belgium (Dufour et al., 2018), Canada (Caron-Beaudoin et al., 2019; Reardon et al., 2019), China (Aimuzi et al., 2020; Aimuzi et al., 2019; Zhang et al., 2018b; Li et al., 2017 Liu, 2020, 6569227; Yang et al., 2016a), Denmark (Dreyer et al., 2020; Inoue et al., 2019; Xiao et al., 2019), Germany (Abraham et al., 2020), Italy (Predieri et al., 2015), Japan (Itoh et al., 2019; Kato et al., 2016), Republic of Korea (Kim et al., 2020a; Kang et al., 2018; Shah-Kulkarni et al., 2016), Taiwan (Tsai et al., 2017; Wang et al., 2014), the United Kingdom (Heffernan et al., 2018), and the United States (Lebeaux et al., 2020; Jain and Ducatman, 2019b; Blake et al., 2018; Byrne et al., 2018; Khalil et al., 2018; Liu et al., 2018a; Preston et al., 2018; Crawford et al., 2017; Lewis et al., 2015; Jain, 2013). Two studies (Itoh et al., 2019; Kato et al., 2016) belonged to the same cohort, the Hokkaido Study on the Environment and Children's Health. While most studies evaluated the relationship between exposure to PFOS and thyroid hormone concentrations, other endocrine outcomes were investigated as well, including: thyroid disease, thyroid antibodies (thyroglobulin antibodies (TgAb) and thyroid peroxidase antibody (TPOAb)), and thyroid hormone-associated proteins (e.g., thyroglobulin, thyroxine-binding globulin).

C.2.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies. First, timing of exposure and hormone concentration measurements was important. Several studies on mother-child dyads examined relationships between maternal serum PFOS measurements and thyroid hormones in both mothers (i.e., a cross-sectional analyses) and in cord blood or children's serum (i.e., a longitudinal analyses). Longitudinal comparisons between maternal PFOS concentrations measured during pregnancy and thyroid hormone levels in cord blood or the child's blood attenuate any concerns for potential reverse causality. Measuring PFOS and thyroid hormone

[6] Itoh et al. (2019) reports thyroid-related hormone levels in a population overlapping with Kato et al. (2016).

concentrations concurrently in maternal serum was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.4 years) (Li et al., 2018), current blood concentrations are expected to correlate well with past exposures. Second, timing of thyroid hormone assessment was a recurring concern due to the diurnal variation in thyroid hormones. Thyroid hormone outcome misclassification due to timing of blood collection is non-differential, however, study sensitivity may be impacted in cases where timing of collection was uncontrolled.

There are 35 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and endocrine effects. Study quality evaluations for these 35 studies are shown in Figure C-13 and Figure C-14.

Of the 35 studies identified since the 2016 assessment, 4 studies were classified as *high* confidence, 15 as *medium* confidence, 9 as *low* confidence, 4 as *mixed* (1 *high/medium*, 1 *medium/low*, 1 *medium/uninformative*, and 1 *low/uninformative*) confidence, and 3 studies (Abraham et al., 2020; Predieri et al., 2015) as *uninformative*.

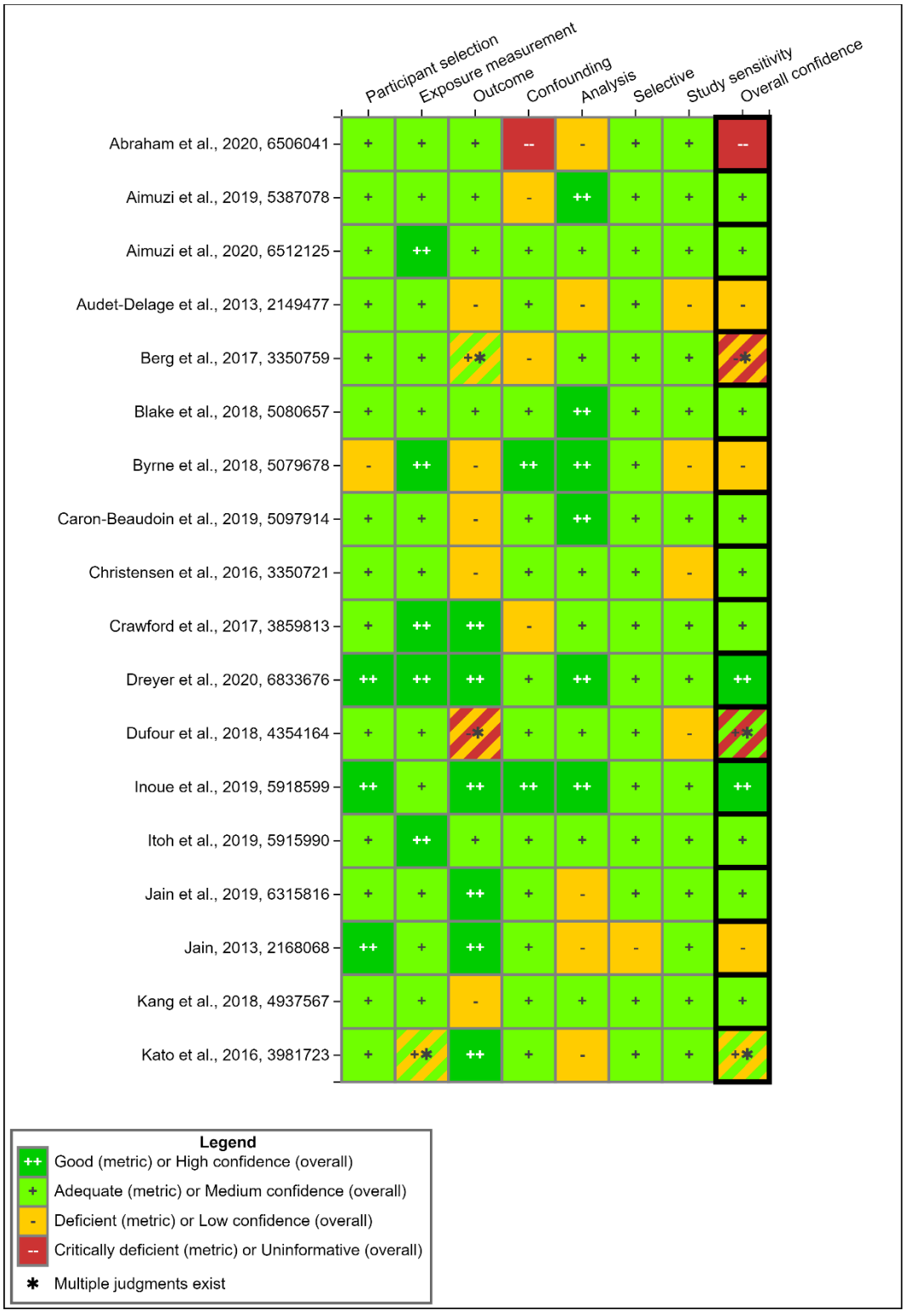


Figure C-13. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Endocrine Effects

Interactive figure and additional study details available on HAWC.

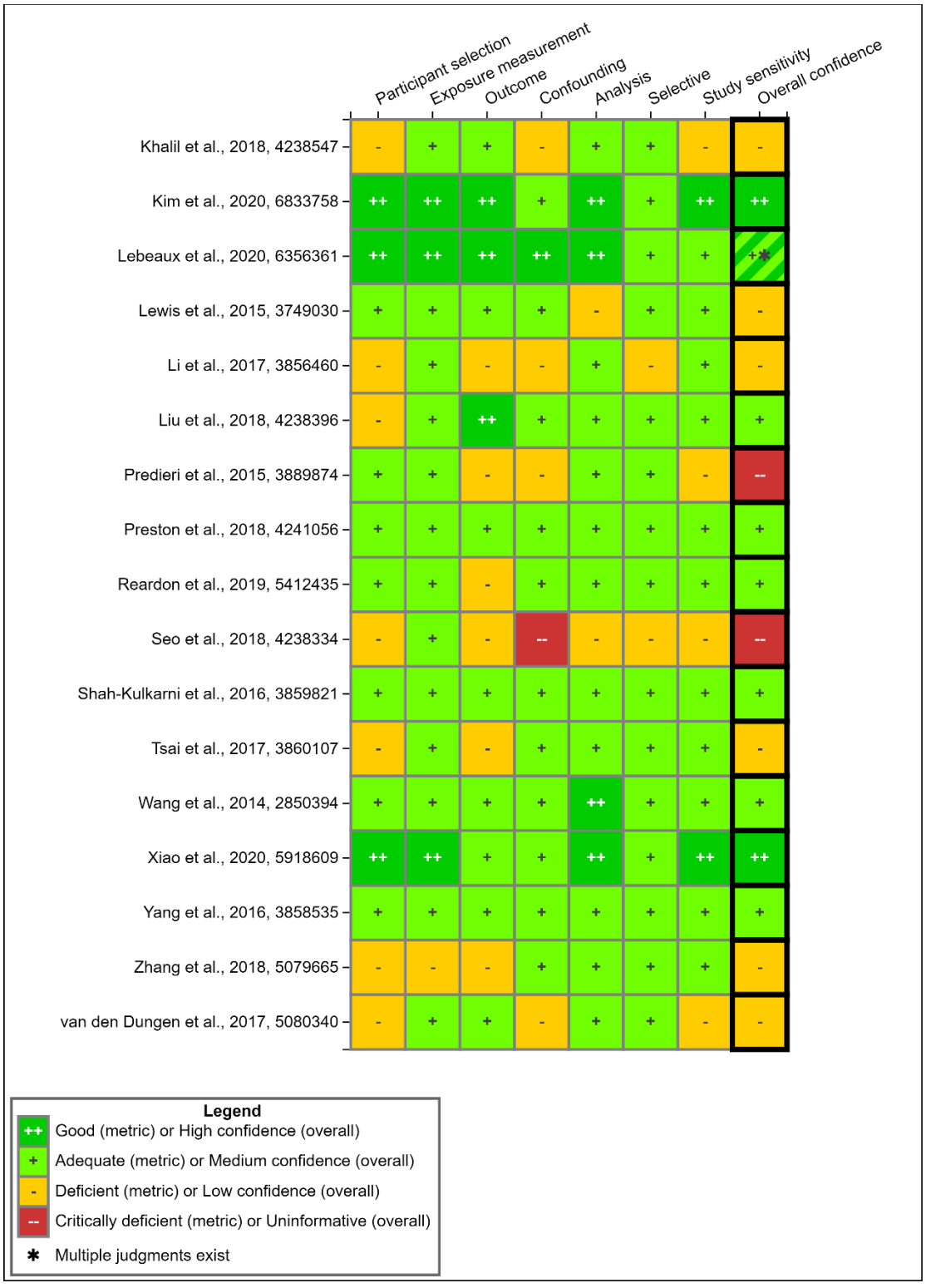


Figure C-14. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Endocrine Effects (Continued)

Interactive figure and additional study details available on HAWC.

The main concerns with *low* confidence and *uninformative* studies included a lack of consideration for outcome sampling time, small sample sizes, or use of statistical methods that did not account for confounding. Other studies rated as *low* or *uninformative* had issues regarding the analysis, including a lack of accounting for population sampling methods (Lewis et al., 2015), or use of statistical methods that did not account for confounding (Abraham et al., 2020). Case-control studies (Kim et al., 2016a; Predieri et al., 2015) were rated *uninformative* and presented issues with insufficient detail regarding participant recruitment and case definitions. However, the largest issues identified in these studies included use of statistical methods that did not account for potential confounding factors, and the sensitivity of both case-control studies was impacted by small sample sizes.

C.2.1.3 Findings From Children

One *high* confidence study (Kim et al., 2020a) observed an inverse association between PFOS concentrations and subclinical hypothyroidism (defined by reference thyroid hormone levels) at age six which was consistent after additional adjustment for dietary iodine intake. The association was observed in boys, but not in girls. A positive association was also observed for PFOS and T3 at six years of age which was significant among boys but not girls, before and after adjustment for dietary iodine intake.

Thyroid hormone levels were examined in 19 studies (Abraham et al., 2020; Kim et al., 2020a; Lebeaux et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Itoh et al., 2019; Xiao et al., 2019; Dufour et al., 2018; Kang et al., 2018; Khalil et al., 2018; Preston et al., 2018; Tsai et al., 2017; Kato et al., 2016; Kim et al., 2016a; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Predieri et al., 2015; Wang et al., 2014) and five observed significant effects (Appendix D). One *high* confidence study (Xiao et al., 2019) on children from the Faroe Islands showed a large, significant positive association between maternal third trimester PFOS concentrations and cord serum TSH. The effect size for TSH was similar in both sexes but was no longer significant in female infants. Additionally, sex-stratified analyses showed positive associations between maternal PFOS and the free thyroxine index (FTI) in cord serum for girls. A *medium* confidence study (Kato et al., 2016) on infants in Sapporo, Japan from the Hokkaido Study observed positive associations with infant TSH which were consistent after stratifying by the infant's sex. Analyses by quartile revealed a significant increasing trend (p for trend = 0.024) for infant TSH and maternal blood. A related *medium* confidence study (Itoh et al., 2019) of a separate Japanese cohort from the same region also found a significant positive association between maternal serum PFOS and TSH among boys. When stratifying by the mother's thyroid antibody (TA) status, the effect remained among boys born to TA-negative mothers. No effect was seen in TA-positive mothers, but the sample size was small (n = 48).

Other *medium* confidence cross-sectional studies in newborns (Aimuzi et al., 2019) showed significant inverse associations with TSH in single pollutant models. These associations remained for girls after stratifying by sex. A significant positive association was observed for free T3 (FT3) among this study sample, but a sensitivity analysis including only those infants with detectable free FT3 concentrations was conducted due to the low detection rate. Associations between PFOS and FT3 were no longer significant after removing participants with non-detectable levels. A *medium* confidence study (Preston et al., 2018) in infants did not show significant associations in continuous analyses; however, a significant inverse association was

found for T4 among all infants in the highest PFOS exposure quartile and among boys in in exposure quartile.

A study in Taiwan (Tsai et al., 2017) found significant positive associations for TSH and inverse associations for T4 in cord blood among the entire sample and among boys in continuous analyses. Analyses by exposure quantiles (<30th, 30th–59th, 60–89th, and ≥90th percentile) were consistent in the direction of effect, but only reached significance for each effect comparing the highest PFOS exposure quantile to the reference in the overall population. A significant effect was also seen among boys in the second quantile (30th–59th) for TSH. However, only 27% of the initially recruited population had available PFOS and thyroid measurements, and reasons for missing data were not provided. This limited the sample size (n = 118) and raised concern for potential selection bias, contributing to a *low* confidence rating.

C.2.1.4 Findings From Pregnant Women

Thyroid hormone levels were examined in six studies (Aimuzi et al., 2020; Inoue et al., 2019; Itoh et al., 2019; Reardon et al., 2019; Berg et al., 2017; Shah-Kulkarni et al., 2016) and five observed significant effects (Appendix D). One *high* confidence study (Xiao et al., 2019) observed a positive association between third trimester PFOS concentrations and maternal TSH in mothers giving birth to girls. This association was not seen in the analysis of the entire cohort or in mothers of boys only. A *medium* confidence study (Reardon et al., 2019) on a Canadian cohort of pregnant women investigated associations between multiple PFOS isomers and thyroid hormones at several timepoints during and after pregnancy. Accounting for all timepoints, a significant positive association was observed for increasing branched PFOS concentrations and TSH. The same association was not observed for linear PFOS, except at 3 months postpartum. In this study, the authors note linear PFOS contributed to 69.0% of exposure concentrations while branched PFOS constituted only 31.0%. Total PFOS exposure was not assessed. A *medium* confidence cross-sectional study (Preston et al., 2018) observed a significant inverse association for maternal TSH among TPOAb-positive mothers. One *low* confidence analysis (Kato et al., 2016) of mothers in Sapporo, Japan from the Hokkaido Study observed significant decreases for maternal TSH concentrations with increasing serum PFOS, which were also observed after stratifying by the infant's sex. Analyses by quartile confirmed this decreasing trend ($p < 0.001$). No significant effects were observed in mothers from the other Hokkaido cohort (Itoh et al., 2019). Another *low* confidence study (Berg et al., 2017) from Norway showed positive associations between maternal PFOS concentrations and TSH levels during the second trimester. Analysis by quartile showed significant associations for the two highest exposure groups, suggesting a consistent trend.

One cross-sectional study (Dufour et al., 2018) on mother-child dyads showed evidence of increased risk of hypothyroidism in mothers. Analysis by quartile showed a consistent effect, but only reached significance for mothers in the third PFOS exposure quartile. This study contained a great deal of uncertainty regarding timing of outcome ascertainment and the method of disease classification which diminish confidence in the findings for maternal hypothyroidism.

One *high* confidence study examined adrenal hormones among pregnant women in the OCC and showed a significant decrease in diurnal urinary (dU) -cortisone and increase in dU-cortisol/cortisone with twofold increases in serum PFOS concentrations (Dreyer et al., 2020). However, dU- and serum cortisol showed non-significant decreases.

C.2.1.5 Findings From the General Adult Population

Thyroid function was examined in 13 studies (Lebeaux et al., 2020; Jain and Ducatman, 2019b; Blake et al., 2018; Byrne et al., 2018; Liu et al., 2018a; Seo et al., 2018; Zhang et al., 2018b; Crawford et al., 2017; Li et al., 2017; van den Dungen et al., 2017; Christensen et al., 2016b; Lewis et al., 2015; Audet-Delage et al., 2013; Jain, 2013) and six observed significant effects (Appendix D). A *medium* confidence study (Blake et al., 2018) in individuals residing near a uranium processing facility in an area with PFAS-contaminated drinking water (Fernald Community Cohort (FCC)) reported a positive association for TSH in whole study sample. Stratifying by sex showed a difference in direction of effect between men and women, however, the interaction term did not reach significance (sex interaction p-value = 0.12). In men, the association for TSH was consistent and was accompanied by a significant inverse association with total T4; no significant associations were observed for women.

Results were mixed in three overlapping NHANES studies (Jain and Ducatman, 2019b; Lewis et al., 2015; Jain, 2013). One *low* confidence study (Lewis et al., 2015) showed several significant and borderline significant results among NHANES (2011–2012) participants. Significant positive associations were found between TSH in males (12–20 years old) and females (20–40 years old), but other results were not consistent among the same stratified groups (by sex and age). There is no evidence that the NHANES complex sampling design was accounted for in the analysis which contributed to a *low* confidence rating. Jain (2013), another *low* confidence study, did not find any significant effects among NHANES (2007–2008) participants. A *medium* confidence follow-up study (Jain and Ducatman, 2019b) examined effects on thyroid hormones stratified by glomerular filtration (GF) stage in a pooled NHANES dataset (2007–2012). A significant effect was found for total T4 in those individuals with stage 3A GF, the second most severe stage. Associations for total T4 among other stages were non-significant and inconsistent in direction of effect.

One additional cross-sectional study (Byrne et al., 2018) of Alaska Natives found a significant sex interaction for free T3. Women showed a positive association between serum PFOS and free T3 while an inverse association was found in men. Borderline significant inverse associations for TSH and total T3 were also observed among men ($p = 0.085$ and $p = 0.08$, respectively). The sensitivity of the study, however, was limited by the population size (total n = 85; male n = 38) and resulted in a *low* confidence rating. Another *low* confidence study (Li et al., 2017) conducted in China found significant associations for TSH, free T3, and free T4 among a population oversampled for thyroid conditions (70%). Inverse associations were observed for free T3 and free T4, while a positive association was found for TSH amongst the whole population. Associations were not significant when stratified by thyroid disease state (i.e., normal, hypothyroidism, Hashimoto's disease). The study was found to be *low* confidence due to missing information on recruitment and participation, especially considering this was a convenience sample. Additionally, there were concerns for selective reporting and residual confounding because individuals (n = 202) varied greatly by age (1 month to 90 years) and lifestyle factors were not addressed.

A case-control study (Zhang et al., 2018b) examined women with and without POI and observed positive associations for TSH among both cases and controls. Additionally, inverse associations were found among cases for free T3 and free T4. The thyroid hormone concentrations were within normal ranges in both cases and controls. The study was rated as *low* confidence due to

insufficient information on control recruitment and potential for reverse causation from irregular menstruation (a PFOS elimination route) for those women with PCOS.

C.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 4 studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and 10 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and endocrine effects. Study quality evaluations for these 14 studies are shown in Figure C-15.

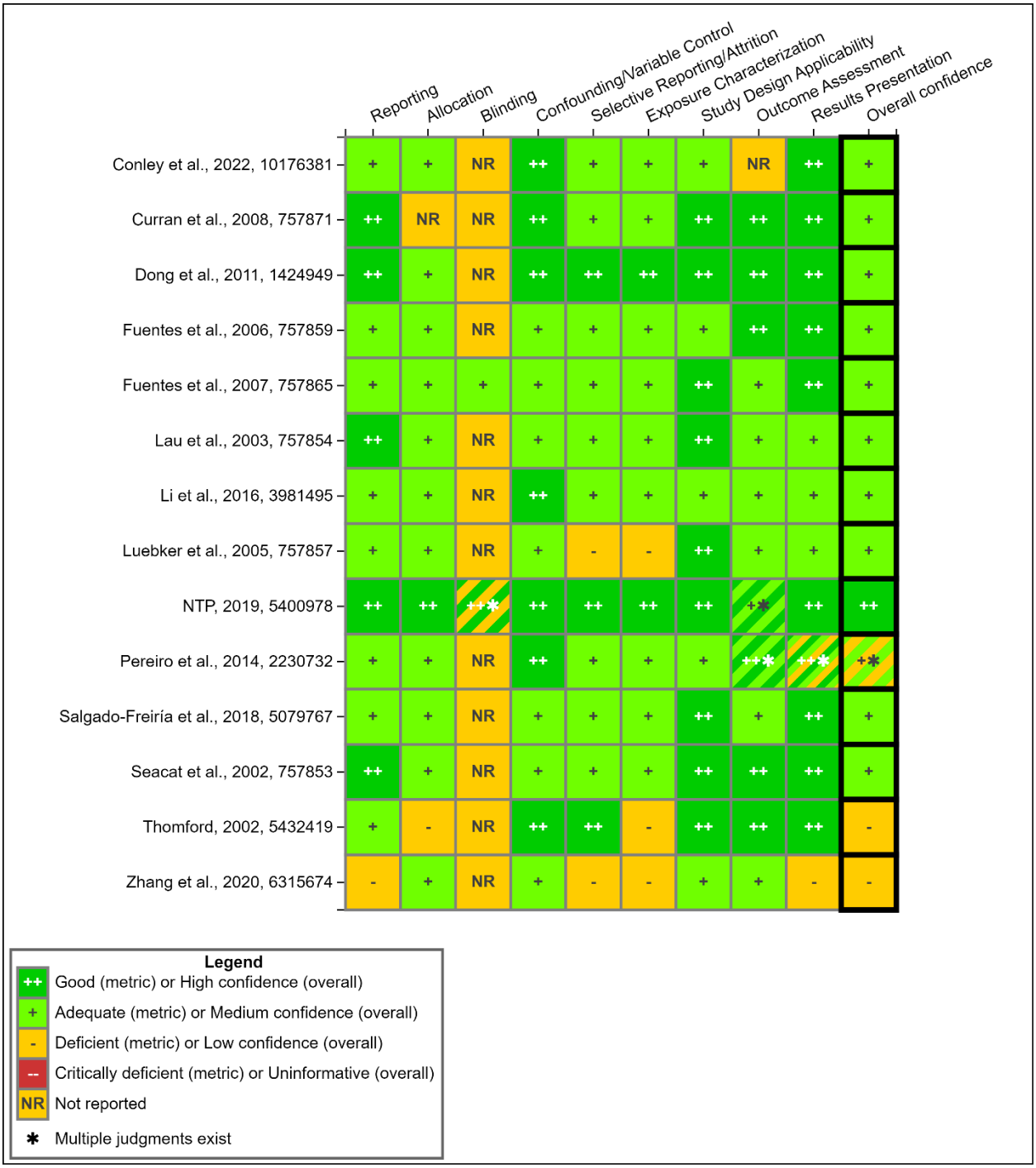


Figure C-15. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Endocrine Effects

Interactive figure and additional study details available on HAWC.

Animal studies suggest that exposure to PFOS can result in adverse effects to the endocrine system. Overall, studies of varying durations in rodent models and a single study in cynomolgus monkeys (Seacat et al., 2002) have reported reductions in endocrine hormone levels and changes

in endocrine organ weights. There are insufficient data to support non-neoplastic lesions (histopathology), and potential neoplastic lesions (see Toxicity Assessment, (U.S. EPA, 2024)). Moreover, reductions were observed in thyroid hormone levels, including total and free thyroxine (TT4 and FT4) and total and free triiodothyronine (TT3 and FT3) (NTP, 2019; Luebker et al., 2005b; Lau et al., 2003), as well as reductions in adrenocorticotropic hormone (ACTH), corticosterone, and/or corticotropin releasing hormone (CRH) (Salgado-Freiría et al., 2018; Pereiro et al., 2014). Absolute and relative adrenal gland weights were reduced in rats (NTP, 2019), however adrenal glands subject to histopathologic examination appeared normal (Pereiro et al., 2014; Chang et al., 2009; Luebker et al., 2005b) (see Toxicity Assessment, (U.S. EPA, 2024)).

C.2.2.1 Thyroid and Thyroid-Related Hormone Levels

Several 28-day studies provide evidence that exposure to PFOS can result in adverse effects on rat thyroid hormone levels (Table C-3). Male and female rats were fed PFOS at doses of 0, 2, 20, 50, or 100 ppm (equivalent to 0, 0.14, 1.33, 3.21, or 6.34 mg/kg/day in males and 0, 0.15, 1.43, 3.73, or 7.58 mg/kg/day in females) for 28-days (Curran et al., 2008). In both males and females, serum TT4 levels were significantly reduced at doses of ≥20 ppm. Serum TT3 was decreased at the 100 ppm and ≥50 ppm dose groups in males and females, respectively (Curran et al., 2008). In another study in rats, male and female Sprague-Dawley rats were exposed to PFOS at doses of 0 mg/kg/day, 0.312 mg/kg/day, 0.625 mg/kg/day, 1.25 mg/kg/day, 2.5 mg/kg/day, or 5 mg/kg/day via oral gavage (NTP, 2019). At study termination, TT4 and FT4 levels were decreased in all male and female dose groups. In addition, TT3 was significantly decreased in males and females treated with ≥0.625 mg/kg/day. No treatment-related effects were seen on TSH levels (NTP, 2019). Yu et al. (2009a) exposed male Sprague-Dawley rats to 0 mg/L, 1.7 mg/L, 5.0 mg/L, or 15.0 mg/L-PFOS in drinking water for 91 days (drinking water consumption was not reported). Significant dose-dependent reductions in TT4 were noted in animals treated at ≥ 1.7 mg/L; however, FT4 was only decreased in the 5.0 mg/L group. A statistically significant increase in TT3 was observed in the 1.7 mg/L dose group, though TT3 in the 5 mg/L and 15 mg/L groups returned to control levels. No treatment-related effects were seen in TSH (Yu et al., 2009a).

A number of reproductive/developmental studies investigated the effect of PFOS on thyroid hormone production in parental and F_1 rodents (Table C-3).

Lau et al. (2003) analyzed thyroid hormones in offspring of pregnant rats exposed by gavage to PFOS at 0 mg/kg/day, 1 mg/kg/day, 2 mg/kg/day, or 3 mg/kg/day from GD 2–GD 21. The authors reported statistically significant reductions in TT4 and FT4 on PND 5 in rat pups treated with 2 mg/kg/day and 3 mg/kg/day during gestation. Signs of recovery in TT4 were noted at weaning, while reduced FT4 persisted through PND 35. No effects were noted in serum TT3 nor TSH of pups when compared with controls (Lau et al., 2003). In a cross-fostering study conducted by Yu et al. (2009b), pregnant Wistar rats were fed a diet containing 0 mg/kg/day or 3.2 mg/kg/day PFOS throughout gestation and/or lactation. PFOS-exposed groups consisted of pups treated with PFOS during gestation only, pups treated with PFOS during lactation only, and pups treated with PFOS during gestation and lactation. Pups in all exposure groups had significant decreases in TT4 on PND 21 and PND 35. In contrast, TT3 and reverse T3 (rT3) were not affected with PFOS exposure in rat pups (Yu et al., 2009b). Another study measured serum TSH in pups and dams (GD 20, PND 4, or PND 21) following oral gavage exposure of pregnant

Sprague-Dawley rats to PFOS (0 mg/kg/day, 0.1 mg/kg/day, 0.3 mg/kg/day, or 1.0 mg/kg/day) from GD 0–PND 20. No statistically significant effects were observed in dams or offspring at any timepoint assayed (Chang et al., 2009).

Luebker et al. (2005b) exposed pregnant Female Crl:CD®(SD)IGS VAF/Plus rats to 0.4 mg/kg/day, 0.8 mg/kg/day, 1.0 mg/kg/day, 1.2 mg/kg/day, 1.6 mg/kg/day, or 2.0 mg/kg/day for 42 days prior to mating through LD 4. Exposed dams showed decreased TT4 and TT3 at doses ≥ 0.4 mg/kg/day and ≥ 1.2 mg/kg/day, respectively, although no perturbations were seen in TSH or FT4 levels. In the pups, no perturbations were noted in TT3, FT4, or TSH, however, TT4 was reduced at doses ranging from 0.4 mg/kg/day to 1.6 mg/kg/day (2.0 mg/kg/day group not assessed due to high pup mortality). The authors noted that the contributions of prenatal versus postnatal effects of PFOS on thyroid hormones were not clear (Luebker et al., 2005b). The authors also conducted follow-up analyses due to potential for negative bias from immeasurable levels of FT3 and FT4 using equilibrium dialysis-radioimmunoassay (ED-RIA) methods and measurements of TT3 and TT4 with chemiluminometric methods to ensure the validity of their initial radioimmunoassay (RIA)-based results. While the ED-RIA reference method indicated potential bias in the results for FT4 in pups, a true comparison could not be made due to insufficient sample sizes (Luebker et al., 2005b). Conley et al. (2022b) also determined levels of thyroid hormones in maternal serum following gestational exposure to PFOS. The authors reported TT3 and TT4 on GD 18 in Sprague-Dawley rats exposed to PFOS at 0 mg/kg/day, 0.1 mg/kg/day, 0.3 mg/kg/day, 1 mg/kg/day, 3, 10, or 30 mg/kg/day from GD 14–GD 18. PFOS significantly reduced TT3 and TT4 at 10 and 30 mg/kg/day. Non-significant decreases ranging from 7% to 34% in TT3 and 3%–24% in TT4 were observed in dams exposed to doses below 10 mg/kg/day. Fuentes et al. (2006) examined the effects of PFOS on thyroid hormones in CD1 mice. The dams were exposed during gestation from GD 6–GD 18 to 0 mg/kg/day, 1.5 mg/kg/day, 3 mg/kg/day, or 6 mg/kg/day. At GD 18, the dams had an overall percent reduction ranging from 11% to 57% in TT3, 36%–57% in FT3, and 42%–67% in FT4. Conversely, increases in TT4 levels ranged from 158% to 188%. Nonetheless, the differences between the exposed and control dams were not statistically significant due to high variability.

Only one study was included that investigated the effects of PFOS exposure on hormone levels during development in mice. Lau et al. (2003) exposed pregnant CD-1 mice to 0 mg/kg/day, 1 mg/kg/day, 5 mg/kg/day, 10 mg/kg/day, 15 mg/kg/day, or 20 mg/kg/day PFOS from GD 1–GD 17 and evaluated TT4 in sera of pooled mouse pups of each sex at several timepoints across postnatal development. Because of mortality in the 15 mg/kg/day and 20 mg/kg/day groups, TT4 was only assessed in the 1 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day groups. TT4 levels varied across the different time points with different trends based on treatment group. On PND 7, PND 14, and PND 28 there was a general trend for decreased TT4 in the 5 mg/kg/day and 10 mg/kg/day exposure groups when compared with control animals (Lau et al., 2003). However, this was not observed on PND 3 or PND 21. Results were not significant at any time point but may be limited by small sample size (3–7 determinations per group).

Male and female cynomolgus monkeys (4–6/sex/group) were orally exposed to PFOS at doses of 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day for 182 days (Seacat et al., 2002). Recovery animals from the 0 mg/kg/day, 0.15 mg/kg/day, and 0.75 mg/kg/day dose groups were then monitored for an additional year. On the last day of dosing (day 182), thyroid hormone levels, including TSH, TT3, and TT4 were evaluated. In males, TT3 was significantly

reduced across all dose groups while TSH and TT4 remained unaffected. In females, significant reductions in TT3 were noted in animals treated with 0.15 mg/kg/day and 0.75 mg/kg/day. Significant reductions in TT4 were noted in the mid-dose group only (0.15 mg/kg/day). TSH remained unaffected in females. Sixty-one days after cessation of treatment there was still a trend for decreased TT3 in 0.15 mg/kg/day males and 0.75 mg/kg/day males and females. Because there were only 2 animals per group at this time, statistical analyses were not appropriate. TT4 and TSH results were not reported in the recovery period (Seacat et al., 2002).

Table C-3. Summary of Results for Thyroid and Thyroid-Related Hormones in Toxicological Studies Following Exposure to PFOS

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
Total Thyroxine (TT4)							
Seacat et al. (2002)[b]	Cynomolgus Monkey	Chronic (26 wk)	Adult	M	0	4.38 ± 0.61	NA
					0.03	4.72 ± 0.68	7.8
					0.15	3.99 ± 0.62	−8.9
					0.75	5.34 ± 1.57	21.9
				F	0	5.66 ± 0.89	NA
					0.03	4.33 ± 1.46	−23.5
					0.15	3.91 ± 0.62*	−30.9
					0.75	5.61 ± 1.00	−0.9
Fuentes et al. (2006)[c]	CD-1 Mice	Developmental (GD 6–18)	P_0 Adult (GD 18)	F	0	0.50 ± 0.13	NA
					1.5	1.29 ± 0.59	158
					3	1.41 ± 0.39	182
					6	1.44 ± 0.57	188
Conley et al. (2022b)[c]	Sprague-Dawley	Developmental (GD 14–18)	P_0 Adult (GD 18)	F	0	3.27 ± 0.83	NA
					0.1	2.49 ± 0.43	−24
					0.3	2.42 ± 0.35	−26
					1	3.18 ± 0.95	−3
					3	2.49 ± 0.42	−24
					10	1.67 ± 0.47	−49
					30	1.04 ± 0.50	−68
Lau et al. (2003)[c,d]	CD-1 Mice	Developmental (GD 1–17)	F_1 Pups (PND 28)	M/F	0	4.2 ± 0.9	NA
					1	3.8 ± 0.5	−9.5
					5	3.6 ± 0.5	−14.3
					10	3.5 ± 0.3	−16.7
Curran et al. (2008)[b]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	6.27 ± 0.92	NA
					0.14	5.19 ± 1.14	−17.3
					1.33	1.11 ± 0.32*	−82.3
					3.21	1.00 ± 0.21*	−84.1
					6.34	1.03 ± 0.20*	−83.6
				F	0	2.92 ± 1.19	NA
					0.15	2.51 ± 0.81	−14.1
					1.43	1.52 ± 0.19*	−48.0

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					3.73	1.17 ± 0.15*	−60.1
					7.58	1.27 ± 0.36*	−56.5
NTP (2019)[c]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	3.51 ± 0.3	NA
					0.312	1.33 ± 0.19*	−62.1
					0.625	0.53 ± 0.09*	−84.9
					1.25	0.26 ± 0.07*	−92.6
					2.5	0.22 ± 0.04*	−93.7
					5	0.48 ± 0.07*	−86.3
				F	0	2.21 ± 0.24	NA
					0.312	1.11 ± 0.12*	−49.8
					0.625	0.55 ± 0.07*	−75.1
					1.25	0.33 ± 0.07*	−85.1
					2.5	0.35 ± 0.09*	−84.2
					5	0.38 ± 0.05*	−82.8
Yu et al. (2009a)[c]	Sprague-Dawley Rats	Subchronic (91 d)	Adult	M	0	4.09 ± 0.18	NA
					0.0017	2.39 ± 0.13*	−41.6
					0.005	1.64 ± 0.54*	−59.9
					0.015	0.85 ± 0.16*	−79.2
Lau et al. (2003)[c,d]	Sprague-Dawley Rats	Developmental (GD 2–21)	F_1 Adult (PND 35)	M/F	0	4.3 ± 0.5	NA
					1	3 ± 0.2	−30.2
					2	2.5 ± 0.2*	−41.9
					3	2 ± 0.1*	−53.5
Luebker et al. (2005b)[b]	Crl:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80 d (42 d pre-mating, GD 0–21, LD 1–4))	P_0 Adult (LD 5)	F	0.0	1.5 ± 0.63	NA
					0.4	0.81 ± 0.41*	−46.0
					0.8	0.6 ± 0.44*	−60.0
					1.0	0.73 ± 0.24*	−51.3
					1.2	0.28 ± 0.32*	−81.3
					1.6	0.27 ± 0.17*	−82.0
					2.0	0.24 ± 0.15*	−84.0
			F_1 Pups (PND 5)[e]	M/F	0.0	0.54 ± 0.22	NA
					0.4	0 ± 0	−100.0
					0.8	0 ± 0	−100.0
					1.0	0.02 ± 0.05	−96.3
					1.2	0.01 ± 0.02	−98.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					1.6	0.01 ± 0.0	−98.1
					2.0	–[f]	–
			F_1 Pups (PND5)[g]	M/F	0.0	2.1 ± 0.6	NA
					0.4	1.6 ± 0.4	−23.8
					0.8	1.5 ± 0.7	−28.6
					1.0	1.5 ± 0.5	−28.6
					1.2	–	–
					1.6	–	–
					2.0	–	–
Yu et al. (2009b)[c,h]	Wistar Rats	Reproductive (GD 0–PND 35)	F_1 Pups (PND 14)	M/F	0	6.78 ± 0.35	NA
					3.2 (Gestation Only)	6.36 ± 0.25	−6.2
					3.2 (Lactation Only)	5.97 ± 0.39	−11.9
					3.2 (Gestation and Lactation)	4.29 ± 0.17*	−36.7
			F_1 Pups (PND 21)	M/F	0	5.81 ± 0.31	NA
					3.2 (Gestation Only)	4.63 ± 0.27*	7.9
					3.2 (Lactation Only)	4.15 ± 0.26*	−3.3
					3.2 (Gestation and Lactation)	4.38 ± 0.24*	2.1
			F_1 Pups (PND 35)	M/F	0	6.75 ± 0.35	NA
					3.2 (Gestation Only)	5.44 ± 0.33*	−19.4
					3.2 (Lactation Only)	4.33 ± 0.30*	−35.9
					3.2 (Gestation and Lactation)	4.23 ± 0.22*	−37.3
Free Thyroxine (FT4)							
NTP (2019)[c]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	0.00253 ± 0.00022	NA
					0.312	0.00095 ± 0.0001*	−62.5
					0.625	0.00047 ± 0.00005*	−81.4
					1.25	0.0004 ± 0.00002*	−84.2

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					2.5	0.00036 ± 0.00005*	−85.8
					5	0.00033 ± 0.00001*	−87.0
				F	0	0.00174 ± 0.00023	NA
					0.312	0.00107 ± 0.00009*	−38.5
					0.625	0.0007 ± 0.00003*	−59.8
					1.25	0.00064 ± 0.00005*	−63.2
					2.5	0.00056 ± 0.00005*	−67.8
					5	0.00048 ± 0.00003*	−72.4
Yu et al. (2009a)[c]	Sprague-Dawley Rats	Subchronic (91 d)	Adult	M	0	1.9 ± 0.13	NA
					0.0017	1.67 ± 0.14	−12.1
					0.005	1.26 ± 0.15*	−33.7
					0.015	1.73 ± 0.11	−8.9
Fuentes et al. (2006)[c]	CD1 Mice	Developmental (GD 6–18)	P_0 Adult (GD 18)	F	0	0.078 ± 0.038	NA
					1.5	0.045 ±0.007	−42%
					3	0.060 ±0.011	−23%
					6	0.026 ±0.014	−67%
Lau et al. (2003)[c,d]	Sprague-Dawley Rats	Developmental (GD 2–21)	F_1 Adult (PND 35)	M/F	0	0.02 ± 0.002	NA
					1	0.014 ± 0.000	−30.0
					2	0.009 ± 0.001	−55.0
					3	0.011 ± 0.001	−45.0
Luebker et al. (2005b)[b]	Crl:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80 d (42 d pre-mating, GD 0–21, LD 1–4))	P_0 Adult (LD 5)	F	0.0	0.00236 ± 0.00061	NA
					0.4	0.00212 ± 0.00058	−10.2
					0.8	0.00261 ± 0.00056	10.6
					1.0	–	–
					1.2	0.00248 ± 0.00022	5.1
					1.6	0.00259 ± 0.00082	9.7
					2.0	–	–
			F_1 Pups (PND 5)	M/F	0.0	0.0019 ± 0.0009	NA
					0.4	0.0013 ± 0.0004	−31.6
					0.8	–	–
					1.0	–	–
					1.2	–	–
					1.6	–	–
					2.0	–	–

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
Free Triiodothyronine (FT3)							
Fuentes et al. (2006)[c]	CD1 Mice	Developmental (GD 6–18)	P_0 Adult (GD 18)	F	0	0.014 ± 0.003	NA
					1.5	0.009 ± 0.001	−36
					3	0.006 ± 0.001	−57
					6	0.008 ± 0.003	−43
Luebker et al. (2005b)[b]	Crl:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80 d (42 d pre-mating, GD 0–21, LD 1–4))	F_1 Pups (LD 5)	M/F	0.0	0.00019 ± 0.00002	NA
					0.4	0.0002 ± 0.00003	5.3
					0.8	0.00015[i]	−21.1
					1.0	0.00018 ± 0.00006	−5.3
					1.2	–	–
					1.6	–	–
					2.0	–	–
Total Triiodothyronine (TT3)							
Fuentes et al. (2006)[c]	CD1 Mice	Developmental (GD 6–18)	P_0 Adult (GD 18)	F	0	0.105 ± 0.034	NA
					1.5	0.045 ± 0.002	−57
					3	0.051 ± 0.008	−51
					6	0.093 ± 0.017	−11
Conley et al. (2022b)[c]	Sprague-Dawley	Developmental (GD 14–18)	P_0 Adult (GD 18)	F	0	0.106 ± 0.013	NA
					0.1	0.082 ± 0.016	−23
					0.3	0.070 ± 0.001	−34
					1	0.099 ± 0.022	−7
					3	0.079 ± 0.014	−25
					10	0.069 ± 0.015*	−35
					30	0.040 ± 0.006*	−62
Seacat et al. (2002)[b]	Cynomolgus Monkey	Chronic (26 wk)	Adult	M	0	0.16 ± 0.007	NA
					0.03	0.119 ± 0.031*	−25.6
					0.15	0.125 ± 0.015*	−21.9
					0.75	0.066 ± 0.027*	−58.8
				F	0	0.135 ± 0.031	NA
					0.03	0.12 ± 0.024	−11.1
					0.15	0.097 ± 0.008*	−28.1
					0.75	0.085 ± 0.012*	−37.0
Curran et al., 2008, 757871[b]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	10.39 ± 2.14	NA
					0.14	11.75 ± 1.23	13.1
					1.33	8.83 ± 1.69	−15.0

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					3.21	8.38 ± 8.38	−19.4
					6.34	7.86 ± 1.49*	−24.4
				F	0	11.88 ± 1.10	NA
					0.15	11.17 ± 0.91	−6.0
					1.43	11.36 ± 1.75	−4.4
					3.73	9.15 ± 1.43*	−23.0
					7.58	8.25 ± 1.30*	−30.6
NTP (2019)[c]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	0.08737 ± 0.00532	NA
					0.312	0.07781 ± 0.00544	−10.9
					0.625	0.06063 ± 0.00464*	−30.6
					1.25	0.0575 ± 0.00267*	−34.2
					2.5	0.05535 ± 0.00275*	−36.6
					5	0.05[i]*	−42.8
				F	0	0.09305 ± 0.00504	NA
					0.312	0.0814 ± 0.00302	−12.5
					0.625	0.07252 ± 0.00427*	−22.1
					1.25	0.0692 ± 0.00363*	−25.6
					2.5	0.06203 ± 0.00178*	−33.3
					5	0.05157 ± 0.00143*	−44.6
Yu et al. (2009a)[c]	Sprague-Dawley Rats	Subchronic (91 d)	Adult	M	0	0.029 ± 0.004	NA
					0.0017	0.048 ± 0.008*	65.5
					0.005	0.023 ± 0.005	−20.7
					0.015	0.023 ± 0.003	−20.7
Lau et al. (2003)[c,d]	Sprague-Dawley Rats	Developmental (GD 2–21)	F_1 Adult (PND 35)	M/F	0	0.08 ± 0.00	NA
					1	0.09 ± 0.00	12.5
					2	0.09 ± 0.01	12.5
					3	0.11 ± 0.01	37.5
Luebker et al. (2005b)[b]	Crl:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80 d (42 d pre-mating, GD 0–21, LD 1–4))	P_0 Adult (LD 5)	F	0.0	0.0760 ± 0.0185	NA
					0.4	0.0729 ± 0.0135	−4.1
					0.8	0.0638 ± 0.00668	−16.1
					1.0	0.0624 ± 0.0132	−17.9
					1.2	0.0529 ± 0.015*	−30.4
					1.6	0.0470 ± 0.020*	−38.2
					2.0	0.0533 ± 0.0173*	−29.9

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
			F_1 Pups (PND 5)[e]	M/F	0.0	0.054 ± 0.018	NA
					0.4	0.056 ± 0.019	3.7
					0.8	0.049 ± 0.018	−9.3
					1.0	0.048 ± 0.009	−11.1
					1.2	0.045 ± 0.022	−16.7
					1.6	0.033 ± 0.008	−38.9
					2.0	0.033 ± 0.012	−38.9
			F_1 Pups (PND 5)[g]	M/F	0.0	0.0424 ± 0.0057	NA
					0.4	0.0362 ± 0.0062	−14.6
					0.8	0.03[i]	−29.2
					1.0	0.03 ± 0*	−29.2
					1.2	–	–
					1.6	–	–
					2.0	–	–
Yu et al. (2009b)[c,h]	Wistar Rats	Reproductive (GD 0–PND 35)	F_1 Pups (PND 14)	M/F	0	0.057 ± 0.004	NA
					3.2 (Gestation Only)	0.052 ± 0.004	−8.8
					3.2 (Lactation Only)	0.051 ± 0.003	−10.5
					3.2 (Gestation and Lactation)	0.043 ± 0.003	−24.6
			F_1 Pups (PND 21)	M/F	0	0.058 ± 0.003	NA
					3.2 (Gestation Only)	0.065 ± 0.007	12.1
					3.2 (Lactation Only)	0.058 ± 0.004	0.0
					3.2 (Gestation and Lactation)	0.059 ± 0.003	1.7
			F_1 Pups (PND 35)	M/F	0	0.059 ± 0.003	NA
					3.2 (Gestation Only)	0.052 ± 0.003	−11.9
					3.2 (Lactation Only)	0.049 ± 0.004	−16.9
					3.2 (Gestation and Lactation)	0.055 ± 0.002	−6.8

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
Reverse Triiodothyronine (rT3)							
Yu et al. (2009b)[c,h]	Wistar Rats	Reproductive (GD 0–PND 35)	F_1 Pups (PND 14)	M/F	0	–	–
					3.2 (Gestation Only)	–	–
					3.2 (Lactation Only)	–	–
					3.2 (Gestation and Lactation)	–	–
			F_1 Pups (PND 21)	M/F	0	0.025[i]	NA
					3.2 (Gestation Only)	0.025 ± 0.003	0.0
					3.2 (Lactation Only)	0.029 ± 0.001	16.0
					3.2 (Gestation and Lactation)	0.025 ± 0.002	0.0
			F_1 Pups (PND 35)	M/F	0	0.02 ± 0.002	NA
					3.2 (Gestation Only)	0.02 ± 0.002	0.0
					3.2 (Lactation Only)	0.015 ± 0.000	−25.0
					3.2 (Gestation and Lactation)	0.02 ± 0.001	0.0
Thyroid Stimulating Hormone (TSH)							
Seacat et al. (2002)[b]	Cynomolgus Monkey	Chronic (26 wk)	Adult	M	0	0.43 ± 0.52[j]	NA
					0.03	0.34 ± 0.3[j]	−20.9
					0.15	0.74 ± 0.75[j]	72.1
					0.75	0.93 ± 0.57[j]	116.3
				F	0	0.73 ± 1.12[j]	NA
					0.03	0.68 ± 0.82[j]	−6.8
					0.15	1.27 ± 1.52[j]	74.0
					0.75	0.84 ± 0.79[j]	15.1
NTP (2019)[e]	Sprague-Dawley Rats	Short-term (28 d)	Adult	M	0	2.039 ± 0.14	NA
					0.312	1.494 ± 0.174	−26.7
					0.625	1.479 ± 0.12	−27.5
					1.25	2.333 ± 0.294	14.4

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					2.5	2.419 ± 0.338	18.6
					5	1.890 ± 0.239	−7.3
				F	0	1.286 ± 0.073	NA
					0.312	1.476 ± 0.088	14.8
					0.625	1.276 ± 0.085	−0.8
					1.25	1.325 ± 0.115	3.0
					2.5	1.4914 ± 0.195	16.0
					5	1.536 ± 0.073	19.4
Yu et al. (2009a)[c]	Sprague-Dawley Rats	Subchronic (91 d)	Adult	M	0	0.072 ± 0.030	NA
					0.0017	0.067 ± 0.027	−6.9
					0.005	0.112 ± 0.034	55.6
					0.015	0.162 ± 0.067	125.0
Chang et al. (2009)[c,d]	Sprague-Dawley Rats	Developmental (GD 0–PND 20)	P_0 Adult (GD 20)	F	0	1.304 ± 0.102	NA
					0.1	1.202 ± 0.096	−7.8
					0.3	1.061 ± 0.058	−18.6
					1	1.1 ± 0.077	−15.6
			P_0 Adult (PND 4)	F	0	1.036 ± 0.115	NA
					0.1	1.119 ± 0.121	8.0
					0.3	0.863 ± 0.032	−19.3
					1	1.023 ± 0.083	−1.3
			P_0 Adult (PND 21)	F	0	1.714 ± 0.205	NA
					0.1	1.758 ± 0.166	2.6
					0.3	1.483 ± 0.128	−13.5
					1	1.95 ± 0.198	13.8
			F_1 Pups (PND 21)	M	0	0.765 ± 0.060	NA
					0.1	0.994 ± 0.089	29.93
					0.3	0.949 ± 0.080	24.05
					1	0.880 ± 0.045	15.03
			F_1 Pups (PND 21)	F	0	0.880 ± 0.06	NA
					0.1	0.889 ± 0.074	1.0
					0.3	0.865 ± 0.07	−1.7
					1	0.840 ± 0.065	−4.5
			F_1 Pups (GD 20)	M/F	0	1.212 ± 0.134	NA
					0.1	1.053 ± 0.08	−13.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					0.3	0.934 ± 0.075	−22.9
					1	0.969 ± 0.075	−20.0
			F_1 Pups (PND 4)	M/F	0	0.557 ± 0.065	NA
					0.1	0.552 ± 0.02	−0.9
					0.3	0.477 ± 0.07	−14.4
					1	0.542 ± 0.06	−2.7
Lau et al. (2003)[c,d]	Sprague-Dawley Rats	Developmental (GD 2–21)	F_1 Adult (PND 35)	M/F	0	0.62 ± 0.08	NA
					1	0.73 ± 0.16	17.7
					2	0.65 ± 0.06	4.8
					3	0.29 ± 0.02	−53.2
Luebker et al. (2005b)[b]	Crl:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80 d (42 d pre-mating, GD 0–21, LD 1–4))	P_0 Adult (LD 5)	F	0.0	0.163 ± 0.096	NA
					0.4	0.114 ± 0.023	−30.1
					0.8	0.144 ± 0.092	−11.7
					1.0	0.111 ± 0.052	−31.9
					1.2	0.145 ± 0.103	−11.0
					1.6	0.167 ± 0.077	2.5
					2.0	0.153 ± 0.068	−6.1
			F_1 Pups (PND 5)	M/F	0.0	0.102 ± 0.017	NA
					0.4	–	–
					0.8	–	–
					1.0	0.236[i]	131.4
					1.2	0.101 ± 0.025	−1.0
					1.6	0.145 ± 0.034*	42.2
					2.0	0.15[i]	47.1

Notes: wk = weeks; F = female; F_1 = first generation; GD = gestation day; LD = lactation day; M = male; NA = not applicable; P_0 = parental generation; PND = postnatal day; TT4 = total thyroxine; FT4 = free thyroxine; FT3 = free triiodothyronine; TT3 = total triiodothyronine; rT3 = reverse Triiodothyronine; d = days; TSH = thyroid stimulating hormone.

*Statistically significant at $p \leq 0.05$.

[a] Values were converted to µg/dL for Seacat et al. (2002) (ng/dL TT3, FT3, FT4; uU/mL TSH); Curran et al. (2008) (nmol/L T4; nmol/L TT3); NTP (2019) (ng/dL FT4, ng/dL TT3; ng/mL TSH); Yu et al. (2009a) (µg/L TT4; µg/L FT4; µg/L TT3; µg/L TSH); Lau et al. (2003) (ng/mL TT4; pg/mL FT4; ng/mL TT3; ng/mL TSH); Luebker et al. (2005b) (ng/dL FT4; pg/mL FT3; ng/dL TT3; ng/mL TSH); Yu et al. (2009b) (ng/mL TT4; ng/mL TT3; ng/mL rT3); Chang et al. (2009) (ng/mL TSH); Conley et al. (2022b) (ng/mL TT3, TT4); Fuentes et al. (2006) (ng/dL TT3, FT3, FT4).

[b] Data are presented as mean ± standard deviation.

[c] Data are presented as mean ± standard error.

[d] Values were estimated from a figure using a digital ruler.

[e] Analyzed by analog radioimmunoassay (RIA).

[f] Insufficient sample for analysis.
[g] Analyzed by analog chemiluminometric assay (CL).
[h] Cross-foster study.
[i] n = 1.
[j] Units in μU/mL.

C.2.2.2 Hypothalamic, Pituitary, and/or Adrenal Hormone Levels

Effects of PFOS exposure on hormones of the hypothalamus, pituitary gland, and adrenals were available in two rat studies conducted by the same laboratory (Figure C-16). Salgado-Freiría et al. (2018) and Pereiro et al. (2014) investigated the effect of PFOS exposure on hypothalamic CRH, ACTH, and corticosterone of male Sprague-Dawley rats treated at 0 mg/kg/day, 0.5 mg/kg/day, 3.0 mg/kg/day, and 6.0 mg/kg/day for 28 days. Following exposure, decreases in serum CRH and corticosterone concentrations in all dose groups were observed, but there was no dose-related trend. However, a dose-dependent decrease in ACTH was observed. In a reproductive/developmental study, pregnant Sprague-Dawley rats were administered 0 mg/kg/day, 5 mg/kg/day, and 20 mg/kg/day from GD 12–GD 18 via gavage (Li et al., 2016). Fetal serum corticosterone levels were significantly increased in animals treated with 5 mg/kg/day and 20 mg/kg/day.

Three studies in mice have examined the effects of PFOS exposure on serum corticosterone (Dong et al., 2011; Fuentes et al., 2007b; Fuentes et al., 2006). Fuentes et al. (2006) observed 1% and 5% decreases at 1.5 mg/kg and 6 mg/kg respectively; and an 8% increase at 3 mg/kg indicating there was no dose-related trend in pregnant CD1 mice. Dose-dependent increases of approximately 20% and 50% were recorded in male CD1 mice following a 4-week exposure to 3 or 6 mg/kg/day PFOS (Fuentes et al., 2007b). In male C57BL/6 mice exposed to 0 mg/kg/day, 0.008 mg/kg/day, 0.017 mg/kg/day, 0.083 mg/kg/day, 0.417 mg/kg/day, or 0.833 mg/kg/day over the course of 60 days, serum corticosterone decreased by 2%, 13%, and 17% at 0.008, 0.017, and 0.083 mg/kg/day (low doses) and increased by 2% and 19% at 0.417 mg/kg/day and 0.833 mg/kg (high doses), indicating a biphasic dose-response trend (Dong et al., 2011). Although the changes in serum corticosterone seem to be related to exposure, they were not statistically significant, likely due to variability.

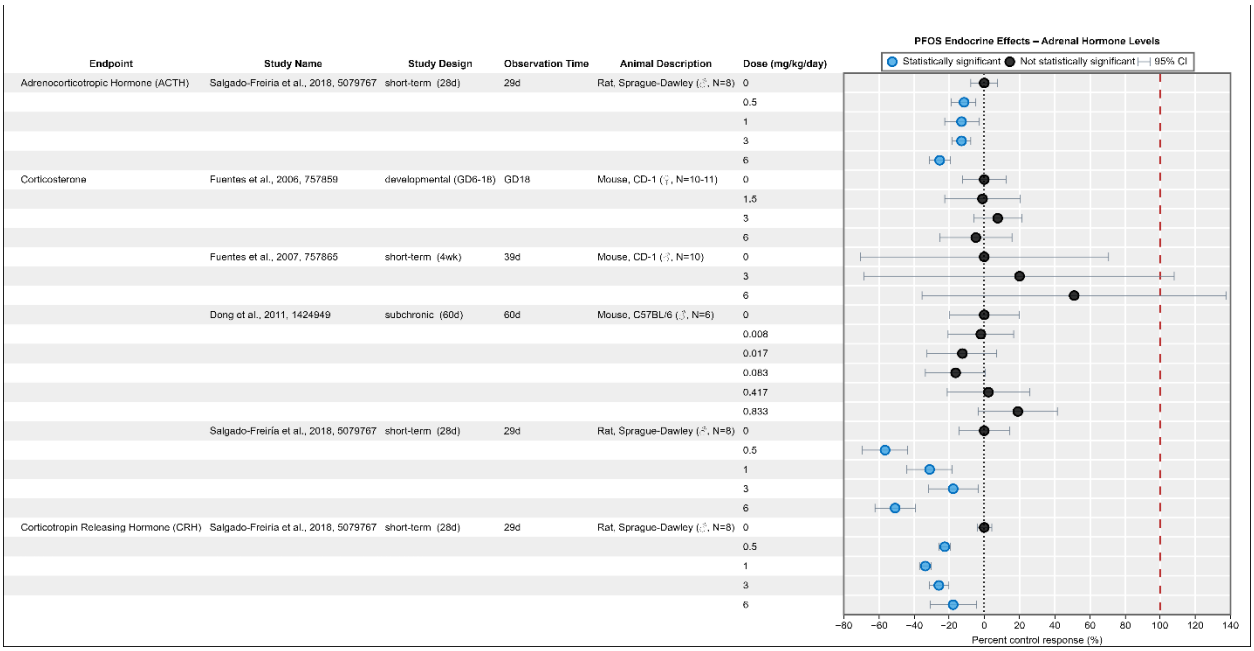


Figure C-16. Percent Change in Adrenal Hormones Relative to Controls in Rodents Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC.
ACTH = adenocorticotropic hormone; CRH = corticotropin releasing hormone; CI = confidence interval.
[a]Pereiro et al. (2014) reported on the same data as Salgado-Freiria et al. (2018) and is not shown in the figure.
[b]The red dashed lines indicate a 100% increase from the control response.

C.2.2.3 Organ Weights

No adverse effects on male and female thyroid weights (Table C-4) were noted in the previously mentioned NTP study (NTP, 2019). In a longer-term study conducted by Yu et al. (2009a), no treatment-related effects were observed on absolute and relative thyroid weights in Sprague-Dawley rats exposed to PFOS in drinking water at doses of 0 mg/L, 1.7 mg/L, 5.0 mg/L, or 15 mg/L for 91 days (Yu et al., 2009a). However, in Sprague-Dawley rats exposed to 2 mg/kg/day, 20 mg/kg/day, 50 mg/kg/day, or 100 mg/kg/day in the diet for 28 days, relative thyroid weight was significantly increased in females and males in the highest dose group. No treatment-related effects were observed on absolute thyroid weight or thyroid weight relative to brain weight (Curran et al., 2008).

PFOS exposure was associated with changes in adrenal gland weighs in rats and non-human primates (Table C-4). In Sprague-Dawley rats, absolute right adrenal gland weights in male rats were reduced at doses ≥ 1.25 mg/kg/day. No effects were observed in females (NTP, 2019). No effects were observed in relative adrenal weights at any dose for either sex after 28 days of exposure to 0 mg/kg/day–5 mg/kg/day via gavage (NTP, 2019). Additionally, relative adrenal gland weight was decreased in male rats treated at doses of ≥ 0.5 mg/kg/day for 28 days (Pereiro et al., 2014). Curran et al. (2008) observed significant trends toward increased adrenal gland weight relative to body weights and increased adrenal gland weight relative to brain weights in male and female Sprague-Dawley rats exposed to 0 mg/kg/day, 2 mg/kg/day, 20 mg/kg/day, 50 mg/kg/day, or 100 mg/kg/day PFOS for 28 days. Seacat et al. (2002) measured absolute and relative adrenal weights in male cynomolgus monkeys exposed to PFOS at doses of

0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day for 182 days. The only significant treatment-related effect was an increase in left adrenal-to-body weight percentages in males of the high dose group (Seacat et al., 2002). No studies were available evaluating the effect of PFOS exposure on mouse organ weights.

Effects on the relative weight of the hypothalamus were observed by Salgado et al. (2015) (see Toxicity Assessment, (U.S. EPA, 2024)).

Table C-4. Associations Between PFOS Exposure and Endocrine Organ Weights in Rodents and Non-human Primates

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
Adrenal Weight, Right, Absolute	NTP (2019)	Sprague-Dawley rat	28 d	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	↓ 1.25–5.0 mg/kg/day
					F	n.s.
Adrenal Weight, Right, Relative	NTP (2019)	Sprague-Dawley rat	28 d	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.
Adrenal Weight Absolute	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 0.14, 1.33, 3.21, 6.34 mg/kg/day	M	n.s.
				0, 0.15, 1.43, 3.73, 7.58 mg/kg/day	F	↑ 1.43 mg/kg/day
Adrenal Weight, Relative to Body Weight	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 0.14, 1.33, 3.21, 6.34 mg/kg/day	M	n.s.
				0, 0.15, 1.43, 3.73, 7.58 mg/kg/day	F	↑ 3.73 mg/kg/day
Adrenal Weight, Relative to Brain Weight	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 0.14, 1.33, 3.21, 6.34 mg/kg/day	M	n.s.
				0, 0.15, 1.43, 3.73, 7.58 mg/kg/day	F	↑ 1.43 mg/kg/day
Adrenal Weight, Relative	Pereiro et al. (2014)	Sprague-Dawley rat	28 d	0, 0.5, 1, 3, 6 mg/kg/day	M	↓ 0.5–6 mg/kg/day
Adrenal Weight, Left, Relative to Body Weight	Seacat et al. (2002)	Cynomolgus monkeys	182 d	0, 0.03, 0.15, 0.75 mg/kg/day	M	↑ 0.75 mg/kg/day
					F	n.s.
Adrenal Weight, Left, Relative to Brain Weight	Seacat et al. (2002)	Cynomolgus monkeys	182 d	0, 0.03, 0.15, 0.75 mg/kg/day	M	n.s.
					F	n.s.

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
Thyroid Weight, Absolute	NTP (2019)	Sprague-Dawley rat	28 d	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.
	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 2, 20, 50, 100 mg/kg/day	M	n.s
	Yu et al. (2009a)	Sprague-Dawley rat	91 d	0, 1.7, 5.0, or 15 mg/L	M	n.s.
Thyroid Weight, Relative to Body Weight	NTP (2019)	Sprague-Dawley rat	28 d	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.
	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 2, 20, 50, 100 mg/kg/day	M	n.s.
	Yu et al. (2009a)	Sprague-Dawley rat	91 d	0, 1.7, 5.0, or 15 mg/L	M	n.s.
	Seacat et al. (2002)	Cynomolgus monkeys	182 d	0, 0.03, 0.15, 0.75 mg/kg/day	M	n.s.
					F	n.s.
Thyroid weight, Relative to Brain Weight	Curran et al. (2008)	Sprague-Dawley rat	28 d	0, 2, 20, 50, 100 mg/kg/day	M	n.s.

Notes: F = female; M = male; n.s. = non-significant.

C.2.2.4 Histopathology

Few histological and morphometric abnormalities were observed in fetal and neonatal thyroid glands in Sprague-Dawley rats that were orally administered PFOS at doses of 0 or 1 mg/kg/day from GD 0–PND 20 (Chang et al., 2009). On GD 20, female fetuses had a significantly higher number of thyroid follicular epithelial cells compared with controls (2.1-fold increase); the number of follicular epithelial cells were not statistically different from controls in male fetuses. No other treatment-related histologic changes in number of follicles present and the distribution of follicle sizes were observed in fetuses at GD 20 or in neonates at PND 4 or PND 21 (Chang et al., 2009). Luebker et al. (2005b) examined the thyroid gland of one male and female Crl:CD®(SD)IGS VAF/Plus pup exposed to 2 mg/kg/day (highest dose group) PFOS through LD4. No microscopic changes were noted (Luebker et al., 2005b).

Pereiro et al. (2014) examined the effect of oral PFOS exposure on the adrenal cortex of male Sprague-Dawley rats treated with 0 mg/kg/day, 0.5 mg/kg/day, 1.0, 3.0 and 6.0 mg/kg/day for 28 days. Fasculata zona cells appeared more activated (presenting spongy cytoplasm due to the presence of liposomes) in animals treated with PFOS when compared with control animals. However, incidence data of non-neoplastic lesions and statistical analysis were not reported/conducted (Pereiro et al., 2014). In contrast, NTP (2019) did not observe histopathological changes in the thyroid, adrenal, or pituitary glands of male or female rats dosed with up to 5 mg/kg/day PFOS for 28 days.

In male and female cynomolgus monkeys orally exposed to PFOS at doses of 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day for 182 days, no treatment-related effect on cell proliferation of the pancreas was observed (Seacat et al., 2002).

C.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse endocrine outcomes is discussed in Sections 3.2.5, 3.3.2, 3.3.6, and 3.4.1.5 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are 29 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to endocrine effects. A summary of these studies is shown in Figure C-17. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to endocrine effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	2	0	2
Cell Growth, Differentiation, Proliferation, Or Viability	3	12	15
Cell Signaling Or Signal Transduction	2	6	8
Extracellular Matrix Or Molecules	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	2	3
Hormone Function	8	13	20
Inflammation And Immune Response	0	1	1
Oxidative Stress	3	0	3
Xenobiotic Metabolism	1	1	2
Other	0	2	2
Not Applicable/Not Specified/Review Article	1	0	1
Grand Total	12	18	29

Figure C-17. Summary of Mechanistic Studies of PFOS and Endocrine Effects

Interactive figure and additional study details available on HAWC.

C.2.4 Evidence Integration

There is *slight* evidence for an association between PFOS exposure and endocrine effects in humans based on studies reporting positive associations for TSH in children and adults. The

2016 PFOS HESD (U.S. EPA, 2016c) included two studies reporting positive associations with thyroid disease in NHANES participants. In this updated review, further evidence on the relationship between PFOS and thyroid disease was limited to two studies, one of which reported an inverse association in children (Kim et al., 2020a) and the other was classified as *uninformative.* The most consistent effects were for TSH in children. Three *medium* confidence studies (Itoh et al., 2019; Xiao et al., 2019; Kato et al., 2016) reported elevated TSH among infants with increasing PFOS exposure, but other studies found the opposite effect (Aimuzi et al., 2019). General population studies in adults also suggested a positive association between PFOS exposure and TSH, but results were limited to one *medium* confidence study, while the rest were *low* confidence. Interestingly, two general population studies identified seemingly sexually dimorphic effects for TSH (Blake et al., 2018) and T3 (Byrne et al., 2018). The 2016 Health Assessment included three studies reporting positive associations between serum PFOS and TSH in pregnant women. In the recent literature, one *high* and one *medium* confidence study reported positive association, while there was inconsistent evidence in *low* confidence studies. Additional uncertainty exists due to the potential for confounding by other PFAS. One study (Aimuzi et al., 2019) on infants reported correlations across PFAS (i.e., PFOA, PFNA, PFDA, perfluoroundecanoic acid (PFUnDA), PFHxS, and PFDoA) and found them to be moderately correlated (r = 0.37–82). Results for PFOS were not significant, however, the direction and magnitude of effect were similar in single-pollutant and multipollutant models.

The animal evidence for an association between PFOS exposure and effects in the endocrine system is considered *moderate* based on effects from 13 *high* or *medium* confidence studies. Decreases in free T4, total T4, and total T3 were observed in rats, mice, and monkeys after PFOS exposure; however, a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology, which is consistent with findings of hypothyroxinemia. Although evidence of thyroid hormone disruption in humans is inconsistent, EPA concluded that the sensitive and consistent changes in thyroid hormone levels in multiple animal models indicate toxicity of relevance to humans.

Reductions in ACTH, corticosterone, and CRH in studies with animal models suggest that exposure to PFOS may interfere with the hypothalamic-pituitary-adrenal axis. However, changes in adrenal weights were inconsistent among studies and among species. More data on the interactions between corticosterone and ACTH are required, as well as potential histological effects in the adrenal gland, to understand the relevance of an effect of PFOS on adrenocortical hormone levels.

C.2.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause endocrine effects in humans under relevant exposure circumstances (Table C-5). This conclusion is based primarily on evidence from animal models showing alterations in circulating thyroid and adrenocortical hormone levels following exposure to doses as low as 0.03 mg/kg/day PFOS. Although a few associations between PFOS exposure and TSH were observed in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-5. Evidence Profile Table for PFOS Endocrine Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.2.1)					⊕⊙⊙ ***Evidence Suggests***
Thyroid and thryoid-related hormones and thyroid disease 3 *High* confidence studies 17 *Medium* confidence studies 10 *Low* confidence studies	In adults, findings indicated significantly increased levels of the thyroid-related hormone TSH (2/11); however, one of the studies was of *low* confidence. Findings for thyroid hormones (i.e., T3 and T4) were generally inconsistent across studies, and considerable differences were observed by sex within studies. TSH was significantly increased among children in three studies (3/19), including a *high* confidence study. However, other studies reported inverse associations for TSH, including one significant finding. Findings for free T4 in children were mixed, but significant decreases (2/6) in T4 and significant increases in T3 (2/6) were reported. Two studies in pregnant women (2/3) reported non-significant positive associations for free T4 and free T3.	• *High* and *medium* confidence studies	• *Low confidence* studies • *Inconsistent direction* of effect in adults which may be influenced by timing of *outcome* sampling (i.e., diurnal variations) • *Imprecision* of *findings*	⊕⊙⊙ *Slight* Evidence for endocrine effects is based on increased TSH in adults, decreased T4 in children, and increased T3 in children. Findings from *medium* confidence studies were frequently inconsistent or imprecise. There was limited evidence reporting effects on thyroid disease. Uncertainties remain regarding diurnal variation of thyroid hormones, differential effects in males and females, and consistency across outcome timing.	*Primary basis*: Animal evidence demonstrated alterations in circulating thyroid and adrenocortical hormone levels. Although a few associations between PFOS exposure and TSH were observed in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistencies across studies and the limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Thyroid hormone antibodies 2 *Medium* confidence studies	Findings for thyroid hormone antibodies were generally imprecise, however, hormone antibody (i.e., TPOAb-negative) status was reported to play a role in the association between exposure and TSH levels in male children.	• *Medium* confidence studies	• *Limited number* of studies examining outcome • *Imprecision* of findings		
Steroid and adrenal hormones 1 *High* confidence study	One study reported decreases in diurnal urinary cortisone among pregnant women, and the diurnal urinary cortisol/cortisone ratio was correspondingly increased.	• *High* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.2.2)					
Thyroid and thyroid-related hormones 1 *High* confidence study 7 *Medium* confidence studies	Reductions in total T4, free T4, and/or total T3 was observed following short-term and developmental exposure in male and female rodents (5/7) and chronic exposure in male and female non-human primates (1/1). No significant change in TSH levels was reported in rats, mice, or non-human primates (4/4).	• *High* and *medium* confidence studies • *Coherent* changes across thyroid hormone levels • *Consistent* findings across species, sex, and study design • Dose-response relationship observed for free T4, total T4, and total T3	• Contributions of prenatal versus postnatal exposure to PFOS on thyroid hormones unclear	⊕⊕⊙ *Moderate* Evidence was based on *high* and *medium* confidence studies that demonstrated decreased thyroid hormone levels (free T4, total T4, total T3). A compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology, which is	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Adrenocortical hormones 5 *Medium* confidence studies[a]	Mixed effects on corticosterone levels were observed in rodent studies but most reported no significant changes (3/5). A dose-dependent decrease in ACTH and a non-monatomic decrease in CRH were reported in male rats (1/1).	• *High* and *medium* confidence studies	• No factors noted	consistent with findings of hypothyroxinemia.	
Organ weights 1 *High* confidence study 3 *Medium* confidence studies	In rodents, absolute (1/2) and relative (1/3) adrenal gland weights were decreased in males while absolute (1/2) and relative (1/2) adrenal gland weights were increased in females following a 28-day exposure in rats. One chronic study in non-human primates reported an increase in relative adrenal weights in males (1/1). No significant changes were observed in absolute or relative thyroid gland weight (4/4).	• *High* and *medium* confidence studies	• *Inconsistent direction* of effect in organ weights across studies • *Limited number* of studies examining outcomes		
Histopathology 1 *High* confidence study 2 *Medium* confidence studies	No significant effects were observed in incidence of non-neoplastic lesions in the thyroid gland, adrenal gland, and/or pituitary	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	gland following exposure to male and female mice, rats, and non-human primates (3/3).				

Notes: TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; TPOAb = thyroid peroxidase antibody; ACTH = adrenocorticotropic hormone; CRH = corticotropin releasing hormone.

[a] Pereiro et al. (2014) reported on the same data as Salgado-Freiría et al. (2018) for adrenocortical hormone measurements.

C.3 Metabolic/Systemic

EPA identified 69 epidemiological and 29 animal studies that investigated the association between PFOS and systemic and metabolic effects. Of the epidemiological studies, 10 were classified as *high* confidence, 36 as *medium* confidence, 14 as *low* confidence, 5 as *mixed* (4 *medium/low* and 1 *medium/uninformative*) confidence, and 4 were considered *uninformative* (Section C.3.1). Of the animal studies, 3 were classified as *high* confidence, 20 as *medium* confidence, 5 as *low* confidence, and 1 was considered *mixed* (*medium/uninformative*) (Section C.3.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.3.1 Human Evidence Study Quality Evaluation and Synthesis

C.3.1.1 Introduction

Diabetes is a category of diseases caused by either insulin resistance or beta-cell disfunction, or both. Type 1 diabetes is characterized by insulin deficiency and beta-cell destruction, while type 2 diabetes is characterized by beta-cell disfunction and insulin resistance. Type 2 diabetes is more common than type 1 diabetes. Gestational diabetes commonly occurs during pregnancy and is a risk factor for developing diabetes later in life. Diabetes can lead to long-term complications in several organ systems, including micro- and macro-vascular complications.

Diagnostic criteria for diabetes include hemoglobin A1c (HbA1c) ≥ 6.5%, fasting plasma glucose ≥ 126 mg/dL, a 2-hour plasma glucose ≥ 127 in an oral glucose tolerance test, or a random plasma glucose ≥ 200 mg/dL (in patients with classic symptoms of hyperglycemia or a hyperglycemic crisis).

Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease (CVD) and diabetes, including abnormalities in triglycerides, waist circumference, blood pressure, cholesterol, and fasting glucose. It is highly prevalent in the general population of the United States. Risk factors for metabolic syndrome include insulin resistance and being overweight or obese.

The 2016 EPA Health Assessment for PFOS concluded that there is no evidence of an association with metabolic syndrome. One study observed an association with gestational diabetes (Zhang et al., 2015a), but no associations were observed with type 1 or type 2 diabetes. Among adults, serum PFOS was significantly associated with increased beta-cell function. Serum PFOS concentration was not associated with metabolic syndrome, glucose concentration, homeostasis model of insulin resistance (HOMA-IR), or insulin levels in adults or adolescents (Lin et al., 2009). Another study reported no association with metabolic syndrome or glucose homeostasis parameters (Fisher et al., 2013). Overall, these studies show a lack of association of PFOS with diabetes, metabolic syndrome, and related outcomes.

For this updated review, 69 new epidemiologic studies examined the association between PFOS and metabolic outcomes. Of these, 32 were cohort studies, six were case-control studies, 27 were cross-sectional studies, two were nested case-control studies, and two were controlled trials. Most studies measured exposure to PFOS using biomarkers in blood. Di Nisio et al. (2019) measured exposure to PFOS using biomarkers in blood and in semen) Shapiro et al. (2016)

measured the exposure to PFOS in urine. Biomarkers in maternal blood were used in 16 studies and cord blood was used in 2 studies. Most studies identified were conducted in the United States and China. Other study locations included Canada, Croatia, Denmark (including the Faroe Islands), France, Italy, Japan, Korea, Norway, Spain, Sweden, Taiwan, the Netherlands, and the United Kingdom.

Twenty-two studies examined diabetes (one in children, nine in pregnant women), and four examined metabolic syndrome in general adult populations. Other metabolic outcomes examined included blood glucose levels or glucose tolerance, HbA1c, insulin or insulinogenic index, insulin resistance, insulin sensitivity, adiponectin, leptin, beta-cell function, proinsulin, insulin-like factor 1, c-peptide, body mass index (BMI) or ponderal index, body weight, gestational weight gain, body fat, and anthropometric measurements (Appendix D).

C.3.1.2 Study Quality

Several criteria were specific to evaluating the quality of studies on metabolic outcomes. Because of concerns for potential reverse causality (where the exposure may be affected by disease status), studies evaluating diabetes were considered critically deficient if exposure and prevalent diabetes were measured concurrently, since the cross-sectional design would not allow for a reliable characterization of exposure before the onset of diabetes. Another concern is for the evaluation of insulin, homeostasis model assessment of beta-cell function (HOMA-B), or HOMA-IR without consideration of diabetes status, as the treatment of diabetes, particularly in those being treated with hypoglycemic medications, influences insulin production and secretion.

There are 69 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and metabolic effects. Study quality evaluations for these 69 studies are shown in Figure C-18, Figure C-19, and Figure C-20.

On the basis of the considerations mentioned, 10 studies were classified as *high* confidence, 36 as *medium*, 14 as *low* confidence, and 4 as *uninformative* for all metabolic outcomes. Five studies have split ratings and were classified as *medium* confidence for one outcome and *low* confidence for other outcomes). One study (Liu et al., 2018a) was considered *uninformative* for insulin resistance and *medium* confidence for other metabolic outcomes. *Uninformative* studies had critical deficiencies in at least one domain. These deficiencies included a lack of control for confounding (Huang et al., 2018; Predieri et al., 2015; Jiang et al., 2014), lack of fasting measures for glucose measurements (Jiang et al., 2014), and treating PFOS as an outcome instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination (Jain, 2020b; Predieri et al., 2015). Other concerns leading to an *uninformative* rating included inadequate reporting of population selection (Jiang et al., 2014), small sample size, and narrow ranges for exposure (Predieri et al., 2015).

The most common reason for a *low* confidence rating was potential for residual confounding, particularly by SES (Fassler et al., 2019; Convertino et al., 2018; Heffernan et al., 2018; Khalil et al., 2018; Koshy et al., 2017; Christensen et al., 2016a; Lin et al., 2013), by adiposity (Lin et al., 2013), by age (Koshy et al., 2017), or by diabetes status (Lind et al., 2014). *Low* confidence studies presented concerns with the outcome measures including potential for outcome misclassification (He et al., 2018; Christensen et al., 2016a; Zong et al., 2016), failing to account

for diabetes status (Lind et al., 2014) or use of medications that would impact insulin levels or beta-cell function (He et al., 2018; Fleisch et al., 2017), analytical methods (Koshy et al., 2017), and failure to establish temporality between PFOS exposure and diabetes (Lind et al., 2014). Other concerns included selection bias (Fassler et al., 2019; van den Dungen et al., 2017), which resulted from self-selection (Christensen et al., 2016a), failure to provide information on control group selection (Heffernan et al., 2018), or differential recruitment for cases and controls (Lin et al., 2013). Small sample size was also a concern in some studies (Heffernan et al., 2018; Khalil et al., 2018; van den Dungen et al., 2017; Christensen et al., 2016a). In the evidence synthesis below, *high,* and *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

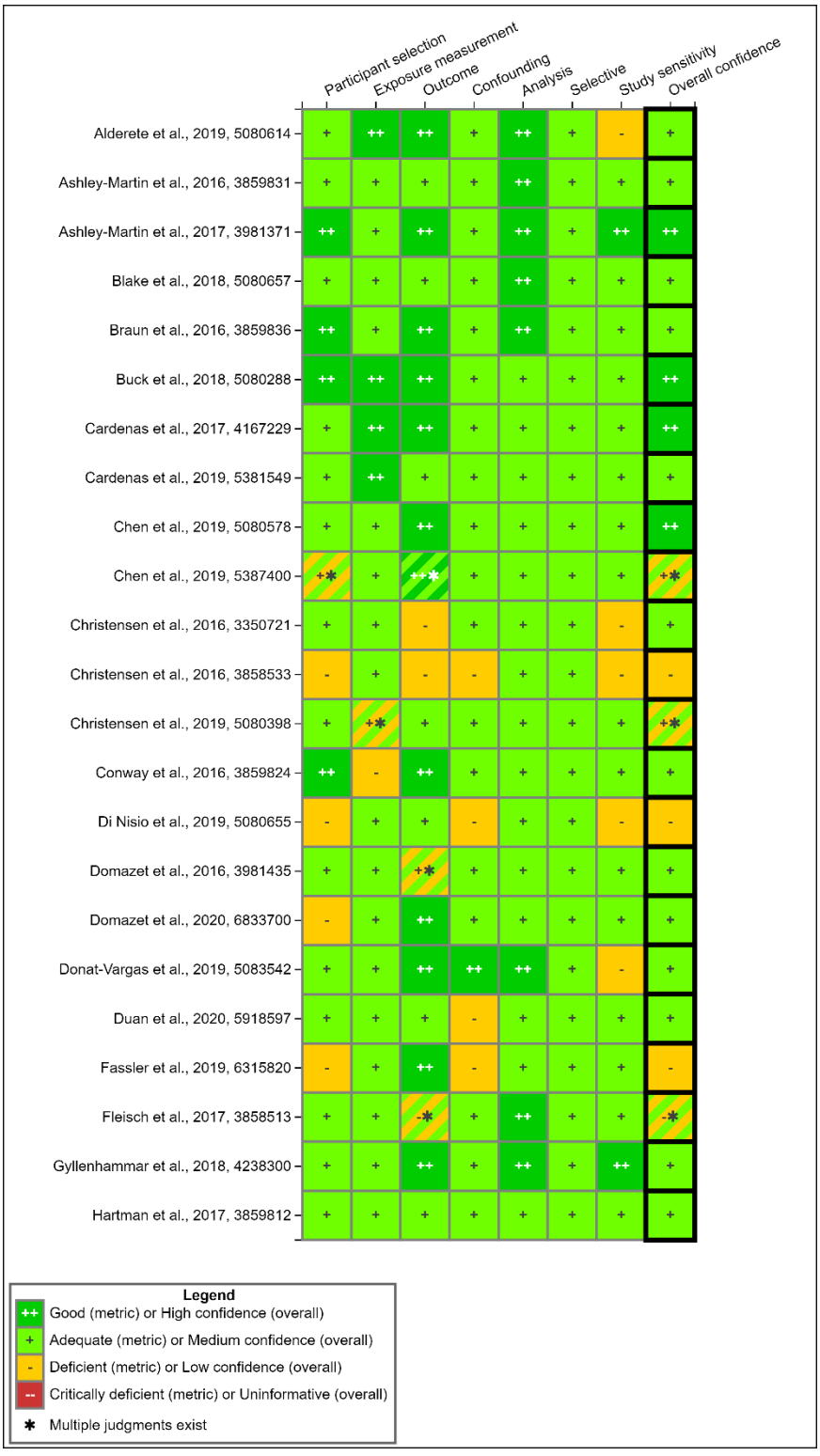


Figure C-18. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Metabolic/Systemic Effects

Interactive figure and additional study details available on HAWC.

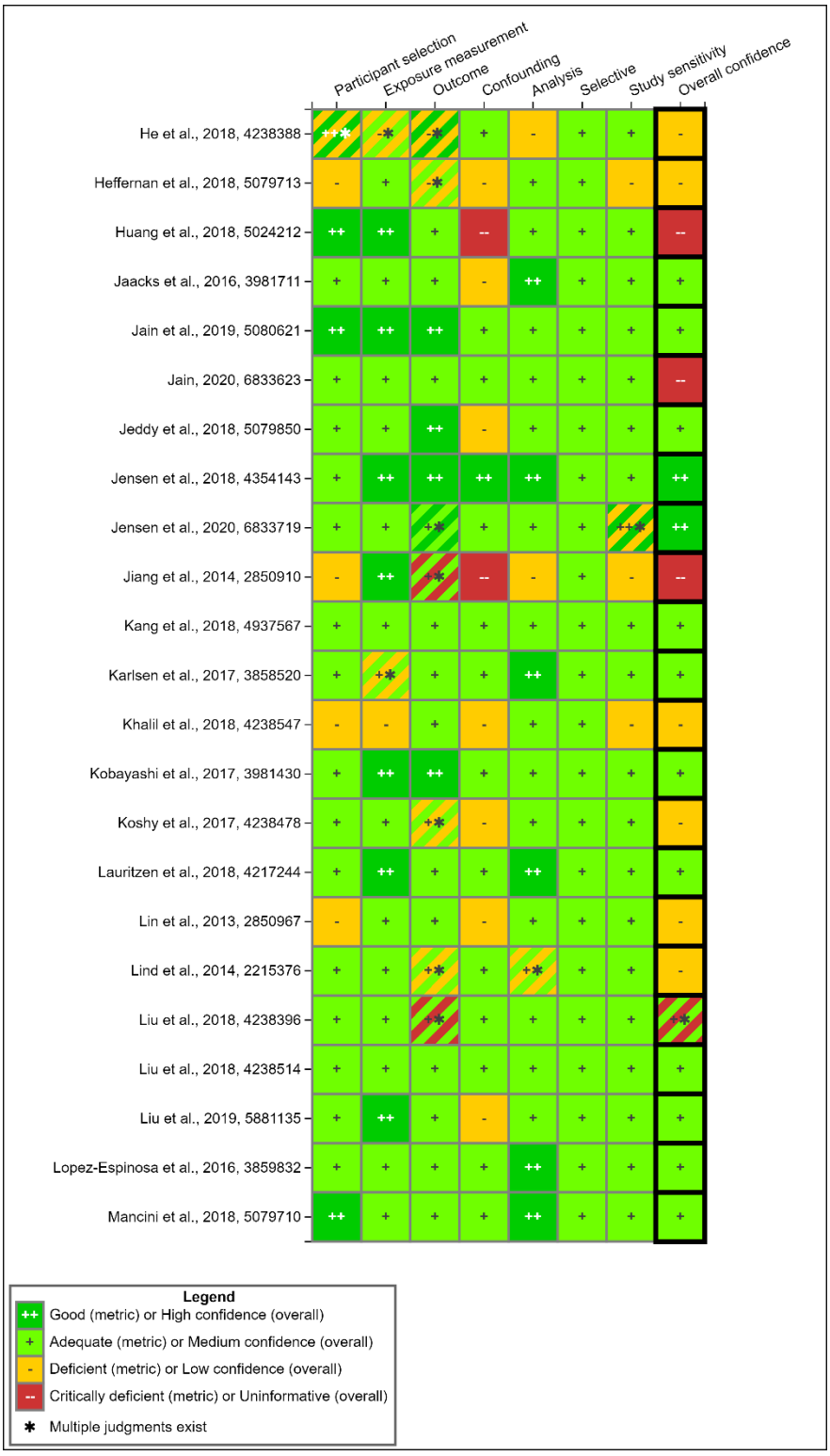


Figure C-19. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Metabolic/Systemic Effects (Continued)

Interactive figure and additional study details available on HAWC.

Figure C-20. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Metabolic/Systemic Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.3.1.3 Findings From Children and Adolescents

Three *medium* confidence studies and two *low* confidence studies evaluated glucose levels in children, with mixed non-significant results. Two *medium* confidence studies (Kang et al., 2018; Domazet et al., 2016) observed positive, non-significant associations with fasting blood glucose. Negative, non-significant associations with fasting blood glucose were observed in three studies, one of *medium* confidence (Alderete et al., 2019), and two of *low* confidence (Fassler et al., 2019; Khalil et al., 2018). Alderete et al. (2019) also reported a positive, non-significant association with 2-hour glucose (Alderete et al., 2019). (Appendix D).

Seven studies examined insulin measures, and two reported statistically significant associations. Insulin resistance, as described by the HOMA-IR, was examined in five studies with mixed results. Fleisch et al. (2017) observed a significant negative association with HOMA-IR in mid-childhood in a study of female children. Five studies (two *medium* and three *low* confidence) reported non-significant negative associations with HOMA-IR (Alderete et al., 2019; Fassler et al., 2019; Khalil et al., 2018; Koshy et al., 2017; Domazet et al., 2016). In a *medium confidence* study, a non-significant decrease in HOMA-IR at age 15 and 21 years per increase in PFOS exposure from 9 years and a non-significant increase in HOMA-IR at 21 per increase in PFOS measured at age 15 (Domazet et al., 2016).

Three studies examined fasting insulin levels. All three of these studies reported negative, non-significant associations with fasting insulin (Fassler et al., 2019; Khalil et al., 2018; Domazet et al., 2016).

A positive non-significant association was observed with insulin sensitivity, measured through both the insulin sensitivity index and the Children's Health and Environmental Chemicals in Korea (CHECK) Index/Quantitative Insulin Sensitivity Check Index (QUICKI) (Fassler et al., 2019).

One *medium* confidence study of reported significant negative associations with insulin-like growth factor 1 (IGF-1) in 6–9-year-old children in the C8 Health Project (Lopez-Espinosa et al., 2016). Significant negative associations for both girls and boys persisted after stratification by sex, and statistically significant decreasing trends across quartiles were also observed (Lopez-Espinosa et al., 2016).

One *medium* confidence study examined HOMA-B. Negative, non-significant associations were observed between PFOS levels at age 9 and beta-cell function at ages 15 or 21, but a positive non-significant association was observed between PFOS levels at age 15 and beta-cell function at age 21(Domazet et al., 2016).

Two *high* and two *medium* confidence studies examined adiponectin and leptin, and one observed significant association. For adiponectin, all studies observed positive associations. A *high* confidence study on the Sapporo Cohort of the Hokkaido Study observed a statistically significant positive association between maternal PFOS and cord blood adiponectin (p-value = 0.028) (Minatoya et al., 2017). Three other studies (one *high* and two *medium* confidence studies) reported positive, non-significant associations with adiponectin (Domazet et al., 2020; Buck et al., 2018; Fleisch et al., 2017). Buck et al. (2018) observed a positive, non-significant association between maternal PFOS and adiponectin, but a negative-non-significant association between mid-childhood PFOS and adiponectin.

Two *medium* and one *high* confidence study reported negative, non-significant association with leptin (Domazet et al., 2020; Fleisch et al., 2017; Minatoya et al., 2017). Minatoya et al. (2017) observed a negative association with leptin among male children and a positive association among female children; the interaction between child sex and PFOS was statistically significant. Another study observed a positive, non-significant association with PFOS; after stratification by sex, a negative non-significant association with leptin was observed among males, but a positive non-significant association was observed among females (Buck et al., 2018).

Six studies examined body fat measures, and one reported a significant negative association. A *medium* confidence study from the Avon Longitudinal Study of Parents and Children (ALSPAC) reported a statistically significant negative association between maternal PFOS and trunk fat percentage in female children (Hartman et al., 2017). One study observed non-significant negative associations with body fat percentage (Braun et al., 2016), and two studies observed a non-significant negative association with body fat mass (Domazet et al., 2020; Jeddy et al., 2018).

A *high* confidence study of 5-year-old children observed positive, non-significant associations with body fat percentage and fat mass; after stratification by sex, the non-significant positive associations persisted for boys, but non-significant negative associations with fat mass and body fat percentage were observed among girls (Chen et al., 2019b). Another study of *medium* confidence observed positive, non-significant associations with mid-childhood total fat mass index, total fat-free mass index, and trunk fat mass index among children from Project Viva (Mora et al., 2017).

Eleven studies examined BMI and related measures with mixed results. In the European Youth Heart Study (EYHS) study, Domazet et al. (2016) observed a positive significant association between PFOS at age 9 and BMI at age 15. Positive, but non-significant associations were observed between PFOS measured at either age 9 or age 15 and BMI measured at age 21 (Domazet et al., 2016). Additionally, two *medium* confidence studies observed significant positive associations with children's BMI (Lauritzen et al., 2018; Mora et al., 2017). Mora et al. (2017) reported a positive, significant association between maternal PFOS and early childhood BMI; the association was positive but not significant for the association with mid-childhood BMI (Mora et al., 2017). After stratification by sex, the association with BMI remained positive (though non-significant) for boys and girls in early childhood and for girls in mid-childhood but was negative and non-significant for boys in mid-childhood (Mora et al., 2017).

Significant negative associations were observed between maternal serum PFOS levels and BMI of girls from the ALSPAC study (Hartman et al., 2017) and between serum PFOS levels and BMI of girls from the Breast Cancer and Environment Research Program (BCERP) study (Fassler et al., 2019). Three studies (one of *high* confidence and two of *low* confidence) reported negative, non-significant associations with BMI (Chen et al., 2019b; Khalil et al., 2018; Koshy et al., 2017). In a sex-stratified analysis, Chen et al. (2019b) observed a negative, non-significant association among girls, but a positive non-significant association among boys.

Di Nisio et al. (2019) reported no difference between BMI between Italian male high school students exposed to PFOS pollution compared with those who were not exposed.

A *medium* confidence study reported a significant negative association between serum PFOS levels and ponderal index at birth in infants from the Hokkaido Study on Environment and Children's Health (Kobayashi et al., 2017).

Seven studies evaluated BMI z-score, and two observed an association with PFOS. In a *medium* confidence study of children from the Faroe Islands, a significant positive association was observed between maternal PFOS and BMI z-score among 18–month old children (Karlsen et al., 2017). In children from the POPUP study, Gyllenhammar et al. (2018b) observed a positive, significant association with BMI z-score among children 4- and 5-years old; the association with BMI z-score among 3-year-old children was positive, but not significant. Three other studies (two *medium* and one *high* confidence) reported positive, non-significant associations with BMI z-score (Jensen et al., 2020a; Manzano-Salgado et al., 2017b; Mora et al., 2017). In an age-stratified analysis, Jensen et al. (2020a) observed a positive, non-significant association with BMI z-score at birth, but a negative, non-significant association with BMI z-score at 3-months and 18-months of age.

Two studies reported negative, non-significant associations with BMI z-score (Koshy et al., 2017; Braun et al., 2016).

Seven studies evaluated the risk of being overweight or obese, and three reported significant associations. A *medium* confidence study reported increased odds of being overweight at 4 years old, with significantly increased odds of being overweight in the 4th quartile of maternal PFOS exposure (Martinsson et al., 2020). Another *medium* confidence study observed significantly increased odds of being overweight with increasing maternal PFOS among 5-year-old children (Lauritzen et al., 2018). A *medium* confidence study of mother-child pairs in the Faroe Islands reported a significantly increased risk of being overweight at 18 months (Karlsen et al., 2017). Two *medium* confidence studies observed an increased, non-significant risk of being overweight (Manzano-Salgado et al., 2017b; Mora et al., 2017). Manzano-Salgado et al. (2017b) observed an increased, non-significant risk of being overweight at age 4, but a non-significant, decreased risk of being overweight at age 7.

Two studies (one *medium* and one *low* confidence) reported non-significant, decreased risks of being overweight or obese (Koshy et al., 2017; Braun et al., 2016). Braun et al. (2016) observed a non-significant decreased risk of being overweight or obese in the second tertile of PFOS exposure, but a non-significant increased risk of being overweight or obese in the third tertile of PFOS exposure.

Six studies examined waist circumference, and two reported an association. A significant, positive association was observed between PFOS exposure at age 9 and waist circumference at age 15 and 21 years old; a positive, non-significant association was reported for PFOS exposure at age 15 and waist circumference at age 21 (Domazet et al., 2016). Two studies, one *high* confidence and one *low* confidence observed negative, non-significant associations with waist circumference (Chen et al., 2019b; Mora et al., 2017). After stratification by sex, Mora et al. (2017) observed negative, non-significant associations with waist circumference among boys, and positive, non-significant associations with waist circumference among girls.

A *medium* confidence study of mother-daughter dyads reported a statistically significant negative association with girls' waist circumference at age 9 (Hartman et al., 2017). In a tertiles analysis,

Braun et al. (2016) observed a negative association with waist circumference in the second tertile of PFOS exposure, but a positive association in the third tertile.

One *low* confidence study reported no statistical difference in waist circumference among PFOS-exposed children compared with non-exposed children (Di Nisio et al., 2019).

Two studies assessed waist circumference z-score among children, and none reported an association. Both studies observed negative, non-statistical associations with waist circumference z-score (Jensen et al., 2020a; Manzano-Salgado et al., 2017b). Manzano-Salgado et al. (2017b) observed a negative, non-significant association with waist circumference z-score at age 4 and a null association at age 7; after stratification by sex, negative, non-significant associations were observed for both boys and girls at age 7. In an age-stratified analysis, Jensen et al. (2020a) reported a positive association with waist circumference z-score at birth, but a negative association at 3-months and at 18-months.

Three studies evaluated waist-to-height ratio among children, and one observed a significant association. A *low* confidence study reported a significant negative association was observed with waist-to-height ratio among 6–8 year-old girls (Fassler et al., 2019).

A *high* confidence study of children from the Shanghai Prenatal Cohort observed negative, non-significant associations with waist-to-height ratio (Chen et al., 2019b). In a *medium* confidence study, a decreased risk of high waist-to-height ratio was observed at age 4, while an increased risk of waist-to-height ratio was observed at age 7 (Manzano-Salgado et al., 2017b).

Two studies examined waist-to-hip ratio in children, with no significant associations reported. A *medium* confidence study observed a positive, non-significant association with waist-to-hip ratio (Fassler et al., 2019), while a null association was observed in a *medium* confidence study (Mora et al., 2017). After stratification by sex, Mora et al. (2017) observed a positive, non-significant association among girls, but a negative, non-significant association among boys.

Three studies examined skinfold thickness metrics, with two studies reporting significant associations. A study from the EYHS reported significant positive associations between PFOS measured at age 9 and skinfold thickness at age 15 and age 21; the association between PFOS at age 15 and waist circumference at age 21 was positive, but not significant (Domazet et al., 2016). Additionally, a significant positive association was observed with tricep skinfold thickness z-score, while associations with subscapular skinfold thickness z-score were positive, but non-significant (Lauritzen et al., 2018).

Mora et al. (2017) observed positive, non-significant associations with subscapular and tricep skin thickness measures in mid- and early childhood. Negative, non-significant associations were observed with the sum of subscapular and tricep skinfold thickness among all children in mid-childhood, as well as with the subscapular-to-tricep skinfold thickness ratio among girls in early childhood (Mora et al., 2017).

C.3.1.4 Findings From Pregnant Women

Ten studies examined diabetes or gestational diabetes and overall results were mixed, with no significant associations (Appendix D).

Positive, non-significant associations with gestational diabetes were reported in four studies (Preston et al., 2020; Liu et al., 2019; Wang et al., 2018a; Matilla-Santander et al., 2017). A *medium* confidences study observed an increased, non-significant risk of gestational diabetes among women with a family history of type 2 diabetes and women were had an overweight pre-pregnancy BMI; a decreased, non-significant risk of gestational diabetes was observed among all women, women without a family history of type 2 diabetes, and with a normal pre-pregnancy BMI (Rahman et al., 2019).

Four *medium* and one *low* confidence studies reported inverse, non-significant associations with gestational diabetes (Xu et al., 2020b; Wang et al., 2018c; Valvi et al., 2017; Shapiro et al., 2016; Zong et al., 2016). With the exception of the *low* confidence study (Zong et al., 2016), gestational diabetes was determined through standard clinical methods. The nested case-control study conducted by Xu et al. (2020b) recruited pregnant women with no history of diabetes and reported inverse, non-significant odds of gestational diabetes across quartiles of PFOS exposure and log-transformed PFOS exposure. Similarly, Shapiro et al. (2016) observed inverse, non-significant odds of gestational diabetes or gestational impaired glucose tolerance, but increased odds of gestational diabetes in the second quartile of PFOS exposure.

Fasting glucose was examined in six studies, and one reported a positive association. A *medium* confidence study observed a significant increase in fasting glucose levels with increasing tertiles of PFOS, but a negative association between PFOS analyzed continuously and fasting glucose (Wang et al., 2018a). Two *high* confidence studies and one *medium* confidence study reported negative, non-significant associations with fasting glucose (Liu et al., 2019; Jensen et al., 2018; Starling et al., 2017). In contrast, two *medium* confidence studies reported positive, non-significant associations with fasting glucose among pregnant women (Ren et al., 2020; Wang et al., 2018c).

Results from oral glucose tolerance tests were assessed in five studies, two of which reported an association. A *high* confidence study from Project Viva observed non-significant positive associations with 1-hour glucose; a significant association with 1-hour glucose was observed in the fourth quartile of PFOS exposure (Preston et al., 2020). Additionally, a *medium* confidence study reported a significant association with 1-hour glucose levels among pregnant women in the Shanghai-Minhang Birth Cohort (Ren et al., 2020). Three studies observed positive, non-significant associations with oral glucose tolerance test results (Liu et al., 2019; Jensen et al., 2018; Wang et al., 2018a).

Three studies examined impaired glucose tolerance among pregnant women. One *low* confidence study reported positive, statistically significant effect estimates between plasma PFOS levels and impaired glucose tolerance among pregnant women from the INMA birth cohort in Spain (Matilla-Santander et al., 2017). A *high* confidence study and a *medium* confidence study both reported positive, non-significant associations with impaired glucose tolerance in the second and third quartiles of PFOS exposure, and a negative, non-significant association with impaired glucose tolerance in the fourth quartile of PFOS exposure (Preston et al., 2020; Shapiro et al., 2016).

Two *high* confidence studies evaluated associations between plasma PFOS levels and hyperglycemia or HbA1c among members of Project Viva. Preston et al. (2020) reported a positive, non-significant association with hyperglycemia. Conversely, Mitro et al. (2020)

observed a negative, non-significant association with HbA1c; negative non-significant associations persisted after stratification by maternal age.

Two studies, one of *high* and one of *medium* confidence observed positive, non-significant associations with both fasting insulin and HOMA-IR in pregnant women (Jensen et al., 2018; Wang et al., 2018c). These studies evaluated members of the OCC in Denmark with high risk of gestational diabetes (Jensen et al., 2018) and women in China in early pregnancy (Wang et al., 2018c). Jensen et al. (2018) reported a negative, non-significant association with insulin sensitivity as reported by the Matsuda index.

One *high* confidence study of members of the OCC examined HOMA-B and levels of fasting c-peptide among pregnant women with high risk of gestational diabetes and reported positive, non-significant associations with both HOMA-B and fasting c-peptide (Jensen et al., 2018).

Two *high* confidence studies compared levels of PFOS and adiponectin or leptin among pregnant women. One *medium* confidence study observed a negative, non-significant association with adiponectin (Mitro et al., 2020) while another *medium* confidence study reported a positive, non-significant association with adiponectin (Ashley-Martin et al., 2017). After stratification by age during pregnancy, Mitro et al. (2020) reported a negative association with adiponectin among women aged 35 and older, and a positive, non-significant association among women under 35.

Among the two *medium* confidence studies examining leptin, one reported a positive, non-significant association (Mitro et al., 2020), while the other reported a negative, non-significant association (Ashley-Martin et al., 2017).

Three *medium* confidence studies examined gestational weight gain, with mixed results.

Jaacks et al. (2016) observed a positive, non-significant association with gestational weight gain among all mothers, and mothers with a BMI < 25, and a negative non-significant association in mothers with a BMI ≥ 25. Increased odds of excessive gestational weight gain and decreased odds of inadequate weight gain were observed and were non-significant (Jaacks et al., 2016).

Ashley-Martin et al. (2016) used data from mother-infant pairs from the MIREC to estimate the odds of having high cord blood PFOS (>0.39 ng/mL) per increase in gestational weight gain. ORs were significant for both 1 kg increase in gestational weight gain and IQR increase in gestational weight gain (Ashley-Martin et al., 2016).

Marks et al. (2019) observed a negative, non-significant association with gestational weight gain. However, a significant interaction was observed between PFOS and pre-pregnancy BMI (Marks et al., 2019).

One *high* confidence study reported a significant positive association with skinfold thickness, as well as a non-significant positive association with waist circumference among pregnant women from Project Viva (Mitro et al., 2020).

In a *high* confidence study, a positive non-significant association was observed between plasma PFOS levels and BMI in pregnant women from the Project Viva study (Mitro et al., 2020).

C.3.1.5 Findings From the General Adult Population

Eleven studies evaluated diabetes in the general population and four reported significant associations with diabetes. A *medium* confidence study of Taiwanese adults aged 20–60 reported a significant positive association with type 2 diabetes (Su et al., 2016). In a quartile analysis, odds of type 2 diabetes significantly increased with increasing quartiles of PFOS (Su et al., 2016). Another *medium* confidence study reported significantly increased odds of type 2 diabetes in the second and third tertile of PFOS exposure among female nurses in the Nurses' Health Study (NHS) II (Sun et al., 2018). A *medium* confidence study from the E3N cohort reported a non-significant increased risk of type 2 diabetes in the 2nd–4th, 6th, 8th–9th deciles of PFOS exposure, and a non-significant decreased risk of type 2 diabetes was observed in the 5th and 10th deciles of PFOS exposure (Mancini et al., 2018) (Appendix D).

Three *low* confidence studies reported non-significant positive associations with diabetes (He et al., 2018; Christensen et al., 2016a; Lind et al., 2014) and prediabetes (Christensen et al., 2016a).

Significant decreased odds of type 1 and type 2 diabetes were observed among 6,889 participants in the C8 Health Project (Conway et al., 2016). The decrease in odds of uncategorized diabetes was not significant. After stratifying by age, significant decreased odds of type 1 diabetes were observed among adults and children (Conway et al., 2016). One *high* confidence cohort study from the Diabetes Prevention Program followed adults at increased risk of type 2 diabetes and observed a decreased non-significant risk of diabetes (Cardenas et al., 2017). After stratification by sex, a significant decreased risk of type 2 diabetes was observed among males, and the decreased risk among females was not significant (Cardenas et al., 2017). Two other *medium* confidence study reported non-significant negative associations with type 2 diabetes (Cardenas et al., 2019; Donat-Vargas et al., 2019a).

Four studies (three *medium* confidence and one *low* confidence) evaluated metabolic syndrome (MetS) and one study reported an association. In an adult population of the island of Hvar (Croatia) Chen et al. (2019a) observed a positive non-significant association with risk of MetS as defined by the Adult Treatment Panel III (ATP III) criteria (OR: 2.19; 95% CI: 0.88, 5.44). Two *medium* confidence studies using overlapping data from NHANES reported non-significant negative associations with MetS. Liu et al., 2018 observed adults aged 20 and older from the 2013–2014 NHANES cycle and Christensen et al. (2019) observed adults aged 18 and older from 2007–2014 NHANES. In a model simultaneously adjusted for PFDE, PFOA, PFHxS, N-methyl-PFOSA (MPAH), PFNA and PFUnDA, Christensen et al. (2019) reported non-significant increased odds of MetS in the third and fourth quartiles of PFOS exposure; the decreased odds observed in the second quartile of PFOS were not significant.

A *low* confidence study observed lower non-significant odds of MetS for participants with serum PFOS >1.90 ng/mL compared with those with serum PFOS ≤ 1.90 ng/mL (Yang et al., 2018). However, concerns for selection bias, outcome misclassification, and residual confounding by SES diminish confidence in the study results.

There were nine studies examining glucose. Three studies reported associations with fasting blood glucose, one reported an association with 2-hour glucose, one reported an association with glucose area under the curve (AUC).

A *medium* confidence study of adults aged 19–87 years from China reported a significant positive association with fasting blood glucose (Duan et al., 2020). Additionally, a study using NHANES 1999–2014 data observed a significant positive correlation between fasting glucose and serum PFOS (Huang et al., 2018). Su et al. (2016) reported a non-significant positive association with fasting glucose; in a quartiles analysis, mean fasting blood glucose significantly increased with increasing quartiles of PFOS. Liu et al. (2018b) reported a negative statistically significant association with fasting blood glucose, but non-significant increased odds of fasting glucose levels ≥ 100 mg/dL.

A *low* confidence study observed a positive, non-significant association with fasting blood glucose (Heffernan et al., 2018), while another reported lower non-significant odds of blood glucose ≥ 1.6 mmol/L for participants with serum n-PFOS >3 ng/mL compared with those with serum n-PFOS ≤ 3 ng/mL (Yang et al., 2018).

Two studies (one *high* confidence and one *medium* confidence) observed non-significant positive associations with 2-hour glucose (Cardenas et al., 2017; Su et al., 2016) and 30-minute glucose (Cardenas et al., 2017). Another *medium* confidence study reported a negative, non-significant association with 2-hour glucose (Liu et al., 2018b).

One *medium* confidence study observed a significant decrease in glucose AUC with increasing quartiles of PFOS and a non-significant negative association between PFOS (measured continuously) and glucose AUC (Su et al., 2016). In the POUNDS Lost clinical trial, a positive, non-significant correlation was observed between PFOS and glucose levels (Liu et al., 2018b).

Blood glucose levels were examined in a *medium* confidence study from NHANES (2007–2014), which reported increased odds of high blood glucose in the second and third quartiles of PFOS, and decreased odds in the fourth quartile of PFOS exposure (Christensen et al., 2019). A *low* confidence study reported a negative association with blood glucose levels (van den Dungen et al., 2017). None of the associations for these two studies reached statistical significance.

Significant associations were reported between resting metabolic rate and PFOS. The association with resting metabolic rate was assessed in the POUNDS Lost trial, a clinical trial of overweight and obese adults aged 30–70. A non-significant negative correlation between PFOS and resting metabolic rate was observed (Liu et al., 2018a). In the first 6 months of the trial, resting metabolic rate decreased non-significantly with increasing tertiles of PFOS exposure for the entire study population, men, and women. The interaction between PFOS and sex were significant (Liu et al., 2018a). In months 6–24 of the trial, a significant positive association was observed with mean resting metabolic rate in all tertiles of PFOS exposure, and average resting metabolic rate significantly decreased with increasing tertiles of PFOS (Liu et al., 2018a). In a sex-stratified analysis, average resting metabolic rate significantly decreased with increasing tertiles of PFOS among men and women (Liu et al., 2018a).

Twelve studies examined insulin resistance measures and one observed significant association with fasting insulin, insulin resistance, fasting plasma insulin, 30-minute insulin, fasting proinsulin, and insulin (corrected response), and one reporting associations with the ratio of proinsulin to insulin.

Four studies measured fasting insulin. One *high* confidence study used a subset of data on 954 adults at high risk of type 2 diabetes from the Diabetes Prevention Program and observed a positive significant association between PFOS and fasting insulin (Cardenas et al., 2017). Two *low* confidence reported non-significant positive associations with fasting insulin (Chen et al., 2019a; Sun et al., 2018), and one reported a non-significant negative association (He et al., 2018). One *medium* confidence study reported a positive, non-significant association with insulin levels (Liu et al., 2018b).

Nine studies examined insulin resistance (measured as HOMA-IR), and one reported a significant association. A *high* confidence study of 956 adults at high risk for type 2 diabetes in the Diabetes Prevention Program reported a significant, positive association with HOMA-IR (Cardenas et al., 2017). A *medium* confidence study of 1871 adults in NHANES observed a non-significant positive association with HOMA-IR (Liu et al., 2018b). However, Donat-Vargas et al. (2019a) reported a non-significant negative association with HOMA-IR in both continuous and tertile analyses. In a sensitivity analysis, a non-significant negative association was observed between HOMA-IR and the third tertile of baseline PFOS, and between HOMA-IR and PFOS measured at the end of follow-up for both the second and third tertile of PFOS exposure. A non-significant positive association with HOMA-IR was reported in the second tertile of baseline PFOS exposure (Donat-Vargas et al., 2019a).

Four *low* confidence studies investigated the association between PFOS and insulin resistance. Of these studies, two reported a positive, non-significant association with insulin resistance (Chen et al., 2019a; Lind et al., 2014; Lin et al., 2013). In a sex-stratified tertile analysis, a non-significant negative association was observed between PFOS and insulin resistance in both males and females; among females, a significant negative association with insulin resistance was observed in the third quartile of PFOS exposure (He et al., 2018). These studies were of *low* confidence due to concerns with the statistical analysis (not accounting for design of NHANES) (He et al., 2018), failure to account for diabetes status (Lind et al., 2014) or medications that could affect insulin levels (Chen et al., 2019a), and concerns for residual confounding and selection bias (Lin et al., 2013).

The association between plasma PFOS and insulinogenic index 1 was investigated in a *high* confidence study from the Diabetes Prevention Program. A non-significant positive association was observed with insulinogenic index among 945 adults at high risk for type 2 diabetes (Cardenas et al., 2017).

In a *high* confidence study, Cardenas et al. (2017) reported significant positive associations between PFOS and fasting plasma insulin, 30-minute insulin, and fasting proinsulin. A non-significant positive association was observed with insulin (corrected response) (Cardenas et al., 2017).

In a *low* confidence study, a non-significant positive association was reported for the ratio of proinsulin to insulin and PFOS (Lind et al., 2014). This study was given a *low* confidence rating due to failure to adjust for diabetes status in statistical analyses.

Four studies measured the association between PFOS and beta-cell function and two reported a significant association. Cardenas et al. (2017) reported a significant positive association with beta-cell function (measured as HOMA-B) in adults at high risk for type 2 diabetes from the

Diabetes Prevention Program. Positive non-significant associations with HOMA-B were reported in adults from NHANES (Liu et al., 2018b) and (Chen et al., 2019a). A *medium* confidence study reported negative, non-significant associations with HOMA-B (Donat-Vargas et al., 2019a).

Four studies examined adiponectin, and none reported significant associations. Two *high* confidence studies reported non-significant positive associations with adiponectin (Buck et al., 2018; Ashley-Martin et al., 2017). In contrast, a non-significant negative association with adiponectin was observed among 945 adults in the Diabetes Prevention Program (Cardenas et al., 2017). A *medium* confidence study reported a negative non-significant correlation between PFOS and plasma adiponectin (Sun et al., 2018).

Three studies examined associations with leptin. One study reported a significant association. Two *high* quality studies measured associations with leptin; one reported a non-significant positive association (Buck et al., 2018), and the other reported a non-significant negative association (Ashley-Martin et al., 2017). A *medium* confidence study reported a positive, non-significant correlation between plasma PFOS and leptin concentrations, and a non-significant, positive correlation with soluble leptin receptors (Liu et al., 2018a).

Nine studies examined HbA1c, and three reported associations. A *high* confidence study on participants in the Diabetes Prevention Program reported a significant positive association with HbA1c (Cardenas et al., 2017). A significant positive association with HbA1c was also reported among adults under age 55 in a *medium* confidence study of adults living in China; the association with HbA1c among adults aged 55 and older was also positive, but not significant (Duan et al., 2020). Two *medium* confidence studies observed positive correlations with HbA1c; one was non-significant (Sun et al., 2018) and the other was significant (Huang et al., 2018). Another *medium* confidence cross-sectional study assessed the association between plasma PFOS and HbA1c in adults aged 20–60 (Su et al., 2016). A positive, non-significant association between HbA1c and continuous PFOS was reported, and a significant increase in average HbA1c was observed with increasing quartiles of PFOS (Su et al., 2016).

In the POUNDS Lost trial, a negative, non-significant correlation was observed between PFOS and HbA1c (Liu et al., 2018a). Additionally, a *medium* confidence study of 1871 adults from NHANES reported a non-significant negative association with HbA1c (Liu et al., 2018b).

One *low* confidence study reported a non-significant negative association with HbA1c (Heffernan et al., 2018). Another *low* confidence study observed a non-significant positive association between PFOS and HbA1c (Chen et al., 2019a). Concerns with measurement of confounders and inclusion of medications that could affect insulin levels (Chen et al., 2019a), as well as concerns with case selection and residual confounding (Heffernan et al., 2018) resulted in *low* confidence ratings.

There were four studies evaluating body weight measures. Associations were observed in one study of body weight, and two studies reported associations with being overweight or obese.

One study, from the POUNDS Lost clinical trial, evaluated body weight and observed a negative, non-significant association with weight loss in the first 6 months of the trial, and a positive, significant association with weight loss in months 6–24 of the trial (Liu et al., 2018a). A

significant increase in average weight gain during months 6–24 of the trial was observed with increasing tertiles of PFOS (Liu et al., 2018a).

Two studies evaluated being overweight, one of which reported an association. A *medium* confidence study reported significantly greater serum PFOS among obese adults compared with non-obese adults (Jain and Ducatman, 2019e). One *medium* confidence study evaluated maternal PFOS and risk of being overweight or obese in their children; this study reported increased, non-significant odds of being overweight at age 4 in the second and third quartiles of PFOS exposure, and significant increased odds of being overweight at age 4 in the fourth quartile (Martinsson et al., 2020).

One *low* confidence study observed significant increased odds of being overweight or obese (Tian et al., 2019c). Another *low* confidence study reported non-significant negative associations with being overweight and obese (Yang et al., 2018).

Five studies evaluated body fat measures, and one reported an association. Four studies of *medium* confidence evaluated body fat. A significant negative association was observed between maternal plasma PFOS and trunk fat in young girls ALSPAC. After stratification by age at menarche, the association remained negative but was not significant in either age group (Hartman et al., 2017). A negative, non-significant association was observed between maternal plasma PFOS and body fat percentage (Hartman et al., 2017).

Three *medium* confidence studies reported positive, non-significant associations with body fat measures (Liu et al., 2019; Mora et al., 2017; Braun et al., 2016).

Two *medium* confidence studies evaluated fat mass; one reported a non-significant negative association with fat mass among children (Jeddy et al., 2018) and a non-significant positive association with fat mass among overweight and obese adults (Liu et al., 2019).

Eleven studies assessed BMI; one significant association was reported for BMI, and one significant association was reported for BMI z-score.

In the Health Outcome Measures of the Environment (HOME) study, a cohort study of 285 mother-child pairs, PFOS exposure was measured during pregnancy and BMI was recorded at age 8 (Braun et al., 2016). Negative, non-significant associations with BMI z-score were observed in the second and third tertile of maternal PFOS exposure (Braun et al., 2016). Liu et al. (2018a) reported a non-significant negative correlation between PFOS and BMI.

One *high* confidence study and two *medium* confidence studies observed positive, non-significant associations with BMI (Chen et al., 2019a; Blake et al., 2018; Cardenas et al., 2017).

In a *medium* confidence cohort study from the ALSPAC, a significant negative association with children's BMI was observed among 312 mother-child pairs (Hartman et al., 2017). Another *medium* confidence study reported non-significant positive association with BMI; in a sex-stratified analysis, a non-significant percent decrease was observed for males, and a non-significant percent increase was observed among females (Blake et al., 2018). In the single *low* confidence study, Tian et al. (2019c) reported a non-significant association with BMI. In a sex-stratified analysis, a non-significant negative association was observed among men and a positive, non-significant association was reported for women. (Tian et al., 2019c). This study

was given a *low* confidence designation due to concerns for PFOS to be potentially related to BMI.

A *high* confidence study measured PFOS in maternal serum and BMI z-score in children. Non-significant negative associations with BMI z-score were observed in children at 3- and 18-months, and a non-significant positive association with BMI z-score was observed at birth. (Jensen et al., 2020a) A *medium* confidence study of 412 mother-child pairs observed a positive, significant association between maternal serum PFOS and 5-year-old child's BMI z-score (Lauritzen et al., 2018).

Five studies examined waist circumference. Two single *medium* confidence studies observed a negative, non-significant association with waist circumference (Liu et al., 2018b; Liu et al., 2018a). One *low* confidence study reported a non-significant positive association with waist circumference (Tian et al., 2019c). Non-significant decreased odds of increased waist circumference were observed among men, and non-significant increased odds were observed for women; the interaction between PFOS and sex was significant but was not significant in continuous analyses (Tian et al., 2019c). In another *low* confidence study, non-significant increased odds of increased waist circumference were observed with increasing quartiles for PFOS; these estimates were adjusted for multiple PFAS (Christensen et al., 2019).

C.3.1.6 Findings From Occupational Studies

No occupational studies examined metabolic outcomes and PFOS.

C.3.2 Animal Evidence Study Quality Evaluation and Synthesis

C.3.2.1 Metabolic Homeostasis

There are three studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and four studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and metabolic effects. Study quality evaluations for these four studies are shown in Figure C-21.

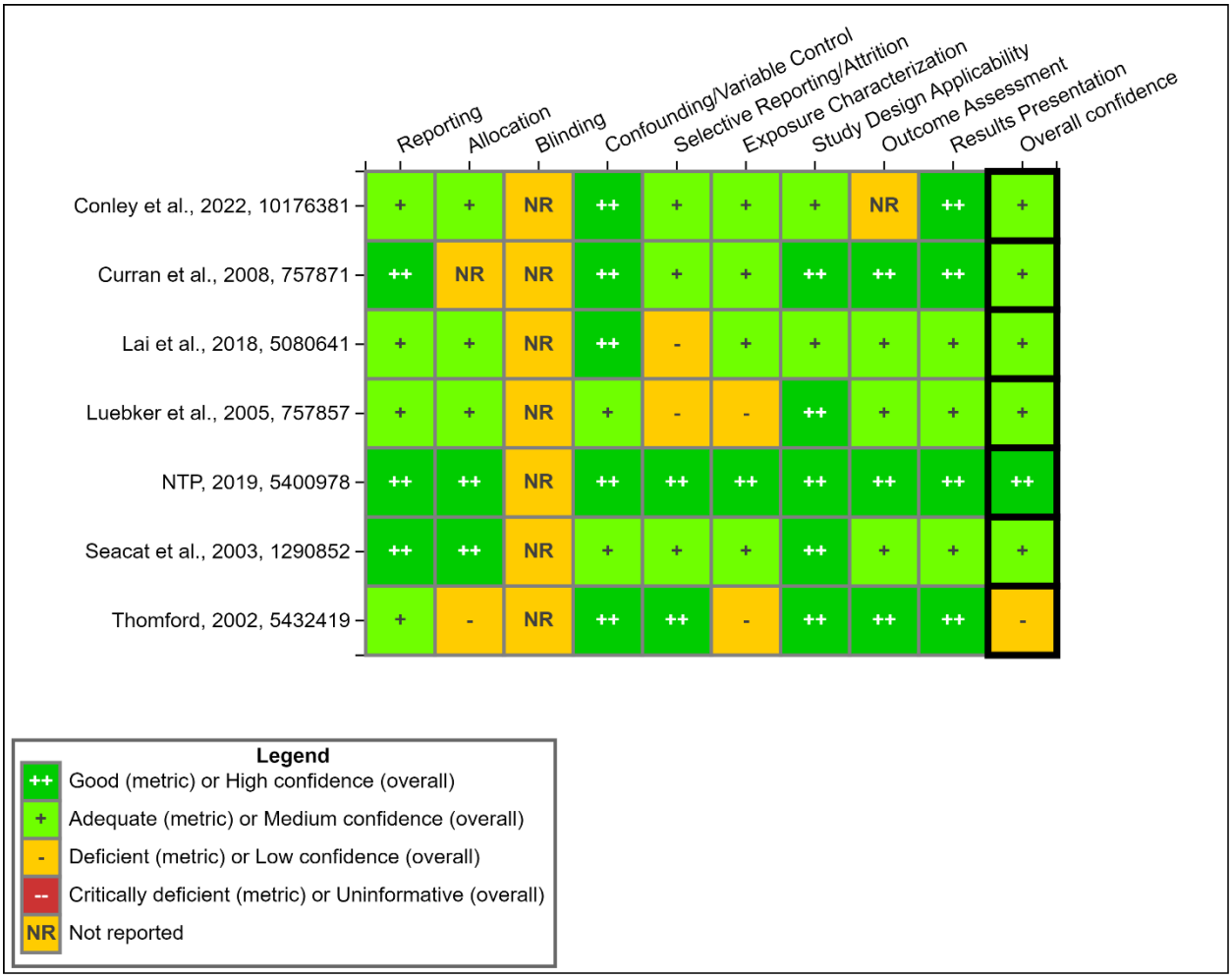


Figure C-21. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Metabolic Effects

Interactive figure and additional study details available on HAWC.

PFOS has been observed to cause perturbations in glucose homeostasis in rodents. Several studies in adult and perinatal rats and mice investigate glucose homeostasis, including serum glucose levels, glucose tolerance, and gluconeogenesis, among other measures. Alterations in these metabolic endpoints were observed, but the data is inconclusive as there are inconsistencies within the literature with too few studies to assess possible difference across life stages, sexes, and species.

NTP (2019) reported no statistical differences in serum glucose in adult male and female Sprague-Dawley rats exposed to PFOS doses up to 5 mg/kg/day for 28 days. In contrast, Seacat et al. (2003) observed a significant decrease in serum glucose in adult male Sprague-Dawley rats compared with controls following 1.51 mg/kg/day PFOS exposure in the diet for 4 weeks. No statistically significant change was seen in females at the 4-week interim timepoint. After 14 weeks, serum glucose concentrations were no longer statistically different in males from any treatment group. In females at 14 weeks, serum glucose was significantly lower in the 0.40 mg/kg/day group, but not in the high dose group (1.56 mg/kg/day).

In a rat reproductive toxicity study, Luebker et al. (2005b) noted significantly higher serum glucose levels on lactational day (LD) 5 in dams treated with 2 mg/kg/day PFOS for 42 days prior to mating until LD 4. This change was not seen in dams sacrificed at GD 21. Serum glucose levels were not significantly altered in fetuses at GD 21 or in pups at LD 5. In a glucose tolerance test, Lv et al. (2013) observed a dose-related increase in serum glucose 10 weeks postweaning in rats perinatally exposed to PFOS from GD 0–PND 20 with significance in the high dose exposure group of 1.5 mg/kg/day. At 15 weeks postweaning, only the low dose (0.5 mg/kg/day) group had significantly elevated serum glucose during the glucose tolerance test. Elevated serum glucose in this test indicates decreased glucose clearance or tolerance. In addition, at 18 weeks postweaning, rats in the high dose group had elevated serum insulin, higher insulin resistance indices, increased leptin levels, and decreased adiponectin levels, all of which indicate dysregulation of glucose homeostasis and insulin resistance, potential signs of prediabetes (Lv et al., 2013).

Wan et al. (2014) exposed CD-1 mouse dams to 0 mg/kg/day, 0.3 mg/kg/day, or 3 mg/kg/day PFOS from GD 3–PND 21. Offspring were then fed either a standard or high-fat diet from PND 21–PND 63. At PND 21, no statistical difference was detected in the fasting serum glucose or insulin levels in dams. However, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was significantly increased in both the 0.3 and 3 mg/kg/day dose groups. Increases in this metric indicate increased risk of insulin resistance, hypertension, and type 2 diabetes (Wan et al., 2014). There was no significant difference in fasting serum glucose or the HOMA-IR index in male or female pups at PND 21, though males from both the 0.3 mg/kg/day and 3 mg/kg/day groups had significantly increased fasting serum insulin levels. No difference was found in fasting serum insulin levels in female pups at PND 21. In pups fed a standard diet, at PND 63, fasting serum glucose levels were significantly higher for males and females at both PFOS doses. Serum insulin and HOMA-IR were significantly increased only at the high dose of 3 mg/kg/day PFOS in both sexes. No significant differences between treatment groups in glucose tolerance were observed in either sex. In the high-fat diet group, fasting serum insulin was increased at PND 63 in the 3 mg/kg/day PFOS group of both sexes. Fasting serum glucose was significantly higher in females dosed with both 0.3 and 3 mg/kg/day, but only for the 3 mg/kg/day males. In the glucose tolerance test, serum glucose was significantly higher only in the high dose group in both sexes, indicating decreased glucose tolerance in these animals. The HOMA-IR index in each sex was elevated in the high dose groups compared with the high-fat diet control group. However, the HOMA-IR indices were significantly higher for the high-fat diet groups compared with the standard diet groups within a specific PFOS treatment group and sex. In contrast, Ngo et al. (2014) did not observe significant changes in blood glucose at PNW 6, PNW 11, or PNW 20 in wild-type or tumorigenic transgenic C57BL/6J-*Min*/+ mice offspring gestationally exposed to 0 mg/kg/day, 0.01 mg/kg/day, 0.1 mg/kg/day, or 3 mg/kg/day PFOS from GD 1–GD 18, though it should be noted that the animals were not fasted prior to serum sample collection.

Lai et al. (2018) exposed CD-1 female mice to 0, 0.3, or 3 mg/kg/day for 7 weeks with conflicting results. The authors conducted an oral glucose tolerance test and an intraperitoneal insulin tolerance test. In both tests, blood glucose levels were significantly lower in the 3 mg/kg/day dose group compared with controls, potentially indicating increased glucose tolerance and reduced insulin resistance, respectively. Pyruvate tolerance was also significantly

decreased in both the 0.3 mg/kg/day and 3 mg/kg/day dose groups which could indicate reduced gluconeogenesis.

C.3.2.2 Survival, Clinical Observations, Body Weight, and Food Consumption

There are 6 studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and 21 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and systemic effects. Study quality evaluations for these 27 studies are shown in Figure C-22 and Figure C-23.

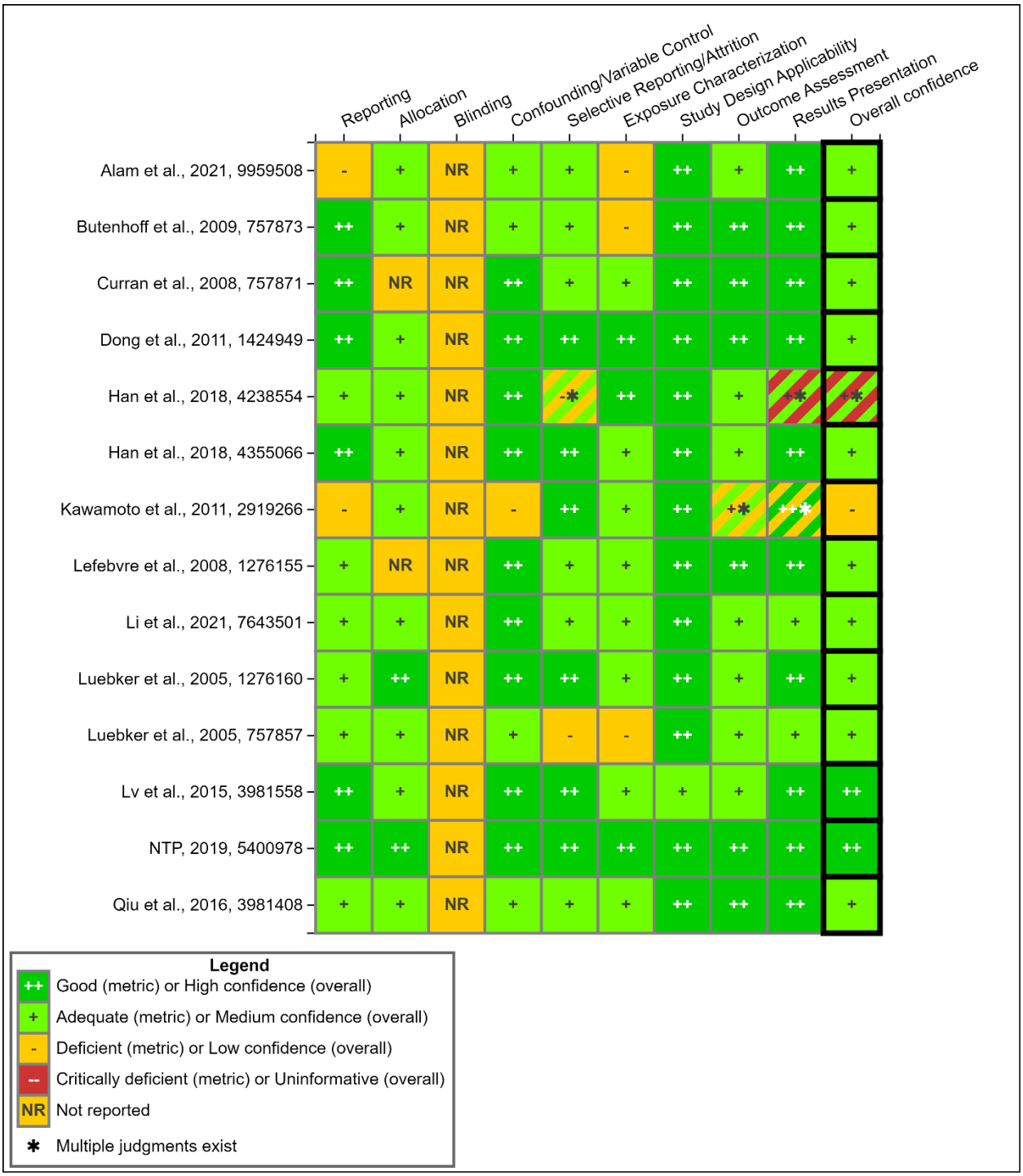


Figure C-22. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Systemic Effects[a]

Interactive figure and additional study details available on HAWC.

[a] Lefebvre et al. (2008) reported on the same animals as Curran et al. (2008).

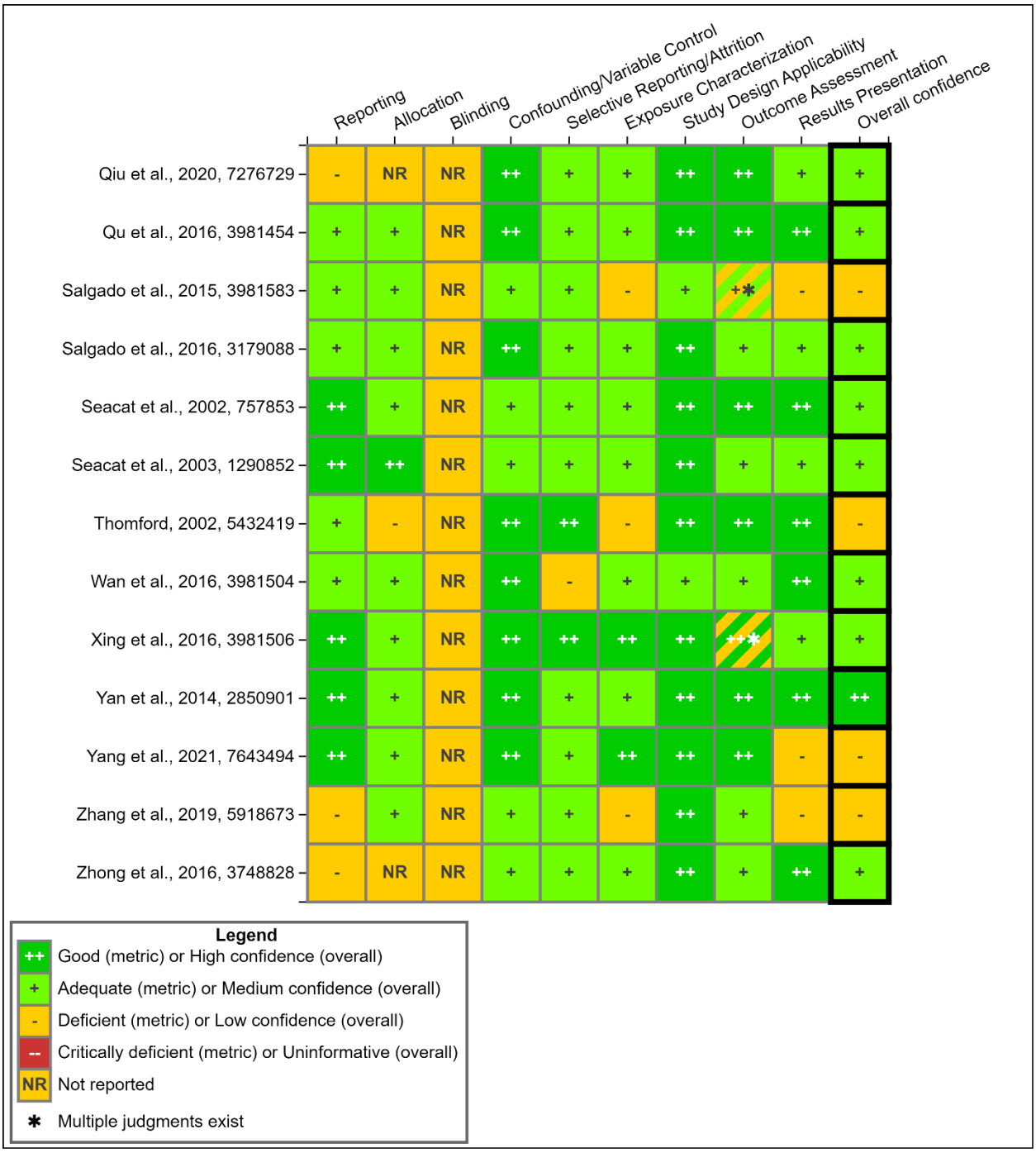


Figure C-23. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Systemic Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.
[a] Lefebvre et al. (2008) reported on the same animals as Curran et al. (2008).

A number of subchronic, chronic, and developmental studies suggest that PFOS exposure can induce whole-body toxicity, which can manifest as decreased body weight, partly due to a reduction in food consumption. These changes were more prominent following high exposures to

PFOS. Although one study in non-human primates suggests PFOS-related mortality, PFOS-induced mortality and clinical observations were not supported by rodent studies.

C.3.2.2.1 Mortality and Clinical Observations

PFOS-related mortality was observed in 2 of 6 male cynomolgus monkeys administered 0.75 mg/kg/day PFOS for 26 weeks. Pulmonary inflammation was identified as the probable cause of death of one monkey that died on day 155 of dosing, and hyperkalemia was suggested for the other monkey that died on day 179 (Seacat et al., 2002). Mortality was not affected in female monkeys administered 0.75 mg/kg/day PFOS or male or female monkeys receiving 0.03 mg/kg/day or 0.15 mg/kg/day PFOS (Seacat et al., 2002).

Rodent studies did not observe mortality with doses up to 10 mg/kg/day and durations up to 60 days. No mortality was observed in C57 male mice exposed to 0.5 mg/kg/day or 10 mg/kg/day PFOS for 5 weeks, but the study did not report if there were any overt clinical observations (Qu et al., 2016). NTP (2019) exposed male and female Sprague-Dawley rats to 0.312–5 mg/kg/day PFOS for 28 days. All rats survived to the end of the study, except for one female Sprague-Dawley rat administered 5 mg/kg/day (NTP, 2019). There were no treatment-related clinical observations reported in male or female rats (NTP, 2019). Similarly, Alam et al. (2021) reported that there was no mortality in male Wistar rats over the course of a 60-day study exposure to 0, 0.015, or 0.15 mg/kg/day PFOS. Xing et al. (2016) did not observe an effect on mortality in C57BL/6J male mice exposed to PFOS at 2.5 mg/kg/day, 5 mg/kg/day, or 10 mg/kg/day for 30 days. Clinical observations such as rough hair, slow movement, and constipation were reported, although neither the exposure group associated with these effects nor incidence were specified (Xing et al., 2016). Study authors indicated that there were no treatment-related clinical signs or mortality in P_0 male Crl:Cd(Sd)lgs rats following 6 weeks of pre-mating exposure to 1.6 mg/kg/day, 2.0 mg/kg/day, or 3.2 mg/kg/day (Luebker et al., 2005a). No mortality was observed in the P_0 females, but timing of the clinical observations (i.e., localized areas of partial alopecia) were not specified when they occurred (Luebker et al., 2005b; Luebker et al., 2005a) (see Toxicity Assessment, (U.S. EPA, 2024)).

C.3.2.2.2 Body Weight in Adults

Many studies with rodent models report reductions in body weight following short-term to subchronic PFOS exposure (Figure C-24). A dose-dependent reduction in body weight change was observed in C57BL/6J male mice exposed to PFOS at 2.5 mg/kg/day, 5 mg/kg/day, or 10 mg/kg/day via gavage for 30 days (Xing et al., 2016). All dose groups had a significant difference in body weight gain when compared with the control with the 10 mg/kg/day group having a 31% reduction in body weight over the study period compared with a 27.75% weight gain in the controls. This reduction may be attributed to reduced food consumption reported across all doses, but the correlation between body weight and food intake was not significant in the treatment groups suggesting that this may not be the only explanation (Xing et al., 2016). Body weight was significantly changed in the highest dose group (50 mg/kg total administered dose equivalent to 0.833 mg/kg/day) in a 60 day study in C57BL/6 mice; this reduction may be attributed to reduced food consumption reported in this group (Dong et al., 2011). C57 male mice exposed to 0 mg/kg/day, 0.5 mg/kg/day, or 10 mg/kg/day by oral gavage for 5 weeks also showed decreased body weight, but only in the 10 mg/kg/day group, which weighed 83% of controls (Qu et al., 2016). In a separate study, although reductions in body weight were observed in male BALB/c mice after 1 week of exposure to 10 mg/kg/day PFOS via gavage, this effect

was attenuated at the end of the exposure period at 3 weeks (Lv et al., 2015). Additionally, a significant increase in body weight was observed in 2.5 mg/kg/day exposure group at the end of the 3-week exposure period (Lv et al., 2015). Food consumption was not reported in these studies (Qu et al., 2016; Lv et al., 2015). No change in body weights were observed across 8 timepoints in male ICR mice exposed to 0.5 mg/kg/day, 5 mg/kg/day, or 10 mg/kg/day by oral gavage for 28 days (Qiu et al., 2016).

Three studies using Sprague-Dawley rats reported decreased body weights following PFOS exposure via oral gavage for 28 days, which usually occurred at the highest dose tested. Of these, Han et al. (2018a) and Wan et al. (2016) exposed males to 1 mg/kg/day or 10 mg/kg/day and observed an approximate 10% reduction in body weight following 10 mg/kg/day. NTP (2019) reported decreased body weights in male and female Sprague-Dawley rats exposed to 5 mg/kg/day PFOS. However, body weights of all dose male and female groups were within 10% of control groups. The decrease in body weights was not associated with reduced food consumption in Han et al. (2018a), and food consumption was not reported in the other studies (NTP, 2019; Wan et al., 2016). Two studies by Salgado et al. (2016; 2015) using the same animals reported no change in body weight variation or food consumption in male Sprague-Dawley rats administered 3 mg/kg/day or 6 mg/kg/day PFOS by oral gavage for 28 days, but data were not provided.

A reduction in body weight was also observed following 6 weeks of PFOS exposure via gavage in male and female Crl:Cd(Sd)lgs Br Vaf rats exposed to 3.2 mg/kg/day (weighing 93 and 88% of control, respectively), which was associated with decreased food consumption (Luebker et al., 2005a). Although a 6-week exposure to 2 mg/kg/day did not reduce body weights in female Crl:CD(SD)Igs Vaf/Plus rats, this dose did reduce mean female body weight gain and food consumption (Luebker et al., 2005b). In a study assessing the dietary PFOS exposure in the same rat strain, no change was observed in body weights or food consumption in male and female Crl:CD(SD)IGS BR rats exposed to PFOS in the diet at concentrations of 0 ppm, 0.5 ppm, 2 ppm, 5 ppm, or 20 ppm (equivalent to 0 mg/kg, 0.05 mg/kg, 0.18 mg/kg, 0.37 mg/kg, or 1.51 mg/kg in males and 0 mg/kg, 0.05 mg/kg, 0.22 mg/kg, 0.47 mg/kg, or 1.77 mg/kg in females) for 4 weeks (Seacat et al., 2003).

Chronic PFOS exposure studies also suggest an effect of PFOS on body weight. Male and female Cynomolgus monkeys exposed to 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day PFOS (equivalent to cumulative doses of 0 mg/kg, 4.6 mg/kg, 22.9 mg/kg, or 114.7 mg/kg) via intragastric intubation for 26 weeks (182 days) showed a reduction in body weight change in the highest dose group (8% reduction in males and 4% reduction in females), although no change in absolute body weight was observed (Seacat et al., 2002). This is in contrast to the 14% and 5% body weight increases in control males and females, respectively. However, chronic (14 weeks) exposure to PFOS in the diet at 0 ppm, 0.5 ppm, 2 ppm, 5 ppm, and 20 ppm (equivalent to 0 mg/kg, 0.05 mg/kg, 0.18 mg/kg, 0.37 mg/kg, and 1.51 mg/kg in males and 0 mg/kg, 0.05 mg/kg, 0.22 mg/kg, 0.47 mg/kg, and 1.77 mg/kg in females) showed had no effect on Crl:CD(SD)IGS BR male or female rats. For 20 ppm dose-group males, terminal body weights appeared to be reduced in a dose-dependent manner, however this difference was not statistically significant (Seacat et al., 2003). In line with reduced body weights, food consumption was significantly decreased in the 20 ppm exposure group, but these data were not shown and the sex of the animals affected was not specified (Seacat et al., 2003).

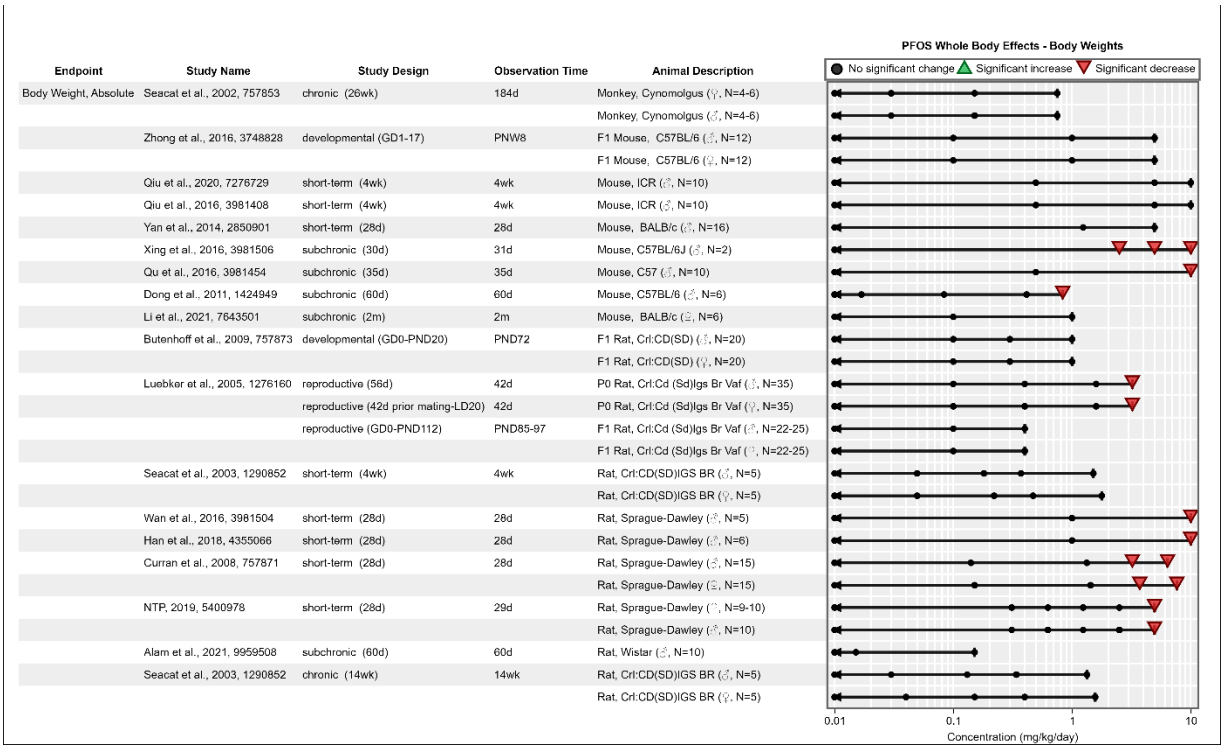


Figure C-24. Effects on Body Weight in Rodents and Non-Human Primates Following Exposure to PFOS (Logarithmic Scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; PND = postnatal day; LD = lactation day; d = day; wk = week.

C.3.2.2.3 Body Weight in Adults Following Developmental Exposure

Offspring body weights during developmental periods have been reported and described (see Toxicity Assessment, (U.S. EPA, 2024)). However, the effects on body weight may not persist into adulthood. No change was observed in adult body weight (PND 85–PND 97) compared with control in male and female Crl:CD(SD)Igs Br Vaf rats exposed perinatally through adulthood to 0.1 mg/kg/day and 0.4 mg/kg/day PFOS (Luebker et al., 2005a). Developmental (GD 1–GD 17) PFOS exposure in C57BL/6 mice at 0.1 mg/kg/day, 1 mg/kg/day, or 5 mg/kg/day was not observed to affect male or female body weight at PNW4 or PNW8 (Zhong et al., 2016). Similarly, body weights from birth to PND 70 were not statistically different from controls in the offspring of female Sprague-Dawley rats exposed to 0 mg/kg/day, 0.1 mg/kg/day, 0.3 mg/kg/day, or 1 mg/kg/day PFOS from GD 0–PND 20 (Butenhoff et al., 2009).

C.3.2.2.4 Food Consumption

Although there is some evidence that short-term and subchronic exposure of rodents to PFOS can lead to reductions in food consumption, this effect is not consistently observed across all exposures and strains tested. Food consumption was decreased in C57BL/6J male mice exposed to 2.5, 5, or 10 mg/kg/day PFOS by oral gavage for 30 days at all three doses (Xing et al., 2016). Decreased food consumption was also observed in female and male Crl:Cd(Sd)lgs Br Vaf rats following a 6 week exposure via gavage to 1.6 mg/kg/day or 3.2 mg/kg/day (Luebker et al.,

2005a), and in female Crl:Cd(Sd)lgs Vaf/Plus rats following a 6 week exposure to 2.0 mg/kg/day (Luebker et al., 2005b) (see Toxicity Assessment, (U.S. EPA, 2024)).

Food and water consumption was not observed to be affected in Sprague-Dawley rats exposed to PFOS via gavage at doses of 1 mg/kg/day or 10 mg/kg/day (Han et al., 2018a), 3 or 6 mg/kg/day (Salgado et al., 2015), nor 0.5 mg/kg/day, 1 mg/kg/day, 3 mg/kg/day, or 6 mg/kg/day (Salgado et al., 2016) for 28 days. Seacat et al. (2003) fed Crl:CD(SD)IGS Br male or female rats 0, 0.5, 2, 5, and 20 ppm PFOS for 4 or 14 weeks (equivalent to 0, 0.05, 0.18, 0.37, and 1.51 mg/kg in males and 0, 0.05, 0.22, 0.47, and 1.77 mg/kg in females). The authors noted that food consumption was slightly reduced in the 20 ppm female dose group during the first 4 weeks of dosing, but these data were not provided (Seacat et al., 2003). By 14 weeks, food consumption was noted to be significantly decreased in the 20 ppm dose group, but these data were not provided and the sex of the animals affected was not specified.

C.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse metabolic outcomes is discussed in Sections 3.2.2, 3.3.2, and 3.3.4 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are 32 and 36 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to metabolic and systemic effects, respectively. A summary of these metabolic and systemic studies is shown in Figure C-25 and Figure C-26, respectively. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to metabolic and systemic effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	2	1	3
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	11	12
Cell Signaling Or Signal Transduction	3	1	8	11
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	7	1	8	15
Hormone Function	1	4	3	8
Oxidative Stress	2	1	2	5
Xenobiotic Metabolism	0	0	2	2
Other	2	0	0	2
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	11	7	16	32

Figure C-25. Summary of Mechanistic Studies of PFOS and Metabolic Effects

Interactive figure and additional study details available on HAWC.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	3	0	2	4
Cell Growth, Differentiation, Proliferation, Or Viability	4	1	11	16
Cell Signaling Or Signal Transduction	2	1	7	10
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	1	9	13
Hormone Function	1	0	0	1
Inflammation And Immune Response	0	0	2	2
Oxidative Stress	5	1	7	13
Xenobiotic Metabolism	3	1	1	4
Other	0	0	4	4
Not Applicable/Not Specified/Review Article	2	0	0	2
Grand Total	10	3	25	36

Figure C-26. Summary of Mechanistic Studies of PFOS and Systemic Effects

Interactive figure and additional study details available on HAWC.

C.3.4 Evidence Integration

There is *slight* evidence of an association between PFOS exposure and metabolic effects in humans based on observed effects for diabetes, gestational weight gain, HOMA-IR, HOMA-B, leptin, and adiponectin in *high* and *medium* confidence studies. Five studies observed non-significant positive associations with gestational diabetes. In the general population, six studies reported positive associations with type 2 diabetes. Three epidemiological studies observed positive associations with gestational weight gain. Seven studies reported non-significant positive associations with HOMA-IR in pregnant women and in general populations, or in adults at high risk for type 2 diabetes. Of the six studies on HOMA-IR in children, only one reported a positive association with HOMA-IR. Four studies reported positive associations with HOMA-B, but an inverse association was observed in children (one study). There is limited evidence suggesting a potential association between PFOS exposure and adiponectin in children, but not adults. Findings for an association between PFOS exposure and MetS were mixed in four general population epidemiological studies identified since 2016: two reported negative associations with MetS, and two reported positive associations.

The animal evidence for an association between PFOS and systemic or metabolic effects is *indeterminate.* Although some alterations related to glucose homeostasis were reported in the available animal toxicity literature, the results from 6 *high* or *medium* confidence studies are inconclusive as there are too few studies to assess possible difference across life stages, sexes,

and species. In addition, the effects on body weight, clinical observations, and mortality from 20 *high* or *medium* confidence studies indicate that the systemic effects occur only at the high doses tested. NTP (2019) and Seacat et al. (2003) reported differing observations on the impact of PFOS on serum glucose in male rats at 4 weeks, which may be explained by differing methods of exposure (gavage and dietary, respectively). Additionally, the statistically significant observations reported by Seacat et al. (2003) and Curran et al. (2008) differ between males and females, are not consistent across timepoints, and sometimes did not follow a linear dose-response relationship. Given the differences noted in timing of measurement, duration of exposure, and differences across sex, the biological significance of the increase or decrease in metabolic endpoints such as serum glucose in these animal models is unclear, especially considering the sensitivity of these parameters to increases in animal stress.

There were also inconsistencies in results reported in developmental studies. Lv et al. (2013) reported dose-dependent increases in serum glucose during a glucose tolerance test at PNW10 in rat offspring. This trend did not continue through PNW 15 in this study. In addition, Wan et al. (2014) did not report significantly altered results of the glucose tolerance test at PND 63 in mouse offspring gestationally exposed to PFOS and fed standard diets. Although multiple studies indicate potential effects of PFOS on glucose homeostasis, the responses were inconsistent and/or transient for specific endpoints across studies and the biological significance of the observed effects is uncertain.

Though the observed metabolic effects were inconsistent, evidence from animal studies suggests that PFOS exposure may induce whole-body toxicity, but only at the higher doses tested. Decreased body weight and food consumption were observed in a number of subchronic and chronic studies using rodents and non-human primates. While signs of decreased body weights can be indicative of poor health in animals and a relevant endpoint demonstrating whole-body toxicity, the effects reported in these studies were generally minimal and only surpassed a >10% change in body weight at the highest doses tested.

C.3.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause systemic and metabolic effects in humans under relevant exposure circumstances (Table C-6). This conclusion is based primarily on diabetes, gestational weight gain, HOMA-IR, HOMA-B, leptin, and adiponectin effects observed in *high* and *medium* confidence studies in humans exposed to median PFOS levels between 5.4 and 35.7 ng/mL. Although there is some evidence of negative effects of PFOS exposure on MetS, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-6. Evidence Profile Table for PFOS Systemic and Metabolic Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.3.1)					⊕⊙⊙ ***Evidence Suggests***
Glucose metabolism 4 *High* confidence studies 13 *Medium* confidence studies 7 *Low* confidence Studies	Findings for FBG in adults were primarily positive (7/12), but only a few reached significance. OGTT results were examined only in studies finding significant increases in FBG and were congruent with FBG findings. In children, decreases in FBG were observed (3/5), but none were significant. Findings for FBG in pregnant women were similarly non-significantly inverse (3/4), however, the three *high* and *medium* confidence studies conducting OGTT observed increases in 1-hr glucose levels, two of which were significant.	• *High* and *medium* confidence studies • *Consistent direction* of effect for FBG in adults	• *Low* confidence studies • Imprecision of findings • Potential for *selection bias* and residual confounding by SES	⊕⊙⊙ *Slight* Evidence for metabolic effects is based on increases in FBG, increased odds of diabetes, and increases in measures of adiposity in adults. Positive associations were reported for heightened glucose levels, effects on insulin regulation, diabetes, and adiposity, but many *medium* and *high* confidence studies presented non-statistically significant results and several studies presented conflicting associations. Uncertainties remain due to mixed results, contrasting findings, and potential for residual confounding in the analysis of outcomes such as glucose metabolism, diabetes, and insulin levels.	*Primary basis*: Human evidence indicted effects on diabetes, gestational weight gain, HOMA-IR, HOMA-B, leptin, and adiponectin and there was limited animal evidence. Although there is some evidence of negative effects of PFOS exposure on MetS, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Diabetes (and gestational diabetes) 3 *High* confidence studies 16 *Medium* confidence studies 5 *Low* confidence studies	Findings in adults were mixed. Among the *high* and *medium* confidence studies (8/11), two reported significant positive associations (2/8), 1 reported a significant inverse association (1/8),	• *High* and *medium* confidence studies	• *Low* confidence studies • Inconsistent direction of effect • *Imprecision* of findings • Potential for *outcome misclassification*, self-selection, residual		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	and 5 reported imprecise associations (5/8). The 3 *low* confidence studies all reported non-significant positive associations and typically relied on self-reported data. Findings for HbA1c were less consistent. In pregnant women, findings for gestational diabetes were mixed. The only study examining diabetes in children was considered *uninformative.*		confounding by SES, and failure to establish temporality		
Insulin levels 2 *High* confidence studies 7 *Medium* confidence studies 10 *Low* confidence studies	Findings from a *high* confidence study in adults reported significant increases in fasting insulin, HOMA-IR, HOMA-B, and insulin responses during an OGTT, however, this population was at high risk for type 2 diabetes. Findings for adults among *medium* and *low* confidence studies were generally mixed, but there were multiple contrasting findings for HOMA-IR, indicating an inverse association (5/9). Studies in children reported mixed and	• *High* and *medium* confidence studies	• *Low* confidence studies • Inconsistent direction of effects • Imprecision of findings • Potential for *residual confounding* by diabetes status or use of medications that would impact insulin levels in some studies		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	generally imprecise findings for measures of insulin resistance. Similarly, findings in studies among pregnant women were imprecise.				
Adiponectin and leptin 5 *High* confidence studies 3 *Medium* confidence studies 1 *Low* confidence study	Inverse associations with adiponectin were reported in two studies of adults (2/2), while one study (1/1) reported increases in leptin. None reached significance. Findings for adiponectin in children were positive (5/6), but only one reached significance. Findings for leptin were mixed among children. Only one study reported findings from pregnant women, observing non-significant increases in both adiponectin and leptin.	• *High* and *medium* confidence studies • *Consistent direction* of effect for adiponectin in children	• *Low* confidence study • Imprecision of findings		
Adiposity 4 *High* confidence studies 17 *Medium* confidence studies 4 *Low* confidence studies	In adults, findings for BMI were primarily positive (4/6), indicating increased BMI. Increases in the odds of being overweight or obese were also reported, which was significant for women in one study. Results were mixed for WC, but one study observed	• *High* and *medium* confidence studies	• *Low* confidence studies • Inconsistent direction of effects • Imprecision of findings		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	differences in direction of effect between men and women. Findings for BMI in children were mixed, with studies of *medium* confidence reporting significant positive and significant inverse associations with measures of BMI. In pregnant women, positive associations were reported for gestational weight gain, but results were inconsistent between studies after stratification of weight status (i.e., under-, normal-, or overweight).				
Metabolic syndrome 4 *Medium* confidence studies 1 *Low* confidence study	In adults, findings for MetS were mixed, and none reached significance (0/4). Significant reduction in the resting metabolic rate were observed in a single study of adults. MetS was not evaluated in children or pregnant women.	• *Medium* confidence studies	• *Low* confidence study • *Inconsistent direction* of effects in *medium* confidence studies • Concern for *selection bias*, outcome misclassification, and residual confounding by SES in *low* confidence study		
Evidence From In Vivo Animal Studies (Section C.3.2)					
Glucose homeostasis 1 *High* confidence study 5 *Medium* confidence studies	Mixed results were reported on glucose levels in rodent studies (6). Of these, 2 reported non-significant effects,	• *High* and *medium* confidence studies	• *Inconsistent direction* and *magnitude* of effects across study designs and sex	⊙⊙⊙ *Indeterminate* Alterations related to glucose homeostasis were	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	and 4 reported significant effects with inconsistent directionality. Reduced glucose levels were reported in female rodents (3/4) at the highest PFOS exposure group tested. No significant effects on glucose levels were observed in males (3/3) and dams (1/2). One study in female mice reported decreased insulin resistance (1/1) and pyruvate tolerance (1/1).		• *Limited number* of studies examining outcomes	reported in 6 *high* or *medium* confidence studies were inconclusive as there are too few studies to assess possible difference across life stages, sexes, and species and results from the existing studies are inconsistent or transient. Systemic effects (e.g., body weight, clinical observations, survival, food consumption, and water consumption) from 20 *high* or *medium* confidence studies indicate that biologically significant effects (e.g., body weight change exceeding 10% of control) tend to occur only at the highest doses tested.	
Body weight 3 *High* confidence studies 17 *Medium* confidence studies	Statistically significant reductions in body weights (9/20) and body weight changes (2/2) were reported in various studies, including studies in rats (11), mice (9), and monkeys (2).	• *High* and *medium* confidence studies • Consistent direction of effects • *Confounding variables* such as food consumption were considered in most studies	• Effects do not follow a *linear dose-responsive* relationship		
Survival and mortality 1 *High* confidence studies 6 *Medium* confidence studies	No effects on survival and mortality were reported in rodent studies (6/6). One study in non-human primates observed increased mortality at the highest dose tested (1/1).	• *High* and *medium* confidence studies • *Consistent direction* of effects across sex, species, and duration of exposure	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Clinical observations 1 *High* confidence study 3 *Medium* confidence studies	Clinical observations were observed in most rodent studies (3/4). Findings found across these studies included: hyperkalemia, rough hair, slow movement, constipation, and localized areas of partial alopecia.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes • Qualitative and subjective data reporting		
Food and water consumption 9 *Medium* confidence studies	Reduced food consumption (6/9) was reported in the higher dose groups in male and female rodents. No significant effects were reported on water consumption in male rats following short-term exposure (2/2).	• *Medium* confidence studies • *Consistent direction* of effects on water consumption	• *Limited number* of studies examining outcomes		

Notes: FBG = fasting blood glucose; hr = hour; OGTT = oral glucose tolerance testing; HbA1c = hemoglobin A1c; SES = social economic status; HOMA-IR = homeostatic model assessment for insulin resistance; HOMA-B = homeostasis model assessment of β-cell function; BMI = body mass index; WC = waist circumference; MetS = metabolic syndrome.

C.4 Nervous

EPA identified 36 epidemiological and 16 animal studies that investigated the association between PFOS and nervous effects. Of the epidemiological studies, 3 were classified as *high* confidence, 28 as *medium* confidence, and 5 were considered *low* confidence (Section C.4.1). Of the animal studies, 1 was classified as *high* confidence, 8 as *medium* confidence, 4 as *low* confidence, 2 as *mixed* (2 *medium/low*) confidence, and 1 was considered *uninformative* (Section C.4.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.4.1 Human Evidence Study Quality Evaluation and Synthesis

C.4.1.1 Introduction

The 2016 Health Assessment (U.S. EPA, 2016c) reviewed studies examining associations between PFOS exposure and neurodevelopmental disorders in children, including attention deficit hyperactivity disorder (ADHD) and learning disabilities and concluded there was limited evidence to suggest an effect. A significant increase in risk of development of cerebral palsy in males was observed in a case-control study of maternal PFOS levels of participants within the DNBC (Liew et al., 2014). One study observed a significant positive association of child PFOS levels with parent-reported ADHD in children aged 12–15 in the general population (Hoffman et al., 2010). No association between maternal plasma PFOS concentrations and Apgar score or between maternal plasma PFOS concentrations and mother reported assessments of fine motor skills, gross motor skills or cognitive skills in children at 6 and 18 months of age were observed in one study of pregnant women and their children (Fei et al., 2008a). No association between parent-reported behavioral or coordination problems in children 7 years of age and prenatal PFOS levels was reported in another study (Fei and Olsen, 2011). No associations were observed between prenatal PFOS and parent-reported motor development scores in children ages 7 to 9; however, the highest PFOS tertile was associated with a 0.5-point higher hyperactivity score for participants within one country with higher exposures, but not for participants within other countries (Høyer et al., 2015). Data interpretations within these studies were limited in some cases by use of a cross-sectional study design (Hoffman et al., 2010; Fei et al., 2008a), potential random misclassification error resulting from using current PFOS levels as proxy measures of etiologically relevant exposures (Hoffman et al., 2010), outcomes defined by parental report (Høyer et al., 2015; Fei and Olsen, 2011; Hoffman et al., 2010; Fei et al., 2008a), and limited sample sizes in some countries (Høyer et al., 2015).

For this updated review, 35 studies (35 publications) investigated the association between PFOS and neurological outcomes that have been identified since the 2016 document. One was conducted in a high-exposure community (Spratlen et al., 2020a). One publication (Vuong et al., 2020b) was conducted in pregnant women. The remainder were conducted in the general population. Study designs included 3 case-control (Shin et al., 2020; Long et al., 2019; Ode et al., 2014), 2 nested case-control (Lyall et al., 2018; Liew et al., 2015), 26 cohort, and 5 cross-sectional studies (Appendix D). The studies measured PFOS in different matrices including blood, serum, plasma, cord blood, breast milk (Lenters et al., 2019; Forns et al., 2015), maternal serum, maternal plasma, and amniotic fluid (Long et al., 2019). Several studies (Vuong et al., 2020b; Vuong et al., 2020a; Vuong et al., 2019; Vuong et al., 2018b; Vuong et al., 2018a; Zhang

et al., 2018a; Vuong et al., 2016; Braun et al., 2014) were conducted on subsets of data from the HOME study. Two studies (Lenters et al., 2019; Forns et al., 2015) utilized data from the Norwegian Human Milk Study (HUMIS). Two studies (Liew et al., 2018; Liew et al., 2015) utilized the DNBC data. The studies were conducted in multiple locations including populations from China, Denmark, the Faroe Islands, Great Britain, Japan, the Netherlands, Norway, Sweden, Taiwan, and the United States (Appendix D). Neurological effects were determined for numerous clinical conditions and by assessing performance on neuropsychological tests assessing various neurological domains, including developmental, general intelligence (i.e., intelligence quotient (IQ)), social-emotional, executive function, ADHD and attention, autism spectrum disorder (ASD) and intellectual disability (ID), and visuospatial performance.

C.4.1.2 Study Quality

There are 34 studies (36 publications)[7] from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and nervous effects. Study quality evaluations for these 36 studies are shown in Figure C-27 and Figure C-28.

Of the 36 studies identified since the 2016 assessment, three (Niu et al., 2019; Harris et al., 2018; Oulhote et al., 2016) were classified as having *high* confidence, 28 studies were classified as *medium* confidence, and five were *low* confidence. Studies rated as *low* confidence had deficiencies including potential residual confounding, exposure misclassification, selection bias, and small sample size. One *low* confidence NHANES study (Berk et al., 2014) had a high likelihood of residual confounding due to the use of an insensitive marker of SES, and the analysis did not account for the population's complex sampling design. Differences in laboratory extraction methods, collection timing, and missing details on storage raised concerns for exposure misclassification in a study on children from the HUMIS cohort (Forns et al., 2015). Additionally, children were only evaluated on some, but not all, test instrument (Ages and Stages Questionnaire (ASQ)) domains, and rationale for domain selection was not provided. Concerns for Lien et al. (2016) included a high loss to follow-up, lack of detail on completion rates of ADHD questionnaires and low detection rate for PFOS. Small sample size, temporality and reporting issues were cited as limitations in Weng et al. (2020). Finally, limitations in Ode et al. (2014) included sensitivity concerns due to the limited number of ADHD cases and potential for residual confounding due to the lack of data on other exposures potentially related to ADHD. In the evidence synthesis below, *high,* and *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

[7] Vuong et al. (2018b) reports score trajectories for the same population and test as Vuong et al. (2016). Vuong et al. (2020a) reports on an overlapping population with the same test as Zhang et al. (2018a).

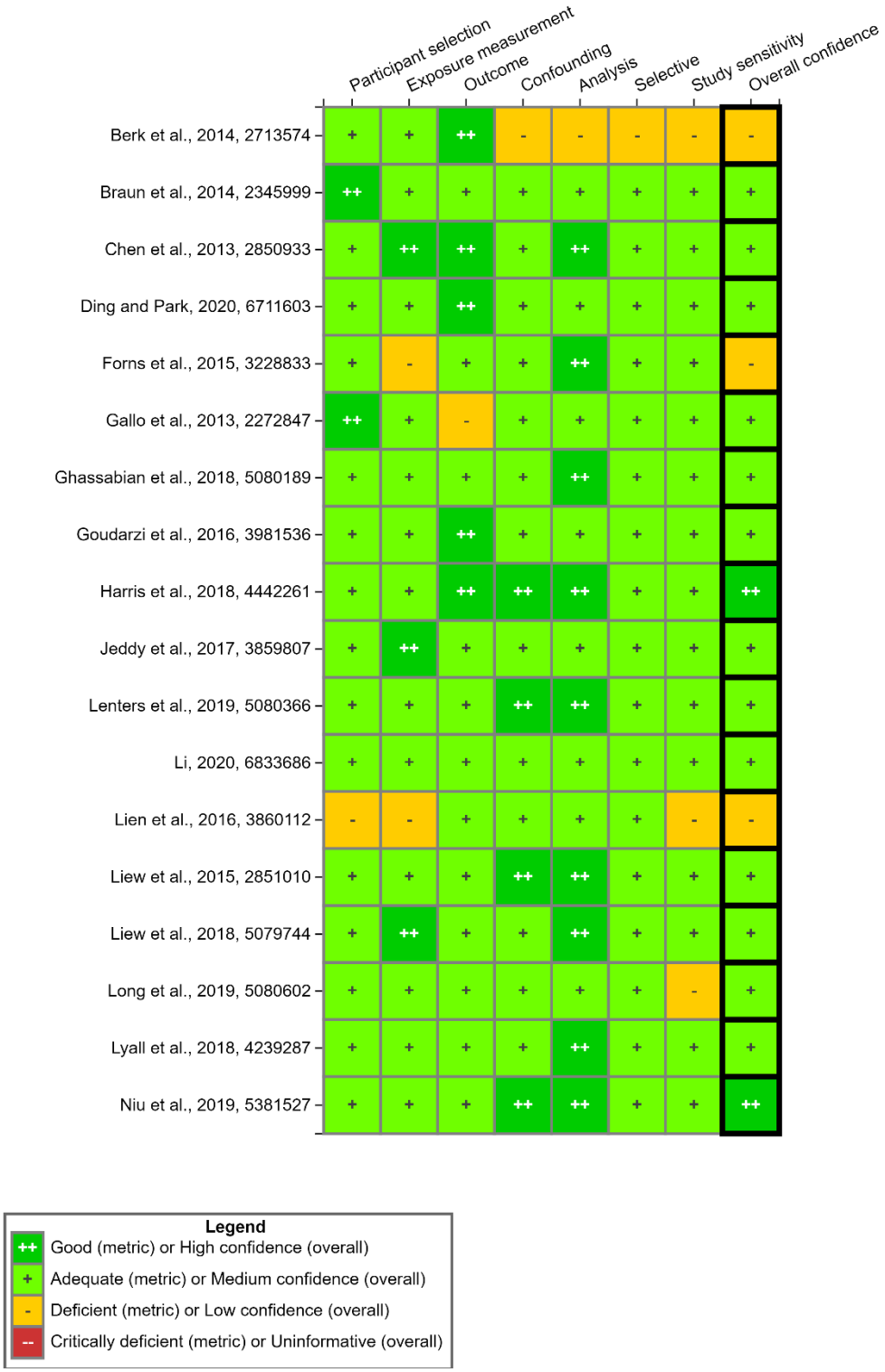


Figure C-27. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Neurological Effects

Interactive figure and additional study details available on HAWC.

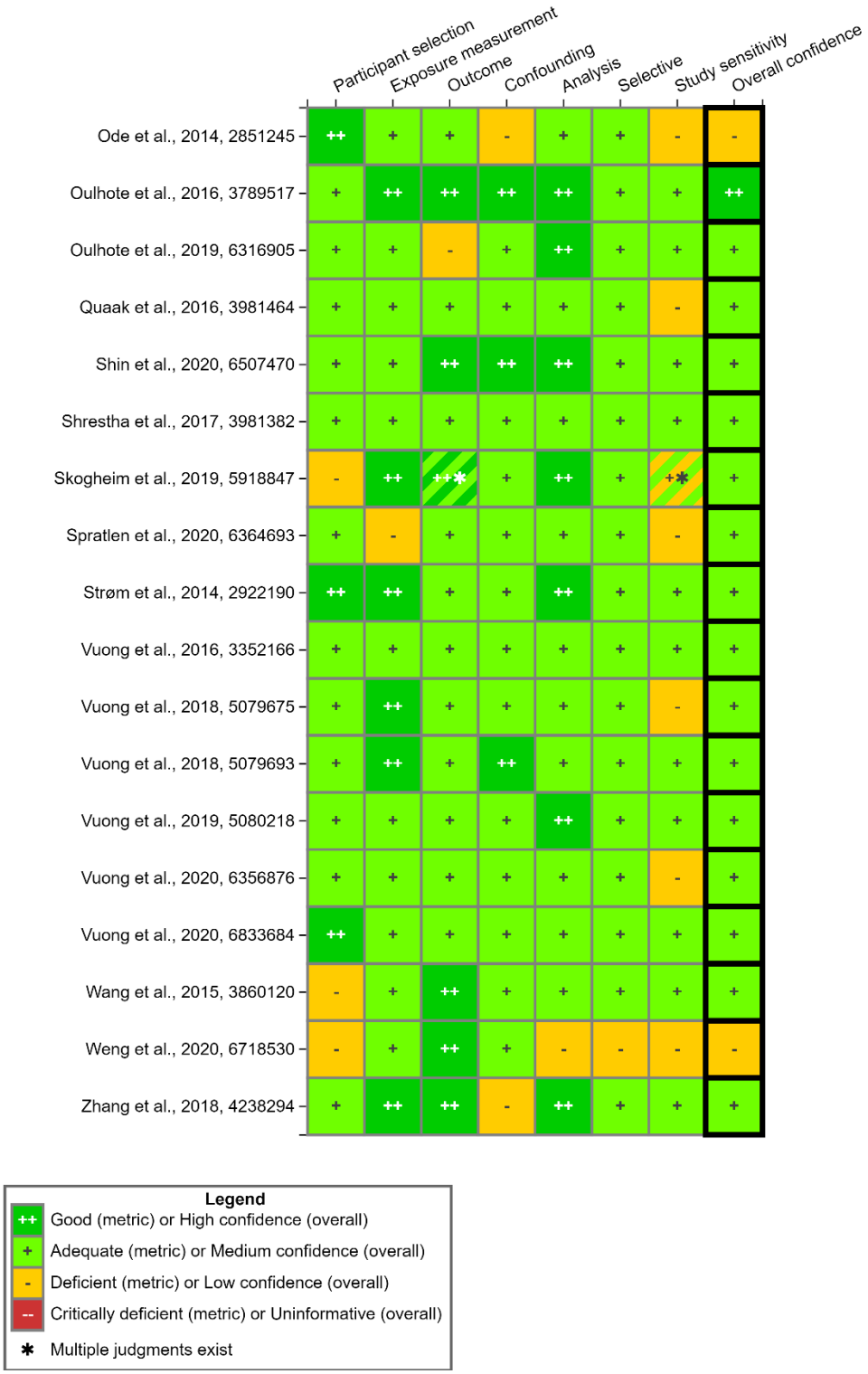


Figure C-28. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Neurological Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.4.1.3 Findings From Children and Adolescents

Six cohort studies (Niu et al., 2019; Jeddy et al., 2017; Shrestha et al., 2017; Goudarzi et al., 2016b; Forns et al., 2015; Chen et al., 2013), and one high-exposure community study (Spratlen et al., 2020a) examined developmental outcomes in children. In a *high* confidence study (Niu et al., 2019) from the Shanghai-Minhang Birth Cohort Study (S-MBCS), maternal PFOS concentrations (median = 10.8 ng/mL) during pregnancy were inversely associated with neuropsychological development (especially for personal-social skills) assessed by the ASQ in 4-year-old children. A *medium* confidence study of data from the Taiwan Birth Panel Study (Chen et al., 2013) observed associations between in utero PFOS (mean = 7.4 ng/mL) and decreases in Comprehensive Developmental Inventory (CDI) developmental quotients in the highest exposure group compared with the lowest exposure group for the whole test as well as for gross motor, fine motor, and self-help domains. Effect sizes were generally greater with increasing PFOS levels. A *medium* confidence study (Jeddy et al., 2017) utilizing data from the ALSPAC observed significant associations between maternal PFOS (median = 19.8 ng/mL) and verbal comprehension scores as assessed by the adapted MacArthur Communicative Development Inventories for Infants (MCDI) in children at 15 months of age, but not for vocabulary comprehension and production, nonverbal communication, or social development. Significant inverse associations were also observed between maternal PFOS and language and intelligibility scores in children at 38 months of age. Results for this study varied by maternal age at delivery. A statistically significant inverse association was reported for vocabulary comprehension and production scores in 15-month infants with mothers <25 years of age. A significant inverse association was observed for intelligibility scores in children 38 months of age with mothers >30 years of age, and a significant positive association was observed for intelligibility scores in children 38 months of age with mothers <25 years of age. Results from a *medium* confidence study (Goudarzi et al., 2016b) reported no significant associations between prenatal PFOS levels (median = 5.7 ng/mL at 6 months; median = 5.8 at 18 months) and Mental (MDI) and Psychomotor (PDI) Development Indices in infants at 6 and 18 months. Similarly, no significant adverse associations or apparent trends between delivery or cord blood PFOS concentrations (median = 6.0 ng/mL) and age 1 mental or psychomotor developmental indices were reported in a high-exposure community study of children prenatally exposed to the World Trade Center (WTC) Disaster, however a significant interaction by sex with MDI at ages 2 and 3, with stronger positive associations for females compared with males was observed (Spratlen et al., 2020a).

Ten studies evaluated cognitive function and IQ measures among children, with most conducted within the general population (Vuong et al., 2020a; Oulhote et al., 2019; Skogheim et al., 2019; Vuong et al., 2019; Harris et al., 2018; Liew et al., 2018; Zhang et al., 2018a; Wang et al., 2015b; Strøm et al., 2014), and one within a high-exposure community (Spratlen et al., 2020a). In a *high* confidence analysis of participants within Project Viva, children born to women with top quartile PFOS (34.9–168.0 ng/mL) concentrations had higher nonverbal IQ scores, although dose-response patterns appeared non-linear (Harris et al., 2018). Positive associations were observed between prenatal PFOS (median = 12.7 ng/mL) and reading skills at age eight years in a *medium* confidence study (Vuong et al., 2020a) which utilized data from the HOME study. Childhood serum PFOS concentrations at ages three and eight years were positively associated with higher children's reading scores at ages five and eight years, respectively in an additional *medium* confidence study of data within the HOME study (Zhang et al., 2018a). No significant

associations were reported between maternal prenatal PFOS (median = 21.4 ng/mL) and offspring scholastic achievement in a *medium* confidence prebirth cohort study of participants within the Danish Fetal Origins 1988 (DaFO88) cohort (Strøm et al., 2014). Maternal prenatal PFOS (median = 27.7 ng/mL) concentrations were associated with lower cognitive function as assessed by the Boston Naming Test in a *medium* confidence study of children aged seven years (Oulhote et al., 2019).

In a *medium* confidence study in a highly exposed community, sex-specific trends between PFOS exposures and some cognitive outcomes (verbal and full-scale IQ only) at 4 and 6 years were observed, suggesting stronger positive associations for females compared with males (Spratlen et al., 2020a). Another *medium* confidence study investigated associations between prenatal exposure to PFOS and IQ at age five in a sample of children from the DNBC with no consistent associations observed (Liew et al., 2018). Consistent adverse associations with age eight cognitive development as assessed by IQ were not observed in an additional *medium* confidence study (Vuong et al., 2019). Similarly, utilizing data from participants within the Taiwan Maternal and Infant Cohort Study, a *medium* confidence prospective cohort study by Wang (Wang et al., 2015b) reported no significant associations between maternal serum PFOS (median = 13.3 ng/mL) and IQ measurements in children five or eight years of age. Evidence was inconsistent, with significant decreases in nonverbal working memory only in the highest quintile and no significant associations with verbal working memory, for the evaluation of the association between prenatal exposure to PFOS (median = 11.5 ng/mL) and cognitive dysfunction in preschool children in a *medium* confidence study from The Norwegian Mother, Father, and Child Cohort Study (MoBa) (Skogheim et al., 2019).

Six studies assessed the relationship between PFOS and behavioral development problems and behavioral regulation problems (Weng et al., 2020; Oulhote et al., 2019; Ghassabian et al., 2018; Vuong et al., 2018a; Oulhote et al., 2016; Quaak et al., 2016). No significant associations between prenatal PFOS (1,650 ng/L) and externalizing problems at age 18 months assessed using the Child Behavior Checklist 1.5–5 (CBCL 1.5–5) were reported in a *high* confidence study utilizing data from the Dutch cohort LINC (Linking Maternal Nutrition to Child Health) (Quaak et al., 2016). No consistent associations in total Strengths and Difficulties Questionnaire (SDQ) behavior scores with serum PFOS (median = 16.8 ng/mL) at age five was observed, but a twofold increase in serum PFOS (median = 15.3 µg/L) in children aged seven years was associated with higher SDQ total behavioral difficulties scores in girls, and lower scores in boys (gender interaction $p < 0.05$) in a *high* confidence study (Oulhote et al., 2016). Maternal prenatal PFOS concentrations (median = 27.7 ng/mL) were positively associated with total scores on the SDQ, indicating more behavioral problems, in a *medium* confidence study of children seven years of age (Oulhote et al., 2019). Higher newborn PFOS levels (median = 1.7 ng/mL) in dried blood spots were associated with increased odds of having behavioral difficulties, driven mostly by problems in conduct and emotional symptoms, as assessed by the maternal completed SDQ at age 7 in another *medium* confidence birth cohort study (Ghassabian et al., 2018). Child sex modified the associations between prenatal PFOS and attention, with males having better performance than females, but not enough evidence was observed to support an overall association between prenatal PFOS (median = 12.9 ng/mL) and inattention and impulsivity as assessed by the Connors' Continuous Performance Test-II in a *medium* confidence study (Vuong et al., 2018a). A *low* confidence study on adolescents reported a significant, inverse correlation

between prenatal PFOS levels (mean = 14.85 ng/mL) and in the right putamen brain region associated with impulsive behavior as assessed by MRI in teenage offspring (Weng et al., 2020).

One *medium* confidence study (Strøm et al., 2014) from the DaFO88 cohort examined the association between prenatal PFOS exposure and depression among offspring with 20 years of follow-up. No significant association was observed between clinical depression and maternal PFOS (median = 21.4 ng/mL) levels.

Three *medium* confidence studies (Vuong et al., 2018b; Shrestha et al., 2017; Vuong et al., 2016) examined the relationship between PFOS concentrations and executive function in children with mixed results. Executive function was assessed with the parent-rated Behavior Rating Inventory of Executive Function (BRIEF) in two studies (Vuong et al., 2018b; Vuong et al., 2016) among HOME study participants at five and eight years of age. Higher BRIEF scores indicate executive function impairments. Maternal serum PFOS concentrations were significantly associated with poorer behavior regulation, metacognition, and global executive functioning, with approximately a 3-point increase in all summary measures with a 1 ln-unit increase in PFOS concentrations (Vuong et al., 2016). Vuong et al. (2018b) again utilized data from the HOME study in a *medium* confidence cross-sectional analysis to examine associations of child PFOS levels measured in children aged eight years with executive function and reported no significant associations between PFOS and executive function.

Five *medium* confidence studies assessed relationships between PFOS exposures and ADHD (Lenters et al., 2019; Skogheim et al., 2019; Quaak et al., 2016; Liew et al., 2015; Strøm et al., 2014). One *medium* confidence study (Lenters et al., 2019) examined early-life high PFOS exposures in breast milk in relation to ADHD among children (range: 7.2–14.1 years old) from the HUMIS and reported significant associations with PFOS concentrations (median = 117.7 ng/L) and increased odds of ADHD (OR = 1.75, 95% CI: 1.11, 2.76) with significant sex-specific effects. Strøm et al. (2014) investigated the association between maternal prenatal PFOS and ADHD among offspring (follow-up to age 20) of participants within the DaFO88 cohort. No significant association between maternal PFOS (median = 21.4 ng/mL) and offspring ADHD was reported in this *medium* confidence study. A *medium* confidence nested case-control study (Liew et al., 2015) within the framework of the DNBC examined prenatal PFOS exposures and ADHD in children. No consistent evidence was observed to suggest that prenatal PFOS exposures (ADHD cases median = 26.8 ng/mL; controls median = 27.4 ng/mL) increase the risk of ADHD. Quaak et al. (2016) explored the relationship between prenatal PFOS exposures and parent-reported ADHD using the CBCL 1.5–5. This *medium* confidence study utilized data from the Dutch cohort, LINC. No significant associations were reported between cord blood PFOS (median = 1,600 ng/L) exposures and ADHD scores in the whole population or in the sex-stratified analyses.

Two *low* confidence studies (Lien et al., 2016; Ode et al., 2014) examined PFOS exposures in relation to ADHD. Ode et al. (2014) investigated the association in a case-control study between cord blood PFOS (median = 6.9 ng/mL for cases, 6.8 ng/mL for controls) exposures and ADHD diagnosis in childhood (age range 5–17 years), but no associations between PFOS and ADHD were observed. Lien, 2016, 3860112 evaluated the association between cord blood PFOS (mean = 4.8 ng/mL) exposures and neurobehavioral symptoms related to ADHD among 7-year-old participants from the Taiwan Birth Panel Study and the Taiwan Early-Life Cohort, but no effects were observed.

One *high* (Oulhote et al., 2016)and five *medium* confidence studies since the 2016 assessment evaluated PFOS exposures in relation to autism, autistic behaviors, and ID (Shin et al., 2020; Long et al., 2019; Lyall et al., 2018; Liew et al., 2015; Braun et al., 2014). A twofold increase in serum PFOS (median = 15.26 µg/L) at age seven was associated with significantly higher SDQ autism screening scores at age seven, with higher autism scores in females than in males, in a *high* confidence study (Oulhote et al., 2016). In a *medium* confidence prospective birth cohort study from the HOME study, increasing maternal serum PFOS concentrations (median = 13 µg/L) were associated with increased autistic behaviors in children 4 to 5 years of age as assessed by maternal completed Social Responsiveness Scale (SRS) scores, although not significantly so, and PFOS levels were positively associated with SRS scores in boys, but not girls (Braun et al., 2014). No consistent evidence of an association between maternal plasma PFOS (median = 25.4 ng/mL for cases; 27.4 ng/mL for controls) and diagnosed childhood autism identified by linkage to the Danish National Hospital Registry was observed in a *medium* confidence nested case-control study of mother-child pairs with an average of ten years of follow-up within the DNBC (Liew et al., 2015). Autism cases had significantly lower PFOS levels in a *medium* confidence case-control study of amniotic fluid PFOS (median = 0.6 ng/mL for cases; 1.4 ng/mL for controls) and diagnosed ASD, with cases identified as born 1982–1999 within the Danish Psychiatric Central Registry (Long et al., 2019). Prenatal maternal serum PFOS (median = 17.5 ng/mL for ASD cases; 15.9 ng/mL for ID cases; 17.9 ng/mL for controls) was inversely associated with ASD and ID in a *medium* confidence study of children aged 4.5–9 years with diagnosed ASD and ID (Lyall et al., 2018). An association was reported in a *medium* confidence study of modeled prenatal maternal PFOS and clinically confirmed ASD from mother-child pairs in the Childhood Autism Risk from Genetics and Environment (CHARGE) study of children ages two to five years, with modeled prenatal maternal PFOS (median = 3.1 ng/mL for cases; 3.3 ng/mL for controls) associated with increased odds of child diagnosis of ASD and among boys when stratified by sex (Shin et al., 2020).

The effects on visuospatial performance were evaluated in one *high* confidence study of participants of Project Viva (Harris et al., 2018). Visual-motor test scores (Wide Range Assessment of Visual Motor Abilities) were consistently lower with increasing prenatal or childhood PFOS exposures. Children in the upper quartile of prenatal PFOS (Q4 = 34.9–168.0 ng/mL) had lower mid-childhood visual-motor scores, and participants in the third quartile of childhood PFOS (Q3 = 6.3–9.7 ng/mL) had significantly decreased visual-motor scores. Participants from the HOME study were assessed using the Virtual Morris Water Maze (VMWM), but no significant effects were observed (Vuong et al., 2018a).

C.4.1.4 Findings From Pregnant Women

No evidence was observed to support an adverse relationship between serum PFOS during pregnancy and maternal depressive symptoms assessed by the Beck Depression Inventory (BDI) from pregnancy to eight years postpartum in a *medium* confidence study based on women from the HOME study (Vuong et al., 2020b).

C.4.1.5 Findings From the General Adult Population

The effects of PFOS on general intelligence and IQ test outcomes were examined in a *medium* confidence study (Shrestha et al., 2017) of adults (ages 55–74 years) in New York state. Findings

indicated higher PFOS was significantly associated with improved performance in tests of delayed recall.

Findings of a *medium* confidence study (Shrestha et al., 2017), described above, indicated no significant associations between serum PFOS in adults and tests of executive function.

Two *medium* confidence studies investigated a possible association between PFOS and depression (Vuong et al., 2020b; Shrestha et al., 2017). No significant associations were observed in a *medium* confidence study of depression assessed by the BDI and serum PFOS (median = 33.7 ng/mL) in a cross-sectional study of adults aged 55 to 74 years (Shrestha et al., 2017). Additionally, no evidence was observed to support a relationship in adults between serum PFOS during pregnancy and maternal depressive symptoms assessed by the BDI from pregnancy to 8 years postpartum in a *medium* confidence study based on women from the HOME study (Vuong et al., 2020b). One *low* confidence study (Berk et al., 2014) of data from adults participating in NHANES reported no adverse associations between PFOS levels and depression as assessed by the nine-item depression module of the Patient Health Questionnaire (PHQ-9).

The effects on visuospatial performance were evaluated in one *medium* confidence cross-sectional study of older adults (Shrestha et al., 2017). A significant association between serum PFOS and improved tests of visual and spatial function results was reported.

Two *medium* confidence studies explored the relationships between PFOS and memory loss. (Shrestha et al., 2017; Gallo et al., 2013). Statistically significant inverse associations between PFOS and memory impairment were reported in a *medium* confidence study of adults in the C8 Health Project (Gallo et al., 2013). No adverse effects of PFOS on memory impairment were again reported in a separate *medium* confidence study of older adults (Shrestha et al., 2017).

Two *medium* confidence cross-sectional studies investigated PFOS and hearing impairment in adult NHANES participants. Li, 2020, 6833686 reported positive correlations between PFOS and hearing impairment, while Ding and Park (2020) observed no significant associations.

C.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and 13 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and nervous effects. Study quality evaluations for these 16 studies are shown in Figure C-29.

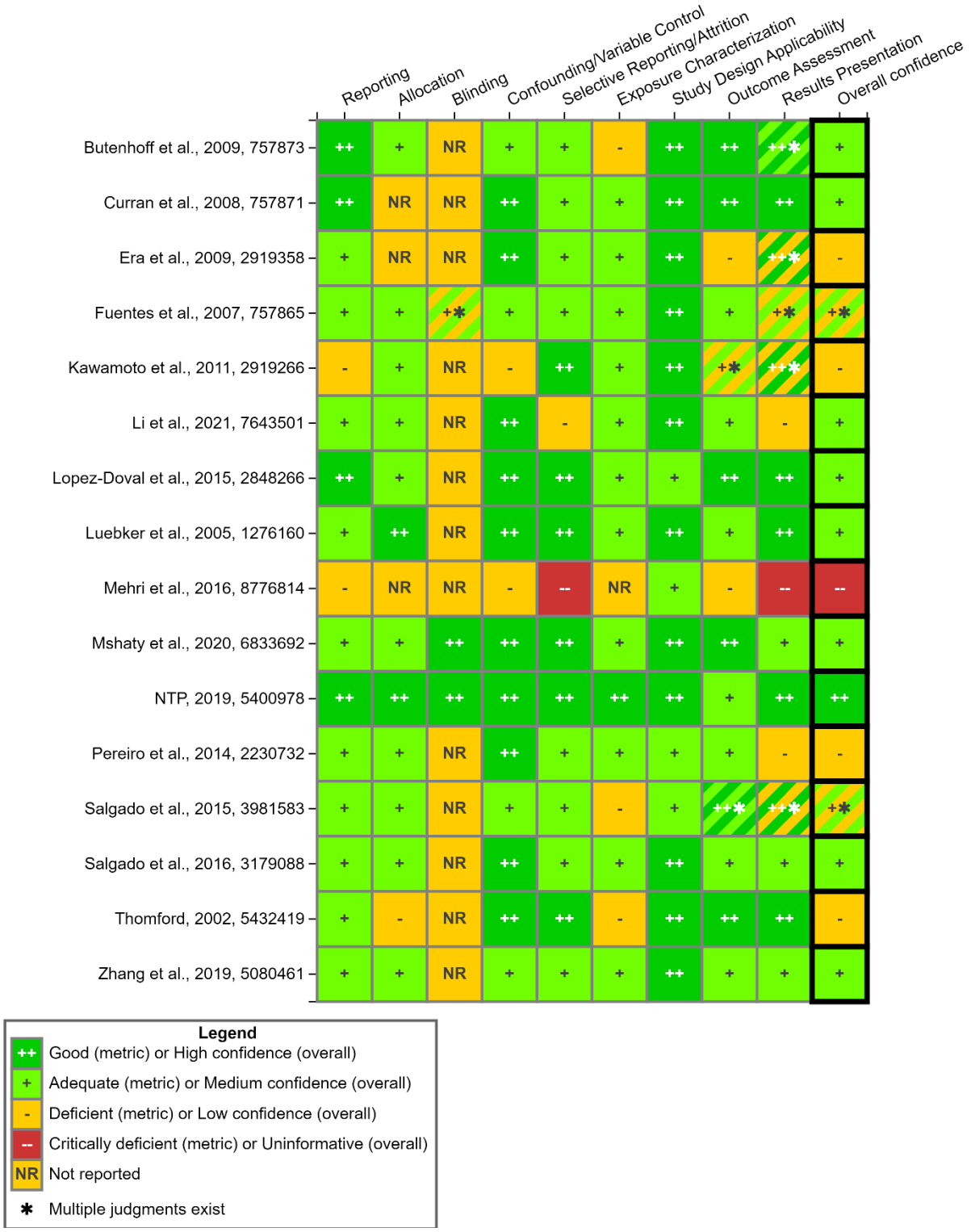


Figure C-29. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Nervous Effects

Interactive figure and additional study details available on HAWC.

There are few studies evaluating neurotoxicity, including neurodevelopmental toxicity, associated with short-term, subchronic, or gestational exposure to PFOS in experimental models (Table C-7). No study indicates morphological changes or damage attributed to PFOS. However, there is some evidence suggesting that PFOS exposure may be associated with neurobehavioral and physiological effects (e.g., impairments in spatial learning and memory, increases in locomotor activity, and changes in neuronal electrophysiology and neurotransmitter levels). Further research may be warranted.

Brain weight was assessed in only one developmental study and two short-term study in rats. Absolute and relative brain weights were unchanged in the offspring of Crl:CD (SD) rats dosed with 0.1–1 mg/kg/day PFOS during gestation and lactation (Butenhoff et al., 2009). In male and female Sprague-Dawley rats exposed to 2 mg/kg–100 mg/kg PFOS in diet for 28 days, relative brain weights were increased in the 50 mg/kg and 100 mg/kg exposure groups in a concentration-dependent manner, which may have been secondary to a decrease in body weights as absolute brain weights were unchanged (Curran et al., 2008). The relative weights of the amygdala, hippocampus, and prefrontal cortex were unchanged in male Sprague-Dawley rats dosed with 0.5 mg/kg/day–6 mg/kg/day PFOS for 28 days (data not provided) (Salgado et al., 2016); the absolute weights of these brain regions were not provided. One developmental and one short-term study examined the gross pathology or histopathology of the brain, and no effects were seen in rats exposed to 0.1 mg/kg/day–5 mg/kg/day PFOS (NTP, 2019; Butenhoff et al., 2009). The authors of a subchronic study in female BALB/c mice dosed with 0.1 mg/kg/day and 1 mg/kg/day PFOS for 2 months noted a small amount of neuron phagocytosis and that neuronal cells were contracted, deeply stained, and lacked clearly defined cytoplasm and nuclei (Li et al., 2021b).

One developmental (Mshaty et al., 2020), one short-term (Fuentes et al., 2007b), and one subchronic study (Long et al., 2013) in mice and several reproductive (Luebker et al., 2005a) and developmental studies (Wang et al., 2015a; Butenhoff et al., 2009; Johansson et al., 2008; Fuentes et al., 2007a) in rats assessed the neurobehavioral effects associated with PFOS. Mshaty et al. (2020) assessed learning and memory in male C57BL/6J mice exposed to 0.1–1 mg/kg/day PFOS from PND 1–PND 14 using the object location test, object recognition test, and pairwise visual discrimination task. The discriminatory index for the object location and recognition memory tests were decreased in mice exposed to 1 mg/kg/day, as was the learning curve for the 1 mg/kg/day group during the visual discrimination task. Spatial learning and memory were also reduced in adult male C57BL6 mice dosed with 2.15 mg/kg/day and 10.75 mg/kg/day but not 0.43 mg/kg/day PFOS for 3 months, as seen by increases in escape latency and decreases in the time spent in the target quadrant using the Morris water maze (Long et al., 2013). Time spent in the target quadrant was also decreased in male CD1 mice dosed with 3 mg/kg/day but not 6 mg/kg/day PFOS for 4 weeks (Fuentes et al., 2007b). In this study, swimming speed was increased in mice exposed to 3 and 6 mg/kg/day and distance traveled was increased in mice exposed to 6 mg/kg/day, whereas no effects on motor activity were seen with the open-field test or rotarod test. Similar effects on spatial learning and memory were seen in the offspring of Wistar rat dams exposed to 15 mg/mL but not 5 mg/mL PFOS in drinking water throughout gestation and/or lactation (drinking water consumption not reported); swimming speed was not affected by exposure (Wang et al., 2015a). However, two studies reported no changes in learning and memory, as tested with the Morris water maze or the Biel swimming maze, in male and female rats exposed to 0.1 mg/kg/day–3.2 mg/kg/day PFOS pre- and postnatally (Butenhoff et

al., 2009; Luebker et al., 2005a). In a two-generation study, Luebker et al. (2005a) also reported no effects on learning, memory, and short-term retention, as measured in a passive avoidance paradigm, and Butenhoff et al. (2009) reported no effects on the acoustic startle response. However, increased motor activity (ambulatory and total locomotor activity) and lack of habituation was seen at PND 17 in males exposed to ≥0.3 mg/kg/day or 1 mg/kg/day, respectively, throughout development (Butenhoff et al., 2009). In male NMRI mice given a single dose of 11.3 mg/kg at PND 10, during a period of development, lack of habituation was also observed at 2 and 4 months of age (Johansson et al., 2008); this effect was not observed with a single dose of 0.75 mg/kg at PND 10. In this study, locomotion, rearing, and total activity was significantly decreased in both the 0.75 mg/kg and 11.3 mg/kg dose groups at 2 months of age. Another development study exposed CD-1 mice to 6 mg/kg/day PFOS from GD 12–GD 18 and assessed neuromotor maturation with surface righting reflex, open-field test, and rotarod test (Fuentes et al., 2007a). Surface righting reflex was delayed at PND 4 and PND 8. Significant effects were also observed during the climb test, with PFOS exposure resulting in diminished resistance to backwards pull and reduced climb ability at PND 10 and PND 11 but not PND 12. Climbing ability and forelimb grip strength was reduced with PFOS exposure at PND 11 but not PND 10 or PND 12. The authors state that these transient effects may support delayed neuromotor maturation due to gestational PFOS exposure. However, no effects were observed with the open-field or rotarod tests at 3 months of age.

A short-term study reported that male CD-1 mice displayed increased anxiety-like behavior in the open-field test, as seen by decreased time in the center of the chamber in the 3 mg/kg/day PFOS group and decreased vertical activity in the 6 mg/kg/day group (Fuentes et al., 2007b). However, in a developmental study by the same authors, no effects on anxiety-like behavior were observed in CD-1 mice exposed to 6 mg/kg/day PFOS from GD 12–GD 18 (Fuentes et al., 2007a). Similarly, no effects on this behavior were observed in a single-dose study in male NMRI mice dosed with 0.75 mg/kg or 11.3 mg/kg PFOS (Johansson et al., 2008).

Table C-7. Associations Between PFOS Exposure and Neurobehavioral Effects in Rodents

Reference	Study Design	Learning and Memory	Acoustic Startle	Anxiety-like Behavior	Motor Activity/ Coordination	Neuromaturation
			Mice			
Fuentes et al. (2007a)[a]	Developmental exposure (GD12–18) to 0 or 6 mg/kg/day	NT	NT	Open field: No effect	Open field: No effect Rotarod: No effect	Surface righting reflex: ↓ at 6 mg/kg/day Grip strength: ↓ at 6 mg/kg/day
Mshaty et al. (2020)[b]	Developmental exposure (PND1–14) to 0, 0.1, 0.25, or 1 mg/kg/day	Object location and recognition test, and pairwise visual discrimination task: ↓ at 1 mg/kg/day	NT	NT	NT	NT
Johansson et al. (2008)[b]	Single dose (PND10) to 0, 0.75, or 11.3 mg/kg	Spontaneous behavior, habituation: ↓ at 11.3 mg/kg	NT	Elevated plus maze: No effect	Spontaneous behavior, total activity: ↓ at ≥ 0.75 mg/kg in first test block; ↑ at 11.3 mg/kg in final test block	NT
Fuentes, et al. (2007b)[b]	Short-term exposure to 0, 3, or 6 mg/kg/day	Morris water maze (acquisition): no effect Morris water maze (probe): ↓ at 3 mg/kg/day	NT	Open field, time in center: ↓ at 3 mg/kg/day; vertical activity: ↓ at 6 mg/kg/day	Open field: No effect Rotarod: No effect[c] Morris water maze (probe), swimming speed: ↑ at ≥ 3 mg/kg/day; distance traveled: ↑ at 6 mg/kg/day	NT
Long et al. (2013)[d]	Subchronic exposure (3 mo) to 0, 0.43, 2.15, or 10.75 mg/kg/day	Morris water maze (acquisition, probe): ↓ at ≥2.15 mg/kg/day	NT	NT	NT	NT

Reference	Study Design	Learning and Memory	Acoustic Startle	Anxiety-like Behavior	Motor Activity/ Coordination	Neuromaturation
			Rats			
Wang et al. (2015a)[e]	Developmental exposure (gestational and/or lactational) to 0, 5, 15 mg/L (0, 0.8, or 2.4 mg/kg/day[f])	Morris water maze (acquisition, probe): ↓ at 15 mg/mL	NT	NT	Morris water maze, swimming speed: No effect	NT
Butenhoff et al. (2009)[a]	Developmental exposure (GD 0–PND 20) to 0, 0.1, 0.3, or 1.0 mg/kg/day	Males, habituation: ↓ at 1 mg/kg/day Biel swimming maze: No effect	No effect	NT	Males, motor activity: ↑ at 0.3 mg/kg/day Females: No effect	NT
Luebker et al. (2005a)[a]	Reproductive exposure (GD 0–PND 112) to 0.0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day	Modified M-maze: No effect Passive avoidance: No effect	NT	NT	NT	NT

Notes: GD = gestation day; NT = not tested; PND = postnatal day.
[a] Males and females analyzed separately.
[b] Study conducted in males.
[c] No quantitative data were presented for this endpoint, which was consequently rated as *low* confidence.
[d] Sexes combined.
[e] Sex was not specified.
[f] Doses in mg/kg/day were derived and presented in the 2016 PFOS HESD (U.S. EPA, 2016c).

Several short-term studies in mice and rats (Salgado et al., 2016; Lopez-Doval et al., 2015; Salgado et al., 2015), one developmental study in mice (Mshaty et al., 2020), and one subchronic study in mice (Long et al., 2013) examined the effects of PFOS on neurotransmitter levels (Table C-8). Glutamine, glycine, and serotonin were each examined in only one study. Neither glutamine nor glycine were altered in the dorsal hippocampus of male C57BL/6J mice exposed to 1 mg/kg/day PFOS from PND 1–PND 14 (Mshaty et al., 2020). Serotonin was increased in the anterior hypothalamus, mediobasal hypothalamus, and the median eminence of male Sprague-Dawley rats dosed with 0.5 mg/kg/day–6 mg/kg/day for 28 days (Lopez-Doval et al., 2015). The effect of PFOS on dopamine and/or gamma-aminobutyric acid (GABA) in various brain regions was examined in three studies (Mshaty et al., 2020; Salgado et al., 2015; Long et al., 2013). A subchronic study found no changes in GABA in the hippocampus of male C57BL6 mice dosed with 0.43–10.75 mg/kg/day PFOS (Long et al., 2013). However, GABA was increased in the dorsal hippocampus of male C57BL/6J mice exposed to 1 mg/kg/day PFOS from PND 1–PND 14 (Mshaty et al., 2020). In adult male Sprague-Dawley rats dosed with 3 and 6 mg/kg/day PFOS for 28 days, GABA was unaltered in the mediobasal hypothalamus and increased in the anterior hypothalamus in both dose groups (Salgado et al., 2015). In male Sprague-Dawley rats dosed with 0.5 mg/kg/day–6 mg/kg/day PFOS for 28 days, dopamine was increased in the hippocampus in the 0.5 mg/kg, 1 mg/kg, and 3 mg/kg groups, but not the 6 mg/kg/day group (Salgado et al., 2016). Increased dopamine levels were also detected in the prefrontal cortex of the 1 mg/kg/day group only and in the anterior hypothalamus of the 3 mg/kg/day and 6 mg/kg/day groups (Salgado et al., 2016; Salgado et al., 2015). No changes in dopamine levels were seen in the mediobasal hypothalamus (Salgado et al., 2015). In male C57BL6 mice dosed with 0.43 mg/kg/day–10.75 mg/kg/day PFOS, dopamine in the caudate putamen was decreased only at the highest dose (Long et al., 2013). In this study, glutamate in the hippocampus was also increased at the highest dose. However, glutamate was increased in the dorsal hippocampus of male C57BL/6J mice exposed to 1 mg/kg/day PFOS from PND 1–PND 14 (Mshaty et al., 2020). Greater sensitivity of the developing brain to PFOS exposure might explain why glutamate increases in the hippocampus were only seen at higher doses in the Long et al. (2013) study compared with increases seen at a lower dose in the Mshaty et al. (2020) study.

Table C-8. Associations Between PFOS Exposure and Neurotransmitters in Rodents

Reference	Study Design	Glutamine/ Glutamate	Glycine	Serotonin	GABA	Dopamine
			Mice			
Mshaty et al. (2020)[a]	Developmental exposure (PND1–14) to 0 or 1 mg/kg/day	Dorsal hippocampus, glutamate: ↑ at 1 mg/kg/day Dorsal hippocampus, glutamine: No effect	Dorsal hippocampus: No effect	NT	Dorsal hippocampus: ↑ at 1 mg/kg/day	NT
Long et al. (2013)[b]	Subchronic exposure (3 mo) to 0, 0.43, 2.15, or 10.75 mg/kg/day	Hippocampus, glutamate: ↑ at 10.75 mg/kg/day	NT	NT	Hippocampus: No effect	Caudate putamen: ↓ at 10.75 mg/kg/day
			Rats			
Salgado et al. (2015)[a]	Short-term exposure (28 d) to 0, 3, or 6 mg/kg/day	NT	NT	NT	Mediobasal hypothalamus: No effect Anterior hypothalamus: ↑ at ≥3 mg/kg/day	Mediobasal hypothalamus: No effect Anterior hypothalamus: ↑ at ≥3 mg/kg/day
Salgado et al. (2016)[a]	Short-term exposure (28 d) to 0, 0.5, 1, 3, or 6 mg/kg/day	NT	NT	NT	NT	Amygdala: No effect Prefrontal cortex: ↑ at 1 mg/kg/day but not at 3 and 6 mg/kg/day Hippocampus: ↑ at 0.5, 1, and 3 mg/kg/day but not at 6 mg/kg/day

Reference	Study Design	Glutamine/ Glutamate	Glycine	Serotonin	GABA	Dopamine
Lopez-Doval et al. (2015)[a]	Short-term exposure (28 d) to 0, 0.5, 1, 3, or 6 mg/kg/day	NT	NT	Mediobasal hypothalamus: ↑ at ≥0.5 mg/kg/day Anterior hypothalamus: ↑ at ≥0.5 mg/kg/day Median eminence: ↑ at ≥0.5 mg/kg/day	NT	NT

Notes: PND = postnatal day; d = days; NT = not tested.
[a] Study conducted in males.
[b] Sexes combined.

Synaptic transmission and plasticity were assessed in one electrophysiology study in SD rats exposed to 0.35 mg/kg/day–2.17 mg/kg/day PFOS throughout development until PND 90 (Zhang et al., 2019). Zhang et al. (2019) observed moderate inhibition of paired pulse facilitation (at highest dose) and the input/output curve (at all doses) in the hippocampus. Long-term potentiation was also decreased in a dose-dependent manner in the 0.72 mg/kg/day and 2.17 mg/kg/day dose groups.

C.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse nervous outcomes is discussed in Sections 3.2.4, 3.2.5, 3.2.6, 3.3.4, 3.3.6, and 3.4.1.4 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are 54 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to nervous effects. A summary of these studies is shown in Figure C-30. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to nervous effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	25	31
Cell Signaling Or Signal Transduction	12	0	21	29
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	0	1	3
Hormone Function	7	0	5	11
Inflammation And Immune Response	1	1	5	6
Oxidative Stress	1	0	10	11
Xenobiotic Metabolism	0	0	1	1
Other	2	0	1	3
Not Applicable/Not Specified/Review Article	3	0	0	3
Grand Total	26	1	32	54

Figure C-30. Summary of Mechanistic Studies of PFOS and Nervous Effects

Interactive figure and additional study details available on HAWC.

C.4.4 Evidence Integration

There is *slight* evidence of an association between PFOS and nervous system effects in humans based on the mostly mixed results. There were no new neurological studies identified that

evaluated cerebral palsy. Outcomes investigated include depression, memory impairment, hearing impairment, ASD, and ID.

Epidemiological studies in this current review provide limited indication of adverse effects of PFOS on neurodevelopment or neuropsychological outcomes (Niu et al., 2019; Jeddy et al., 2017; Chen et al., 2013), cognitive development (Oulhote et al., 2019; Harris et al., 2018), and executive function (Vuong et al., 2016) in human populations. No adverse effects were observed for PFOS and depression or memory impairment, and only one study indicated effects of PFOS on hearing impairment (Li, 2020), however the number of studies was limited. Overall, results from studies of neurodevelopmental, neuropsychological, and cognitive outcomes were mixed.

The recent studies provide limited indication of adverse effects of PFOS on behavioral problems, ADHD, ASD, and ID. The studies reviewed provide some indication of behavioral problems associated with PFOS (Oulhote et al., 2019; Ghassabian et al., 2018; Oulhote et al., 2016), however overall results were mixed. Of the multiple studies examining associations between PFOS and ADHD, only one (Lenters et al., 2019) reported a significant relationship between PFOS and ADHD, with results indicating heterogeneity with respect to gender. No adverse associations of ID with PFOS were reported in the single study reviewed (Lyall et al., 2018). There was an indication of a potential relationship between PFOS and autistic behaviors or ASD diagnosis in some studies (Shin et al., 2020; Oulhote et al., 2016; Braun et al., 2014). However, many studies have methodological concerns, as PFOS exposures in cases and controls within the ADHD and ASD studies were often either similar to or had mean control exposures greater than cases in some studies. A single category outcome for ASD may also not adequately encompass the heterogeneity in terms of developmental history, intelligence, comorbidity, and severity that might be important in accurately revealing associations. The current evidence examining PFOS exposure and neurodevelopmental disorders in children, including ADHD and learning disabilities, is limited.

The animal evidence for an association between PFOS and neurological effects is *moderate*. There are several *medium* confidence studies available where changes in neurobehavioral effects were observed. Although the studies varied by design, endpoints measured, and methods of measurement leading to some inconsistencies across studies, there is evidence of effects on learning and memory. Of the studies available in animal models, no effects were noted for brain weight with limited changes observed for histopathology. Some neurobehavioral effects were observed but these results and the methods used to quantify them were relatively inconsistent. Alterations in neurotransmitter levels and synaptic transmission and plasticity were also observed, though it is often unclear what magnitude of change in neurotransmitters levels can be considered adverse. Notably, Mshaty et al. (2020) observed dose-dependent effects of PFOS in both the object recognition memory test and object location recognition memory test, as well as dose-dependent effects of PFOS across 9 days of a visual discrimination task. These behavioral changes in the 1 mg/kg/day dose group were accompanied by significant increases in hippocampal neurotransmitter concentrations, including glutamate and GABA. Increased hippocampal glutamate levels may cause excitotoxicity which could explain the spatial learning deficits seen by Mshaty et al. (2020). Importantly, the exposure period in this study encompassed a sensitive period of neurodevelopment (i.e., lactation) and the observed effects occurred at relatively low doses. In addition, the deficits in spatial learning and increased hippocampal

glutamate concentrations observed by Long et al. (2013) in PFOS-exposed adult mice support these results.

C.4.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause nervous system effects in humans under relevant exposure circumstances (Table C-9). This conclusion is based primarily on alterations in neurodevelopment, neurobehavior, and neurotransmitter levels in animals following exposure to doses as low as 0.5 mg/kg/day PFOS. Although there is some evidence of adverse effects of PFOS exposure on neurodevelopment or neuropsychological outcomes, cognitive development, executive function and behavioral problems in humans, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-9. Evidence Profile Table for PFOS Nervous System Effect

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.4.1)					⊕⊙⊙ ***Evidence Suggests***
Neurodevelopment 2 *High* confidence study 4 *Medium* confidence studies 1 *Low c*onfidence study	Inverse associations were reported for neurodevelopmental outcomes in three studies of children (3/7). One *high* confidence study observed a significant inverse association with social measures among girls. Of the *medium* confidence studies, one observed significant inverse associations with total neurodevelopment and motor skill measures. Another study reported significant inverse associations with communication measures but a positive association with cognition. The same study reported inconsistent effects when stratified by maternal age. Results reported in the remaining studies were inconsistent.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effects across studies	⊕⊙⊙ *Slight* Evidence of nervous system effects is based on *high* confidence studies reporting significant associations, which varied in magnitude and were inconsistent across neurological outcomes. Uncertainties remain due to inconsistent findings within studies and mixed findings across studies. Studies with mixed findings were primarily of *medium* or *low* confidence.	*Primary basis*: Animal evidence indicated alterations in neurodevelopment, neurobehavior, and neurotransmitter levels. Although there is some evidence of adverse effects of PFOS exposure on neurodevelopment or neuropsychological outcomes, cognitive development, executive function and behavioral problems in humans, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Cognitive function 1 *High c*onfidence studies 9 *Medium* confidence studies	Reported results were largely inconsistent across studies, with both positive and inverse non-significant associations	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across studies • Small magnitude of effect		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	reported. One *high* confidence study observed non-significantly increased nonverbal IQ scores among the highest exposure group. Positive associations with reading scores were observed in some *medium* confidence studies (2/9).				
Social-emotional and behavioral regulation 1 *High* confidence study 4 *Medium* confidence studies 1 *Low* confidence study	One *high* confidence study found no significant associations with behavioral measures at age 5 but observed a positive association among females and a negative association among males at age 7. Of the *medium* confidence studies, two observed positive associations with behavioral difficulties (2/4). Another *medium* confidence study observed that the association with impulsivity was modified by sex, with males performing better than females (1/4). One *low* confidence study of adolescents observed a significant inverse	• *High* and *medium* confidence studies	• *Low* confidence study • *Inconsistent direction* of effects across and within studies		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	correlation with the region of the brain associated with impulsive behavior.				
Depression 3 *Medium* confidence studies 1 *Low* confidence study	One *medium* confidence study reported positive but non-significant results for depression in the general population adults. Another *medium* confidence study explored depression in children followed for 20 yr, reporting no association. An additional study of *medium* confidence reported no association with depression among pregnant women. A *low* confidence study reported no association.	• *Medium* confidence studies	• *Low* confidence study		
Executive function 3 *Medium* confidence studies	Two *medium* confidence studies examined executive function measures, including behavior regulation and metacognition, among children from the HOME study (2/3). One of these studies reported significantly inversed associations with executive function measures, while the other	• *Medium* confidence studies	• *Inconsistent direction* of effects across studies • *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	reported no significant associations. A *medium* confidence study of adults did not observe significant associations.				
Attention 5 *Medium* confidence studies 2 *Low* confidence studies	Studies examining measures of attention reported mixed findings. One *medium* confidence study reported significantly increased odds of ADHD. When stratified by child sex, significant effects remained. The remaining *medium* confidence studies (4/5) did not report significant associations. Additionally, the two *low* confidence studies observed no associations with measures of attention.	• *Medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effects across studies		
Autism, autistic behaviors, and intellectual disability 1 *High* confidence study 5 *Medium* confidence studies	One *high* confidence study observed a positive association with autism scores when measured at age 7. When stratified by sex, higher scores were observed in females compared with males. Findings from the five *medium* confidence studies were mixed. Two	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across studies		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	studies observed positive associations, with one study reporting associations for the overall study population and the other study reporting the association only among males. Another *medium* confidence study reported inverse associations. Other reported results were not significant.				
Visuospatial performance 1 *High* confidence study 1 *Medium* confidence studies	Two studies examined visuospatial performance effects among children. One *high* confidence study among children observed a significant inverse association with visual-motor performance across quartiles of exposure. The *medium* confidence study reported no association with visuospatial performance.	• *High* and *medium* confidence studies • Large magnitude of effect	• *Inconsistent direction* of effects across studies • *Limited number* of studies examining outcome		
Memory impairment 2 *Medium* confidence studies	Two studies reported associations with memory loss among adult populations. One *medium* confidence study observed a significant inverse association with memory impairment. No	• *Medium* confidence studies	• *Inconsistent direction* of effects across studies • Small magnitude of effect • *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	significant effects were reported from the remaining *medium* confidence study.				
Hearing impairment 2 *Medium* confidence studies	Two *medium* confidence studies examined hearing impairment among adults from NHANES. One study observed positive correlations with hearing impairment, while the other reported no associations.	• *Medium* confidence study • Large magnitude of effect	• *Inconsistent direction* of effects across studies • *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.4.2)					
Neurobehavior 4 *Medium* confidence studies	Changes in neurobehavior endpoints were altered and decreases in learning and memory tasks were largely consistent among studies (3/4). Motor activity was found to be increased (2/2), with anxiety-like behavior being decreased (1/1). A single study measured acoustic startle and found no changes (1/1).	• *Medium* confidence studies • *Coherence* of findings in neurotransmitters	• *Limited number* of studies examining specific outcomes • Inconsistent direction of effects	⊕⊕⊙ *Moderate* Several *medium* confidence studies are available where changes in neurobehavioral effects were observed. Although the studies varied by design, endpoints measured, and methods of measurement leading to some inconsistencies across studies, there is evidence of effects on learning and memory. No effects were noted for brain weight and limited changes were observed for histopathology. Alterations in	
Neurotransmitters 3 *Medium* confidence studies	Changes in neurotransmitter levels in short-term studies in male mice included a dose-responsive increase in serotonin (1/1) and	• *Medium* confidence studies • *Coherence* of findings in neurobehavior endpoints	• *Limited number* of studies examining outcome • *Biological significance* of the magnitude of effect is unclear		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	region-specific decreases of GABA (1/1) and dopamine (2/2).	• *Dose-response* relationship		neurotransmitter levels and synaptic transmission and plasticity were also observed, though it is often unclear what magnitude of change in neurotransmitters levels can be considered adverse.	
Organ weights 3 *Medium* confidence studies	No effects were observed on absolute brain weights (2/2). One study reported a significant increase in relative brain weights; however, this increase was confounded by a reduction in body weight.	• *Medium* confidence studies	• *Limited number* of studies examining outcomes • *Confounding variables* such as decreases in body weights		
Histopathology 1 *High* confidence study, 1 *Medium* confidence study	One study found no effects on brain histopathology in male and female rats, whereas some phagocytosis in the brains of PFOS-exposed mice was noted in another study.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes		
Electrophysiology 1 *Medium* confidence study	One developmental study in male and female rats found inhibition of paired pulse facilitation and the input/output curve in the hippocampus. Hippocampal long-term potentiation was also decreased (1/1).	• *Medium* confidence study • *Dose-response* relationship	• *Limited number* of studies examining outcome		

Notes: yr = years; ADHD = attention deficit/hyperactivity disorder; GABA = gamma-aminobutyric acid; HOME = Health Outcomes and Measures of the Environment; IQ = intelligence quotient; NHANES = National Health and Nutrition Examination Survey.

C.5 Renal

EPA identified 19 epidemiological and 12 animal studies that investigated the association between PFOS and renal effects. Of the epidemiological studies, 17 were classified as *low* confidence and two were considered *uninformative* (Section C.5.1). Of the animal studies, two were classified as *high* confidence, eight as *medium* confidence, and two were considered *low* confidence (Section C.5.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.5.1 Human Evidence Study Quality Evaluation and Synthesis

C.5.1.1 Introduction

PFOS has the potential to affect the kidney's function given the saturable resorption from the renal tubules (U.S. EPA, 2016c). Biomarkers of renal function include blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), serum creatinine, and uric acid. eGFR is a marker of non-malignant renal disease.

The 2016 PFOS HESD (U.S. EPA, 2016c) concluded there was evidence of a suggestive association between PFOS and chronic kidney disease (CKD; defined as glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2) based on two studies on the general population (Shankar et al., 2011; Steenland et al., 2010) and two on children (Geiger et al., 2014b; Watkins et al., 2013); however, given the cross-sectional study designs, the potential for reverse causality could not be ruled out.

For this updated review, 19 studies examined the association between PFOS and renal health outcomes. Five studies were in children and adolescents (Khalil et al., 2018; Qin et al., 2016; Kataria et al., 2015; Predieri et al., 2015; Geiger et al., 2013), one study in pregnant women (Nielsen et al., 2020), one study in occupational workers (Rotander et al., 2015), and the remainder of the studies were in the general population. Fifteen of the studies utilized a cross-sectional study design; the remaining study designs included one case-control study (Predieri et al., 2015), and three cohorts (Nielsen et al., 2020; Blake et al., 2018; Conway et al., 2018) (Appendix D). All studies measured PFOS in blood components (i.e., plasma or serum). Two studies conducted in China investigated the same population from the Isomers of C8 Health Project (Wang et al., 2019b; Zeng et al., 2019c). Among the studies investigating populations in the United States, five studies utilized data from the NHANES (Scinicariello et al., 2020b; Jain and Ducatman, 2019a, c; Kataria et al., 2015; Geiger et al., 2013). Outcomes evaluated in these studies include clinical conditions, such as CKD and gout and biomarkers of renal function, including uric acid, eGFR, albumin, and creatinine.

C.5.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies examining kidney function and kidney disease. Since PFOS is removed from the blood by the kidney, cross-sectional analyses using serum PFOS as the exposure measure are problematic if individuals with compromised kidney function are included: PFOS concentrations could be increased in

those individuals and an apparent association with GFR would be observed, even if one did not exist (Dhingra et al., 2017).

There are 19 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and renal effects. Study quality evaluations for these 19 studies are shown in Figure C-31.

Of the 19 studies identified since the 2016 assessment, 17 studies were classified as *low* confidence and the remaining two as *uninformative* (Seo et al., 2018; Predieri et al., 2015). No studies were classified as *high* or *medium* confidence. The main concerns with the *low* confidence studies included potential for residual confounding, selection bias, and reverse causality. Another concern included small sample sizes (Nielsen et al., 2020; Khalil et al., 2018). Additionally, *low* confidence studies utilizing cross-sectional analyses of kidney function with serum PFOS were impacted by the potential for reverse causation.

Deficiencies identified in Predieri et al. (2015) included a small sample size and narrow ranges of exposures which contributed to an *uninformative* rating. Seo et al. (2018) presented bivariate correlations between PFOS exposure and renal outcomes, limiting the ability to interpret the results. Other potential sources of bias were identified, including a lack of information on participant recruitment and selection, unexplained discrepancies in samples sizes, and missing details on outcome assessment methods. Neither *uninformative* study adjusted for key confounders (e.g., age and SES), resulting in a high potential for residual confounding.

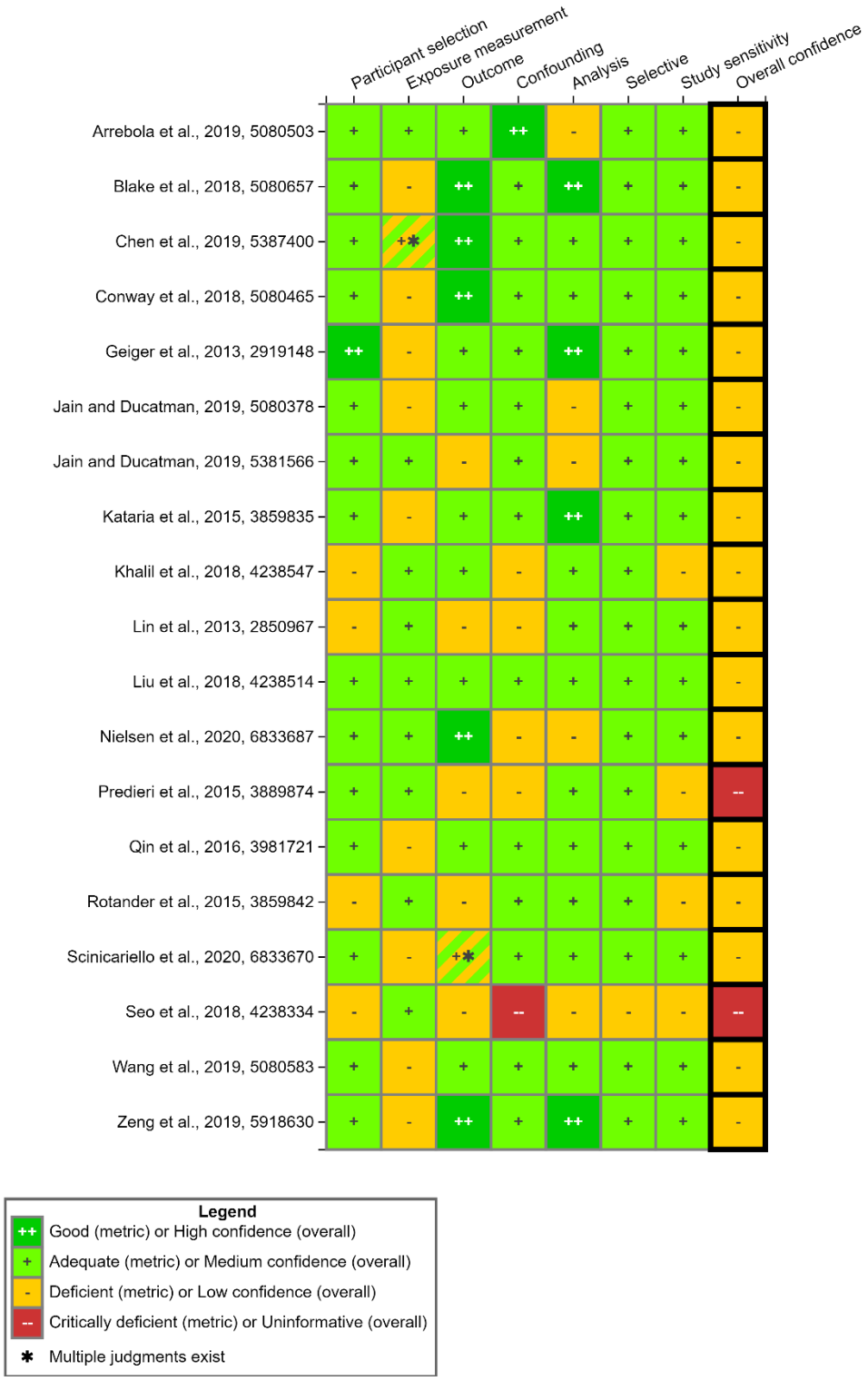


Figure C-31. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Renal Effects

Interactive figure and additional study details available on HAWC.

C.5.1.3 Findings From Children and Adolescents

Three *low* confidence studies reported on uric acid among children and adolescents (Qin et al., 2016; Kataria et al., 2015; Geiger et al., 2013) with two also reporting on hyperuricemia (Qin et al., 2016; Geiger et al., 2013), defined as serum uric acid levels ≥ 6 mg/dL. The three studies reported mixed results. Among adolescents aged 12 to 18 years from NHANES (1999–2008), Geiger et al. (2013) observed statistically significant positive associations between increasing quartiles of PFOS and hyperuricemia (p-trend = 0.0221), and serum uric acid (p-trend = 0.0575). An overlapping NHANES (2003–2010) study (Kataria et al., 2015) also reported a positive association with uric acid levels among adolescents, where the highest PFOS quartile (≥ 19.4 ng/mL) was associated with a 0.19 mg/dL (95% CI: 0.032, 0.34 mg/dL, $p < 0.05$) increase in uric acid levels compared with the lowest PFOS quartile (<7.9 ng/mL). Qin et al. (2016) did not observe significant associations for hyperuricemia or uric acid in children aged 12 to 15 years from the GBCA in Taiwan.

One *low* confidence study (Kataria et al., 2015) reported on GF in children aged 12 to 19 years from NHANES (2003–2010). Significant negative associations were observed for eGFR in the second, third, and fourth quartiles of PFOS exposure compared with the lowest quartile.

Two *low* confidence studies and one *uninformative* study investigated serum creatinine among children and adolescents (Khalil et al., 2018; Kataria et al., 2015; Predieri et al., 2015). One *low* confidence study (Kataria et al., 2015) on NHANES (2003–2010) adolescents (12–19 years old) reported a significant positive association with serum creatinine in the third and fourth quartiles of PFOS exposure. One *low* confidence study (Khalil et al., 2018) examined serum creatinine levels among obese children aged 8 to 12 years, but no significant effect was observed.

C.5.1.4 Findings From the General Adult Population

Two *low* confidence studies examined CKD in the general population (Wang et al., 2019b; Conway et al., 2018) and both observed positive associations. CKD was defined as an eGFR of < 60 mL/min/1.73 m^2. In C8 Health Project participants, Conway, 2019, 5080465 observed significantly elevated odds of CKD among non-diabetic participants; a negative association was observed among participants with diabetes. The prevalence of CKD in the diabetic population was higher (22%) than the non-diabetic population (7%). Wang et al. (2019b) observed non-significantly elevated odds of CKD in participants from the Isomers of C8 Health Project in China. However, a concern for reverse causality makes interpretation of the results difficult in both studies.

Gout was examined in one *low* confidence study (Scinicariello et al., 2020b) in adults from NHANES (2009–2014). Positive associations were observed between serum PFOS and self-reported gout, however, none were significant.

Six *low* confidence general population studies (Scinicariello et al., 2020b; Arrebola et al., 2019; Chen et al., 2019a; Jain and Ducatman, 2019a; Zeng et al., 2019c; Lin et al., 2013) and one *low* confidence occupational study (Rotander et al., 2015) examined PFOS and uric acid levels, and three of those studies evaluated uric acids as they pertained to hyperuricemia (Scinicariello et al., 2020b; Arrebola et al., 2019; Zeng et al., 2019c).

A *low* confidence NHANES (2009–2014) study (Scinicariello et al., 2020b) observed significantly elevated serum uric acid across increasing PFOS exposure quartiles, and the trend was significant (p-trend = 0.003). Higher odds of hyperuricemia among participants in the highest exposure quartile (>11.90 ng/mL) compared with the lowest (≤ 4.43 ng/mL) was also observed, but the trend was not significant (p-trend = 0.15). Results were similar when restricted to participants without CKD. Another *low* confidence study (Zeng et al., 2019c) on participants from the Isomers of C8 Health Project reported significantly elevated uric acid levels with increasing PFOS exposure, and a marginally significant association (OR: 1.17, 95% CI: 0.99, 1.39, p = 0.074) for hyperuricemia., Jain and Ducatman (2019a) examined uric acid by glomerulation stage among NHANES (2007–2014) participants. For males, positive associations with uric acid were observed for stages GF-1 ($p < 0.01$) and GF-2 ($p = 0.05$), but the effect was negative for stages GF-3A ($p = 0.66$) and GF-3B/4 ($p < 0.01$). For females, all associations were positive across stages of GF with significant associations ($p < 0.05$) for GF-1 and GF-3A. Two *low* confidence studies did not observe associations with plasma uric acid in Croatian adults aged 44–56 years (Chen et al., 2019a), or in adolescents and young adults aged 12–30 years in the Young Taiwanese Cohort Study (Lin et al., 2013). Another *low* confidence study (Arrebola et al., 2019) using pooled cohort data (the BIOAMBIENT.ES study) observed a non-significant increase in serum uric acid with increasing PFOS.

One *low* confidence occupational study examined serum uric acid levels among firefighters with past exposure to aqueous film-forming foam (AFFF) (Rotander et al., 2015). No significant association was observed for serum uric acid and increasing PFOS exposure.

Two general population studies evaluated PFOS and eGFR (Wang et al., 2019b; Blake et al., 2018). A *low* confidence study (Blake et al., 2018) assessed participants of the FCC with high exposure to PFAS from their household water supplies. A significant inverse association with eGFR was observed in the latent effects mixed effect model (LME), but not in the repeated measures LME. These results were consistent with the *low* confidence study (Wang et al., 2019b) which assessed participants of the Isomers of C8 Health Project and observed negative association between total PFOS serum concentrations and eGFR.

The evidence of association between PFOS and renal effects among pregnant women was limited. Only one *low* confidence study reported on pregnant women (Nielsen et al., 2020) using a small sample of women (n = 73) from the Pregnancy Obesity Nutrition and Child Health study (PONCH) study. No significant Spearman rank correlations were reported between PFOS and kidney function parameters.

Two studies examined albumin and creatinine as biomarkers for renal function (Chen et al., 2019a; Jain and Ducatman, 2019c). The two *low* confidence studies provided differing conclusions. Jain, 2019, 5381566 utilized NHANES (2005–2014) data and reported statistically significant positive associations with serum and urine creatinine, and serum albumin. Statistically significant negative associations were also reported with urine albumin and urine albumin-creatinine ratios. Stratification by stages of GF was noted as better representing more severe stages of renal failure. For PFOS, stratification by stages of GF had inconsistent effects. One *low* confidence study (Chen et al., 2019a) did not observe significant associations with plasma creatinine in Croatian adults ages 44–56 years.

One *low* confidence study, Liu et al. (2018b) examined serum proteins among NHANES (2013–2014) participants, and positive associations ($p < 0.01$) were observed for serum protein with increasing PFOS exposure. The effect was consistent when stratified by linear and branched PFOS.

C.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There are four studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and renal effects. Study quality evaluations for these 12 studies are shown in Figure C-32.

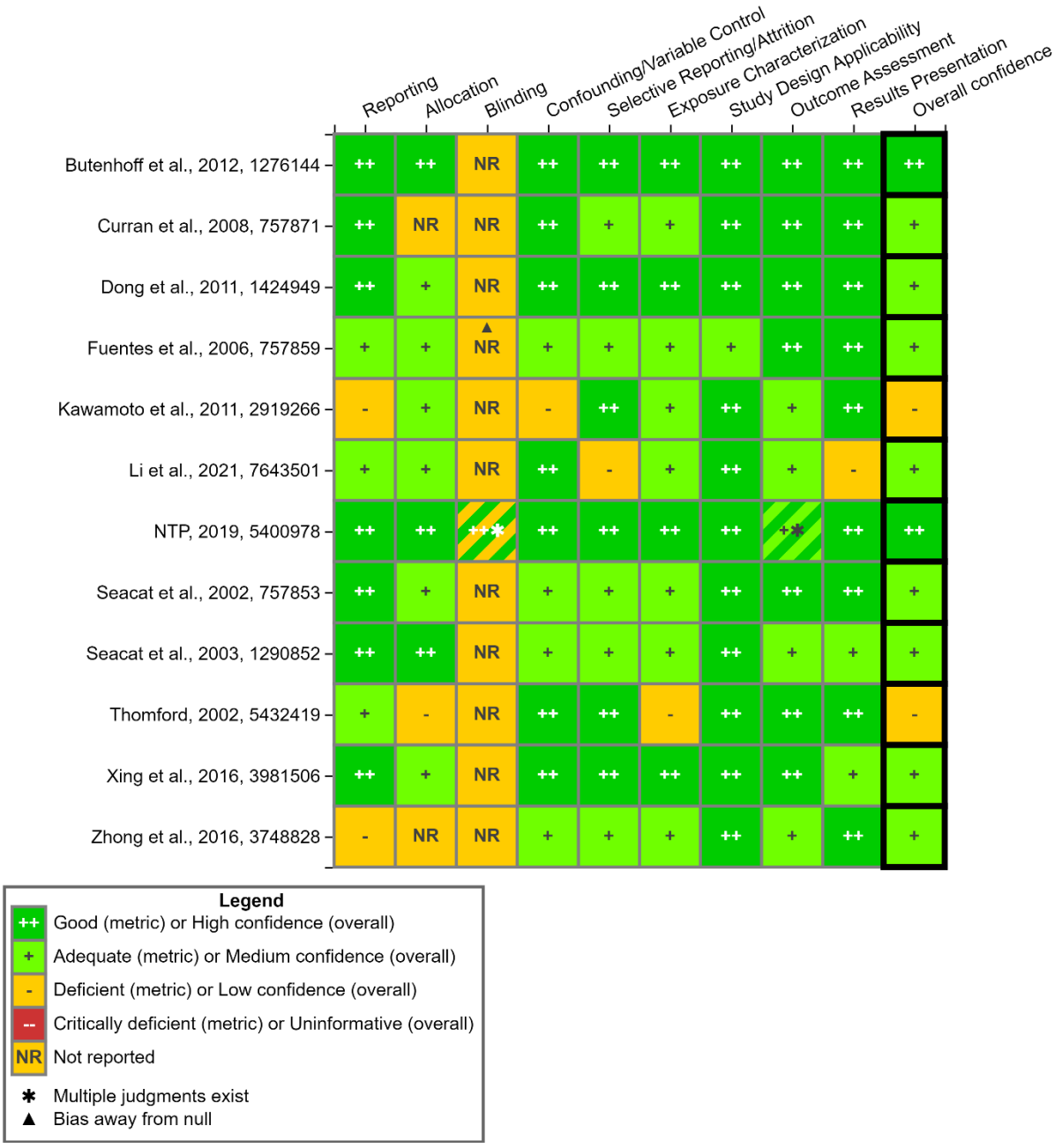


Figure C-32. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Renal Effects

Interactive figure and additional study details available on HAWC.

Few renal effects were observed across multiple studies assessing PFOS toxicity in animal models. Most studies did not observe significant effects of PFOS exposure on kidney weight or histopathology (Li et al., 2021b; Zhong et al., 2016; Dong et al., 2011; Peden-Adams et al., 2008; Yahia et al., 2008; Fuentes et al., 2006; Seacat et al., 2003; Seacat et al., 2002). However, two subchronic studies in male mice reported significant decreases in relative kidney weight with PFOS treatment for 30 days at the highest dose tested of 10 mg/kg/day (approximately 10%

decrease) (Xing et al., 2016) and treatment for 60 days at doses of 0.83 mg/kg/day or 2.083 mg/kg/day (approximately 18% and 16% decreases, respectively) (Dong et al., 2009). Neither of these studies reported absolute kidney weight and, in both studies, PFOS treatment resulted in decreased body weight at these doses which precludes evaluation of the significance of relative weights. One developmental study in mice reported no significant changes in maternal relative or absolute kidney weight (Fuentes et al., 2006).

In contrast to the mouse studies, four short-term/subchronic studies in male rats reported significant increases in relative kidney weight at doses as low as 1.25 mg/kg/day (NTP, 2019), 5 mg/kg/day (Cui et al., 2009), 6 mg/kg/day (Goldenthal et al., 1978), and 6.34 mg/kg/day (Curran et al., 2008). NTP (2019) observed an approximately 14% increase in relative kidney weight at the highest dose tested (5 mg/kg/day) that occurred along with significantly decreased body weight. Small but significant increases (approximately 8%) in relative kidney weight were also observed at 1.25 and 2.5 mg/kg/day; however, no significant changes were observed in absolute kidney weight at any dose level. While Cui et al. (2009) did not provide absolute kidney weight data, no significant difference was observed in body weight in the 5 mg/kg/day dose group; the study authors indicate that the increased relative kidney weight may be due to renal hypertrophy. Body weight was affected in all other dose groups showing changes in relative kidney weight in Goldenthal et al. (1978), Cui et al. (2009), and Curran et al. (2008). Curran et al. (2008) also reported that absolute kidney weight and kidney weight relative to brain weight were both significantly decreased in male rats exposed to 6.34 mg/kg/day, which also indicates that the increase in relative kidney weight in that dose group was driven by decreased body weight.

NTP (2019) also observed small but significant increases (approximately 9%) in relative kidney weight in female rats at doses as low as 0.625 mg/kg/day, but the increase was not significant at the highest dose tested (5 mg/kg/day). Curran et al. (2008) observed a significant increase in relative kidney weight for female rats at doses as low as 3.73 mg/kg/day, but body weights were significantly decreased in the same dose groups and there were no significant changes in absolute kidney weight or kidney weight relative to brain weight. Similarly, a chronic study in female rats reported significant increases in kidney weight relative to body weight with the highest dose tested (1.25 mg/kg/day) but reported no change in kidney weight relative to brain weight at the same dose, indicating these effects were also driven by the significant decreases in body weight seen at this dose (Butenhoff et al., 2012).

Cui et al. (2009) observed altered kidney histopathology in male rats, including turbidness and tumefaction in the epithelia of the proximal convoluted tubule, congestion in the renal cortex and medulla, and enhanced cytoplasmic acidophilia, though only in the highest dose group (20 mg/kg/day). Besides Cui et al. (2009), all other studies reported no treatment-related changes in kidney histopathology (Li et al., 2021b; NTP, 2019; Xing et al., 2016; Butenhoff et al., 2012; Curran et al., 2008; Yahia et al., 2008; Seacat et al., 2003).

Several studies also analyzed clinical chemistry endpoints relevant to renal toxicity. At the highest dose tested in each study (1.3 mg/kg/day–5 mg/kg/day), Seacat et al. (2003) and NTP (2019) (males only) both reported significant increases in BUN in rats after 14-week and 28-day exposures, respectively. Similarly, Curran et al. (2008) observed a significant trend toward increased serum urea in male rats exposed to doses up to 6.34 mg/kg/day for 28 days, although no significant differences were detected between exposure groups. In an extension of the Seacat

et al. (2003) study, Butenhoff et al. (2012) reported increased BUN in both males and females of the high dose group (approximately 0.98 mg/kg/day and 1.25 mg/kg/day, respectively) at 27 weeks and significantly increased BUN in doses ≥0.1 mg/kg/day in males and ≥0.3 mg/kg/day in females at 53 weeks. However, the studies that reported increased BUN did not see concurrent increases in serum creatinine concentrations at the same dose levels and time points (NTP, 2019; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003); NTP (2019) and Butenhoff et al. (2012) consider mild increases in BUN without increases in creatinine to be more consistent with decreased water intake and mild dehydration rather than a direct toxicological effect of chemical exposure, though these studies did not quantify water intake in exposed animals. Additionally, increases in BUN were not seen in male mice treated with up to 10 mg/kg/day PFOS for 30 days (Xing et al., 2016) or in male or female monkeys treated with up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al., 2002). Other clinical chemistry endpoints, including creatine kinase (NTP, 2019; Curran et al., 2008; Seacat et al., 2002), uric acid (Curran et al., 2008), urinary N-acetyl-b-glucosaminidase (NAG) (Xing et al., 2016), and urinalysis parameters including urine pH (Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003; Seacat et al., 2002), were not widely assessed across multiple studies and either showed no significant changes or inconsistent responses between studies.

C.5.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse renal outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There are three studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to renal effects. A summary of these studies is shown in Figure C-33. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to renal effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	2	2	2
Cell Signaling Or Signal Transduction	1	2	2
Extracellular Matrix Or Molecules	1	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	1	1
Inflammation And Immune Response	1	1	2
Oxidative Stress	0	1	1
Renal Dysfunction	1	1	2
Xenobiotic Metabolism	0	1	1
Grand Total	3	2	3

Figure C-33. Summary of Mechanistic Studies of PFOS and Renal Effects

Interactive figure and additional study details available on HAWC.

C.5.4 Evidence Integration

There is *slight* evidence for an association between PFOS exposure and renal effects in humans based on observed effects on measures of renal function and kidney disease in 17 *low* confidence studies. The 2016 PFOS HESD (U.S. EPA, 2016c) concluded there was evidence of a suggestive association between PFOS and CKD. The epidemiological evidence in this review observed positive associations between serum PFOS concentrations and CKD only in *low* confidence studies (Wang et al., 2019b; Conway et al., 2018). There is suggestive evidence of associations with decreased kidney function, although reverse causality (i.e., increases in serum perfluoroalkyl levels could be due to a decrease in GF and shared renal transporters for perfluoroalkyls and uric acid) cannot be ruled out. There were mixed results across the measures of renal function. Results were more consistent for eGFR, in which inverse associations were reported by two *low* confidence studies (Wang et al., 2019b; Blake et al., 2018). Regarding hyperuricemia and uric acid levels, results varied across glomerular function and sex. Among children, there were mixed results for associations with creatinine and uric acid. One *low* confidence study reported a statistically significant decrease in eGFR in adolescents across PFOS quartiles (Kataria et al., 2015). Additionally, given the limited evidence, conclusions cannot be drawn between PFOS and renal effects among pregnant women and occupational workers.

The animal evidence for an association between PFOS exposure and effects on renal toxicity is considered *indeterminate* based on 10 *high* or *medium* confidence animal studies. The renal system does not appear to be sensitive to PFOS toxicity. Effects on kidney weight were

inconsistent between species and mainly consisted of changes in relative kidney weights occurring at relatively high doses where body weights were also decreased. These changes in relative kidney weight are considered a reflection of changes in body weight rather than adverse effect on the kidney. Additionally, changes in clinical chemistry parameters such as increased BUN without further evidence of kidney dysfunction (e.g., increased serum creatinine) are not generally considered adverse and may be more reflective of changes in water consumption than effects on the kidney.

C.5.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause renal effects in humans under relevant exposure circumstances (Table C-10). This conclusion is based primarily on effects on measures of kidney function observed in studies in humans exposed to median PFOS ranging from 3.5 ng/mL to 11.9 ng/mL. Although there is some evidence of negative effects of PFOS exposure on CKD, there is considerable uncertainty in the results due to inconsistency across studies, mixed findings, limited number of studies, and potential for reverse causation.

Table C-10. Evidence Profile Table for PFOS Renal Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.5.1)					⊕⊙⊙ ***Evidence Suggests***
Uric acid 10 *Low* confidence studies	Increases in uric acid were observed in both children (3/3) and adults (4/7). Significant increases in uric acid were observed in adults (2/7). Results were consistently stratified by CKD status, but the direction of effect was less consistent when stratified by eGFR. Increases in uric acid led to increased odds of hyperuricemia in all studies that assessed hyperuricemia (5/5).	• *Consistent direction* of effects among children and adults	• *Low* confidence studies	⊕⊙⊙ *Slight* All studies were of *low* confidence, which found evidence of decreased kidney function in adults and children, including increased uric acid, hyperuricemia, and decreased eGFR. In adults, studies found evidence of increased albumin and total serum proteins, and children studies reported evidence of decreased creatinine. Overall, inconsistent findings in direction of effect and imprecision were observed for most outcomes. The limitation of only *low* confidence studies, mixed results, and risk of high bias leaves uncertainty regarding renal outcomes and PFOS exposures.	*Primary basis*: No evidence in animals and human evidence indicted effects on kidney function. Although there is some evidence of negative effects of PFOS exposure on CKD, there is considerable uncertainty in the results due to inconsistency across studies, mixed findings, limited number of studies and potential for reverse causation. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Serum and urinary biomarkers 5 *Low* confidence studies	Significant increases in serum albumin were observed in adults (2/2), while albumin was not analyzed in children. Creatinine was significantly increased in children (2/3), but two studies in adults reported inconsistent directions of effect. A study in adults from NHANES observed significant positive associations of serum proteins with PFOS and when linear and branched PFOS were analyzed separately.	• No factors noted	• *Low* confidence studies		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Chronic kidney disease 2 *Low* confidence studies	Two studies examined CKD in adults. Odds of CKD was increased among general population adults (2/2), with one reporting a significant increase. The direction of effect was not consistent after stratification by diabetes status.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining outcome		
Glomerular filtration rate 4 *Low* confidence studies	One study in children reported significantly decreased eGFR in all exposure groups (1/1). In adults, significant decreases in eGFR were observed (2/2), but results were less consistent after stratification by sex. Results in pregnant women (1/1) were not significant.	• Consistent direction of effects	• *Low* confidence studies		
Gout 1 *Low* confidence study	No significant associations were observed in the overall study population, or in analyses stratified by CKD status.	• No factors noted	• *Low* confidence study • *Limited number* of studies examining outcome • Potential outcome misclassification due to self-reported outcome		
Evidence From In Vivo Animal Studies (Section C.5.2)					
Kidney weight 2 *High* confidence studies 7 *Medium* confidence studies	Relative kidney weight was increased in rats (3/4), mainly occurring at relatively high dose levels that also resulted in	• *High* and *medium* confidence studies	• *Inconsistency* of findings across species	⊙⊙⊙ *Indeterminate* Evidence was based on 10	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	decreased body weight. Relative kidney weight was decreased in mice (1/4) and absolute kidney weight was decreased in rats (1/4), both at dose levels that also resulted in decreased body weight. One study in monkeys reported no effects on kidney weight.		• Changes in body weight may limit ability to interpret these responses	*high* and *medium* confidence studies. The renal system does not appear to be sensitive to PFOS toxicity. Effects on kidney weight were inconsistent between species and mainly consisted of changes in relative kidney weights occurring at relatively high doses with body weights also decreased. There were no apparent exposure-related changes observed in kidney histopathology or urinalysis endpoints. Changes in clinical chemistry parameters such as increased BUN without further evidence of kidney dysfunction (e.g., increased serum creatinine) are not generally considered adverse and may be more reflective of changes in water consumption than effects on the kidney.	
Histopathology 2 *High* confidence studies 4 *Medium* confidence studies	None of the studies that examined kidney histopathology (0/6) found evidence of morphological damage or exposure-related lesions following short-term, subchronic, or chronic exposure to PFOS.	• *High and medium* confidence studies • *Consistent* effects across study design, sex, and species	• No factors noted		
Serum biomarkers 2 *High* confidence studies 4 *Medium* confidence studies	Serum BUN was increased (3/6) mainly at the highest dose tested and only in rats (1 study each in monkeys, rats, or mice found no effects on BUN). One *high* confidence study with chronic exposure observed increased BUN in male and female rats at several timepoints throughout the study with a dose response evident in female rats after 53 wk of exposure. No significant changes in serum creatinine were	• *High* and *medium* confidence studies	• *Incoherence* of findings in serum biomarkers of renal function		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	observed (5/5), including all studies that observed increased BUN. No exposure-related changes were observed for serum uric acid (1/1) or creatine kinase (2/2).				
Urinalysis 1 *High* confidence study 4 *Medium* confidence studies	No exposure-related changes were observed for urinalysis endpoints (5/5). Urine pH was increased or decreased (2/5), but the changes were not exposure-related. One subchronic study in mice found no changes in urinary N-acetyl-b-glucosaminidase.	• *High* and *medium* confidence study	• No factors noted		

Notes: BUN = blood urea nitrogen; CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; NHANES = National Health and Nutrition Examination Survey; wk = weeks.

C.6 Hematological

EPA identified eight epidemiological and five animal studies that investigated the association between PFOS and hematological effects. Of the epidemiological studies, three were classified as *medium* confidence, two as *low* confidence, and three were considered *uninformative* (Section C.6.1). Of the animal studies, one was classified as *high* confidence, three as *medium* confidence, one was considered *low* confidence (Section C.6.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.6.1 Human Evidence Study Quality Evaluation and Synthesis

C.6.1.1 Introduction

The mechanisms for PFOS effects on hematological parameters might include immune suppression, shifts in nutrients absorbed from the diet, or the influences related to other health outcomes such as cardiometabolic or kidney dysfunction (Abraham et al., 2020; Jain, 2020a; Chen et al., 2019a). PFOS has been implicated in endocrine disruption, which may affect vitamin D homeostasis (Etzel et al., 2019). It could also alter epigenetics via DNA methylation (van den Dungen et al., 2017). The effects of PFOS on hematological outcomes may differ by characteristics such as age, gender, race, and genetics.

Hematological health outcomes in humans were previously reviewed in the 2016 PFOS HESD (U.S. EPA, 2016d). Six occupational studies and one general population study, published prior to 2010, provided hematology data. No statistically significant associations between PFOS exposure and hematology parameters were identified. The 2016 PFOS HESD did not specifically discuss or draw conclusions about these parameters independent of other health outcomes.

For this updated review, eight studies examined the association between PFOS hematological health outcomes (Appendix D). The specific hematological parameters investigated included hematology tests (calcium, erythrocytes, ferritin, fibrinogen, hematocrit, hemoglobin, iron), blood coagulation tests, Vitamin D levels and deficiency and anemia.

All studies assessed exposure to PFOS using biomarkers in blood. Samples were taken from participating pregnant women, children, adolescents, or adults. All included studies were cross-sectional designs. Four were from the United States, three from Europe, and one from Asia. Three studies used overlapping data from a large, ongoing survey in the United States, NHANES (Jain, 2020a, b; Etzel et al., 2019). Etzel et al. (2019) used 2003–2010 NHANES data for adolescents and adults 12 years and older, and Jain (2020a) and Jain (2020b), used 2003–2016 NHANES data for adults 20 years and older. Also in the United States, Khalil et al.(2018) included 48 obese children 8–12 years old from a hospital lipid clinic in Dayton, Ohio. Abraham et al.(2020) included 101 healthy one-year-old German children in the Berlin area, including 27 children living near a former copper smelting site. Jiang et al.(2014) recruited 141 pregnant women in Tianjin, China. Chen et al.(2019a) conducted a pilot study with 1,430 male and female adults from the island of Hvar, off the coast of Croatia. A study conducted by van den Dungen et al.(2017) included 80 men aged 40–70 years in the Netherlands who regularly consumed eel.

C.6.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies on hematological parameters. Important considerations included the influence of diet, supplement or medication use, adiposity (due to lipid binding), disease status, and SES on both PFOS exposure and hematology. In particular, the duration of breastfeeding is expected to be associated with both PFOS exposure and nutrition intake (Abraham et al., 2020). The blood matrix (whole blood versus plasma or serum) could also affect the interpretation of results. Measuring PFOS and serum lipids concurrently was considered adequate in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.5 years) (Li et al., 2018), current blood concentrations are expected to correlate well with past exposures.

There are eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and hematological effects. Study quality evaluations for these eight studies are shown in Figure C-34.

On the basis of the considerations mentioned, three studies were classified as *medium* confidence, two as *low* confidence and three as *uninformative*. Two *low* confidence studies had deficiencies in participant selection, confounding, or sample size. Khalil et al. (2018) was affected by a small sample size, the cross-sectional design, and potential residual confounding attributable to differences in participants' SES. Van den Dungen et al. (2017) was affected by a small sample size, concerns about selection bias, and a lack of information on key confounders such as SES.

Three studies were rated as *uninformative* for hematological outcomes. For Jain (2020b), the use of PFOS as the dependent variable and health outcomes as the independent (predictive) variable rendered the study *uninformative* for hazard assessment (Jain, 2020b). Abraham et al. (2020) and Jiang et al. (2014) only performed unadjusted correlation analyses and therefore did not consider the influence of potential confounding factors.

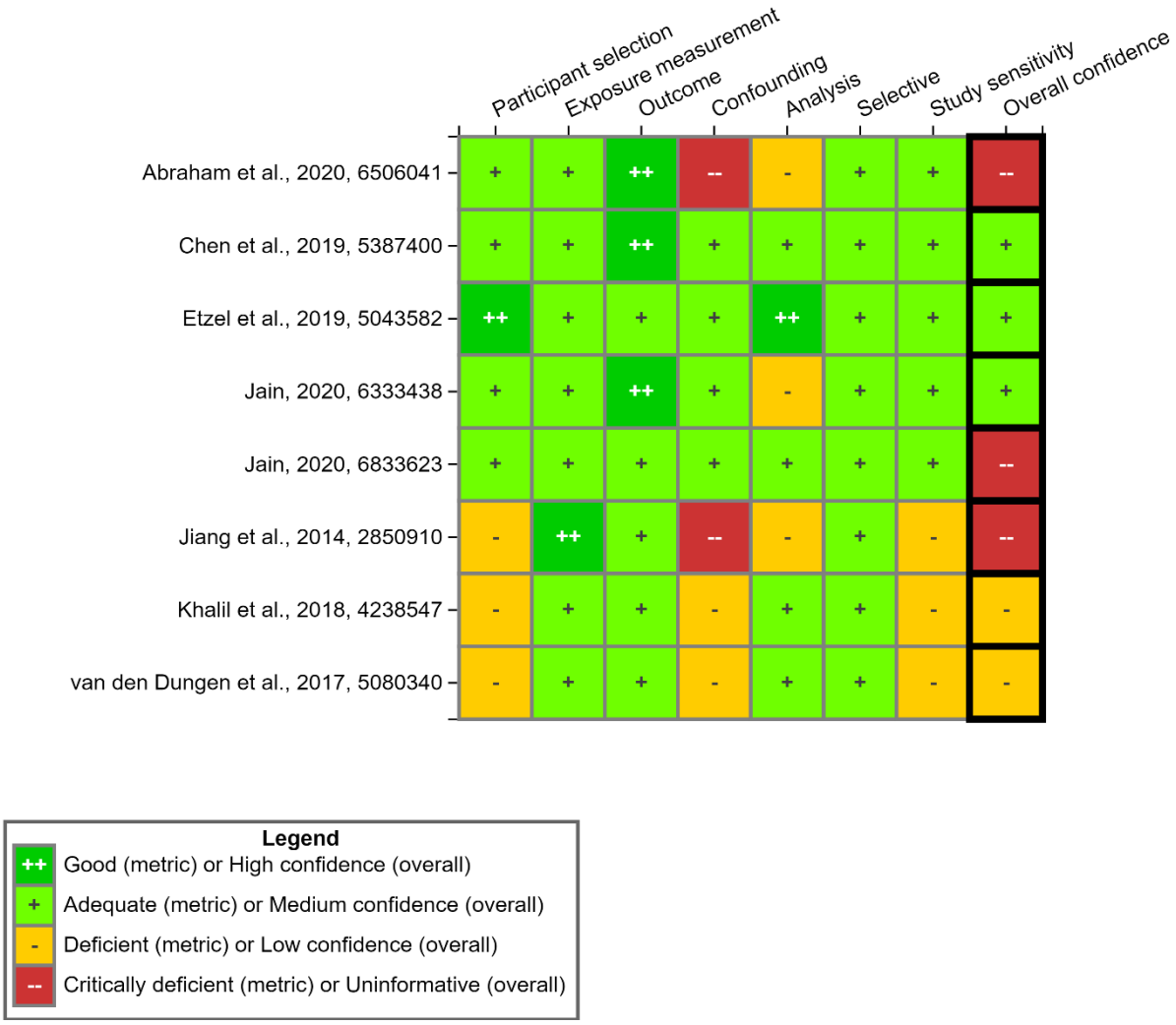


Figure C-34. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Hematological Effects

Interactive figure and additional study details available on HAWC.

C.6.1.3 Findings

Two studies examined levels of 25-hydroxy vitamin D and vitamin D deficiency and a significant association was observed in one study (Etzel et al., 2019). In adolescents and adults from NHANES (2003–2010), Etzel et al.(2019) observed a statistically significant decrease in total serum 25-hydroxy vitamin D per a 2-fold increase in PFOS and comparing the top quintile of PFOS exposure (25.9 ng/mL–435.0 ng/mL) to the lowest quintile. Statistically significant decrease in total serum 25-hydroxy vitamin D were also observed in participants 60 and older. A positive non-significant association with prevalence ORs for vitamin D deficiency was also observed. In 8–12-year-old U.S. children, Khalil et al. (2018) also observed a decrease in 25-hydroxy vitamin D levels, but it did not reach significance.

In adults from NHANES (2003–2016), Jain (2020a) observed small statistically significant increases in whole blood hemoglobin levels (WBHGB) with increased PFOS exposure among

adult males or females ≥ 20 years (Appendix D). This was true for subgroups with or without anemia, although the magnitude of the effect was larger among those defined as anemic. Anemia was defined as WBHGB concentrations < 12 g/dL for females or < 13 g/dL for males. Jain (2020a) also evaluated the impact of deteriorating kidney function, by stratifying results by stages of GF. For anemic males, association between WBHGB and PFOS concentrations were uniformly positive across worsening stages of renal failure. For anemic females, association between WBHGB and PFOS concentrations were positive except at GF-1 (eGFR ≥ 60 mL/min/1.73 m^2). Overall, the association between WBHGB and PFOS followed U-shaped distributions. Hemoglobin levels were also examined in pregnant women (Jiang et al., 2014). Small significant positive correlations were observed between total PFOS and hemoglobin levels ($r = 0.280$, $p < 0.01$) as well as total PFOS and red blood cell count (RBC) ($r = 0.206$, $p < 0.01$), although these results did not consider the influence of confounding factors and should be interpreted with caution. In high-exposed population (van den Dungen et al., 2017), observed non-significant decreases in hemoglobin and hematocrit levels, and non-significant increases in retinol.

Chen et al.(2019a) found that serum calcium levels among Croatian adults were statistically significantly decreased in association with an increase in the natural log of PFOS exposure.

C.6.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOS HESD (U.S. EPA, 2016c) and two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and hematological effects. Study quality evaluations for these five studies are shown in Figure C-35

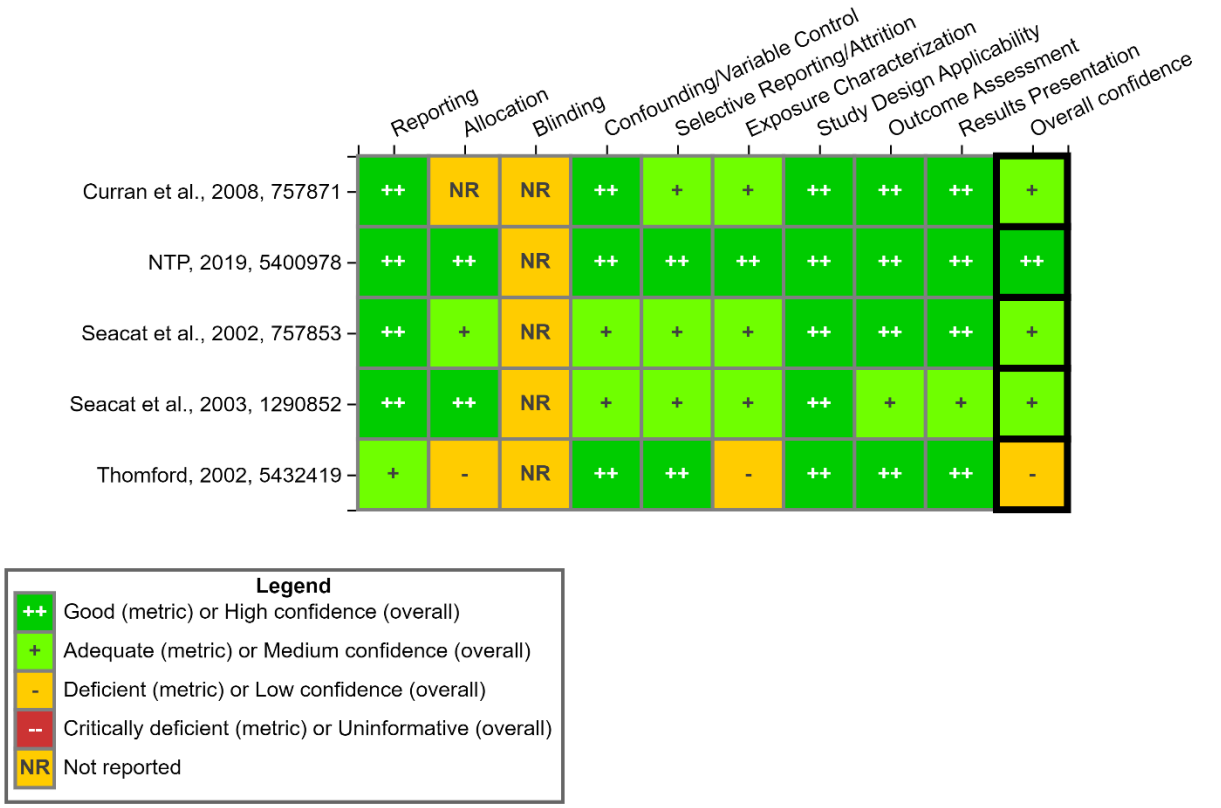


Figure C-35. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Hematological Effects

Interactive figure and additional study details available on HAWC.

Hematological measures, along with other biomarkers or histopathological findings, may be informative for assessment of the health and function of blood-forming tissues such as the spleen and bone marrow. The focus of this section is clinical hematological endpoints including alterations in hemoglobin and hematocrit levels and changes in red blood cell production and structure. Four oral studies in rodents or monkeys with short-term to chronic exposure durations evaluated the effects of PFOS on the hematological system (see Toxicity Assessment, (U.S. EPA, 2024)).

Significantly decreased reticulocyte counts were observed in male and female Sprague-Dawley rats following 28-day oral gavage exposure to 2.5 mg/kg/day or 5 mg/kg/day (NTP, 2019). The percent decrease from control was 42% and 49% in the 5 mg/kg/day dose group for males and females, respectively, indicating potential deficiencies in red blood cell maturation. Increased incidences of decreased splenic hematopoiesis, as well as increased bone marrow hypocellularity characterized by minimal increases in the number of adipocytes and reductions in hematopoietic cells, were observed in both males and females at these doses (see Toxicity Assessment, (U.S. EPA, 2024)). NTP (2019) suggests that a combination of these findings may indicate a suppression in erythropoiesis.

No other effects on hematocrit, hemoglobin, mean cell volume, platelet count, and red blood cells were reported in male or female Sprague-Dawley rats in the NTP (2019) report or in male

or female Sprague-Dawley rats administered up to 20 ppm PFOS (equivalent to 1.51 mg/kg/day or 1.77 mg/kg/day in females and males, respectively) in feed for 28 days (Seacat et al., 2003). In a third 28-day study, female Sprague-Dawley rats exposed to 100 mg/kg of PFOS in diet (highest dose tested, equivalent to 7.58 mg/kg/day), displayed significantly reduced red blood cell numbers, hemoglobin levels, hematocrit, and mean cell hemoglobin concentrations, though these effects were generally within 10% of control levels (Curran et al., 2008). In male rats, there was a trend toward reduced red blood cell distribution widths (i.e., decreased range in the volume and size of erythrocytes) with increasing PFOS dose. Circulating blood platelet numbers were unaffected, but mean platelet volume was significantly reduced in male rats at 6.34 mg/kg/day (100 mg/kg of PFOS in the diet) and in female rats at 3.73 mg/kg/day (50 mg/kg of PFOS in the diet). In both males and females exposed to 100 mg/kg PFOS in the diet, equivalent to 6.34 mg/kg/day and 7.34 mg/kg/day, respectively, the red blood cell deformability index was significantly reduced over a range of shear stress levels. Effects on blood electrolyte levels were also noted in these rats. Notably, the sodium/potassium ratio was increased in males and females at 100 mg/kg PFOS in the diet (7.34 mg/kg/day) while inorganic phosphate was decreased in females only at this same dose (Curran et al., 2008).

Other reported hematologic effects following subchronic or chronic exposure to PFOS appear to be minimal in the low dose range. For example, male and female Sprague-Dawley rats exposed to 0.5–20 ppm PFOS in feed (equivalent to 0.03 mg/kg/day–1.33 mg/kg/day and 0.04 mg/kg/day–1.56 mg/kg/day in males and females, respectively) for 14 weeks showed no effects on hematocrit, hemoglobin, mean cell volume, platelet count, and red blood cells (Seacat et al., 2003). Hemoglobin levels were decreased in male Cynomolgus monkeys following a chronic 182-day exposure to 0.75 mg/kg/day, although no changes were observed in female monkeys. While the hemoglobin levels in males reported by Seacat et al. (2002) are statistically significant, they are within 10% of control and no other hematologic changes were reported in the study.

C.6.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse hematological outcomes is discussed in Section 3.1.1.1 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to hematological effects. A summary of these studies is shown in Figure C-36. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOS leads to hematological effects.

Mechanistic Pathway	Animal	Human	Grand Total
Atherogenesis And Clot Formation	0	1	1
Other	1	0	1
Grand Total	1	1	2

Figure C-36. Summary of Mechanistic Studies of PFOS and Hematological Effects

Interactive figure and additional study details available on HAWC.

C.6.4 Evidence Integration

The evidence evaluating an association between PFOS exposure and hematological effects in humans is *indeterminate.* The limited number of studies reporting on hematological effects of PFOS in humans is limited and relevant outcomes were not studied in more than in one study, hence coherence is impossible to evaluate. There is evidence for an association between increased PFOS and slightly increased WBHGB levels (Jain, 2020a), particularly among anemic adults in a large NHANES study. Increases in hemoglobin and RBC may also affect pregnant women (Jiang et al., 2014). However, it is unclear whether the observed changes are clinically adverse. The two studies that examined 25-hydroxy vitamin D levels reported mixed non-significant effects; three studies examined hemoglobin and also reported mixed effects.

There is *indeterminate* animal evidence of an association between PFOS exposure and hematological effects. Although the available 28-day studies in rats observed some hematological effects, the alterations were generally within 10% of control, except for reduced reticulocyte counts observed by NTP (2019). These reductions in reticulocyte counts support histopathological changes in the spleen (splenic extramedullary hematopoiesis) that have been identified as notable immune endpoints (see Toxicity Assessment, (U.S. EPA, 2024)). Reticulocyte counts do not appear to be as sensitive as the corresponding histopathological findings in the spleen; decreases in reticulocytes were observed at doses ≥ 2.5 mg/kg/day whereas histopathological alterations were observed at a slightly lower dose of 1.25 mg/kg/day and higher. Further, the available subchronic and chronic studies measured hematology at various timepoints did not observe any consistent effect of treatment on red blood cells.

C.6.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOS exposure can cause hematological effects in humans under relevant exposure circumstances (Table C-11).

Table C-11. Evidence Profile Table for PFOS Hematological Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.6.1)					⊙⊙⊙ ***Inadequate Evidence***
25-hydroxy vitamin D 1 *Medium* confidence study 1 *Low* confidence study	Two studies observed decreases in 25-hydroxy vitamin D. One of the studies observed a significant decrease among the whole study population. Results were similar in all stratifications and study authors reported increased vitamin D deficiency.	• *Medium* confidence study • Consistent direction of effects	• *Low* confidence study • *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence for hematological effects is based on two studies reporting decreased 25-hydroxy vitamin D and one study reporting increased WBGHB. Considerable uncertainty due to limited number of studies and unexplained inconsistency across studies and endpoints.	*Primary basis*: Evidence in humans and animals were limited and largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Anemia and whole blood hemoglobin (WBHGB) 1 *Medium* confidence study 1 *Low* confidence study	One study (1/2) observed significantly increased WBGHB, and one study (1/2) observed non-significant decreases in hemoglobin.	• *Medium* confidence study	• *Low* confidence study • Inconsistent direction of effects		
Serum electrolytes 1 *Medium* confidence study	One study observed significantly decreased serum calcium among adults.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.6.2)					
Complete blood count 1 *High* confidence study 3 *Medium* confidence study	One short-term study in rats found evidence of decreased reticulocyte counts in male and female following PFOS exposure (1/1). Hematocrit levels were decreased in female rats	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent direction* of effect for reticulocyte, hematocrit, hemoglobin, and RBC levels	⊙⊙⊙ *Indeterminate* Evidence was limited, inconsistent with direction of effect, and largely non-significant for hematological	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	at the highest dose tested following short-term exposure (1/4). Decreased hemoglobin (2/4) was observed in male monkeys following chronic exposure (1/4) and in female rats following short-term exposure (1/4). No effects on hemoglobin were found after short-term and chronic exposure in rats (2/4). RBC was decreased (1/4) in females at the highest dose tested and only in rats (2 additional studies in rats and 1 study in monkeys found no effects on RBC). No significant changes In mean cell volume (2/2) and red cell distribution width (1/1) were observed. An increase in the RBC deformity index associated with increased PFOS dose and log shear stress in both male and female rats in a short-term study (1/1). Decreased mean platelet volume (1/1) was observed in male and female rats following			endpoints in animal models.	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	short-term exposure to PFOS. No significant exposure-related changes were observed in platelet count (3/3).				
Serum electrolytes inorganic phosphate, chloride, and Na/K ratio 2 *Medium c*onfidence studies	Inorganic phosphate levels were decreased (1/2) in female rats chronically exposed to the highest dose tested (1/1) but no significant findings were observed in a short-term study for male or female monkeys (1/1). In a chronic rat study, increased Na/K ratio (1/1) was observed in males and females and no exposure-related changes were observed in chloride levels (1/1).	• *Medium* confidence studies	• *Limited number* of studies examining outcome		

Notes: WBHGB = whole blood hemoglobin; RBC = red blood count; Na/K = sodium/potassium ratio.

C.7 Respiratory

EPA identified five epidemiological and five animal studies that investigated the association between PFOS and respiratory effects. All five of the epidemiological studies were classified as *medium* confidence (Section C.7.1). Of the animal studies, one was classified as *high* confidence, three as *medium* confidence, and one was considered *low* confidence (Section C.7.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.7.1 Human Evidence Study Quality Evaluation and Synthesis

C.7.1.1 Introduction

Respiratory health can be ascertained by several measurements. The most informative are measurements of pulmonary function (e.g., lung volume and air flow measures determined by spirometry, as well as respiratory sounds, sputum analysis, and blood gas tension) or pulmonary structure (e.g., lung weight, histopathology, and chest radiography), while respiratory symptoms (shortness of breath, cough/presence of sputum, chest tightness), history of respiratory illnesses, and respiratory mortality have low specificity and sensitivity.

The 2016 Health Assessment for PFOS (U.S. EPA, 2016c) did not examine any epidemiological evidence of association between exposure to this chemical and respiratory health effects.

For this updated review, five epidemiological studies investigated the association between PFOS and respiratory outcomes. All studies measured PFOS using biomarkers in blood. Three studies were mother-child cohort studies conducted in Europe (Agier et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018), one was a cross-sectional case-control study (cross-sectional analyses were performed in asthmatic cases and non-asthmatic controls) conducted in Taiwan (Qin et al., 2017); and one was a cross-sectional study of adolescents and young adults residing near the WTC (Gaylord et al., 2019). The five available studies examined lung function measures in children and young adults, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, forced expiratory flow at 25%–75% ($FEF_{25\%–75\%}$), peak expiratory flow rate (PEF), lung volume, resistance at oscillation frequencies of 5 Hz or 20 Hz, lung function at birth, and severity of obstructive airways disease (Appendix D).

Studies that examined respiratory illnesses or symptoms reflecting immune system responses (e.g., asthma and allergies) and respiratory tract infections (e.g., cough) are analyzed in the immune system section.

C.7.1.2 Study Quality

There are five studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and respiratory effects. Study quality evaluations for these five studies are shown in Figure C-37. The five general population studies identified since the last assessment were all classified *medium* confidence. These studies had minor deficiencies, including concerns that co-exposures in the WTC disaster could confound the results (Gaylord et al., 2019), reduced

sensitivity because of low exposure levels and narrow ranges (Impinen et al., 2018), or concerns with potential bias in selection of non-asthmatic controls (Qin et al., 2017).

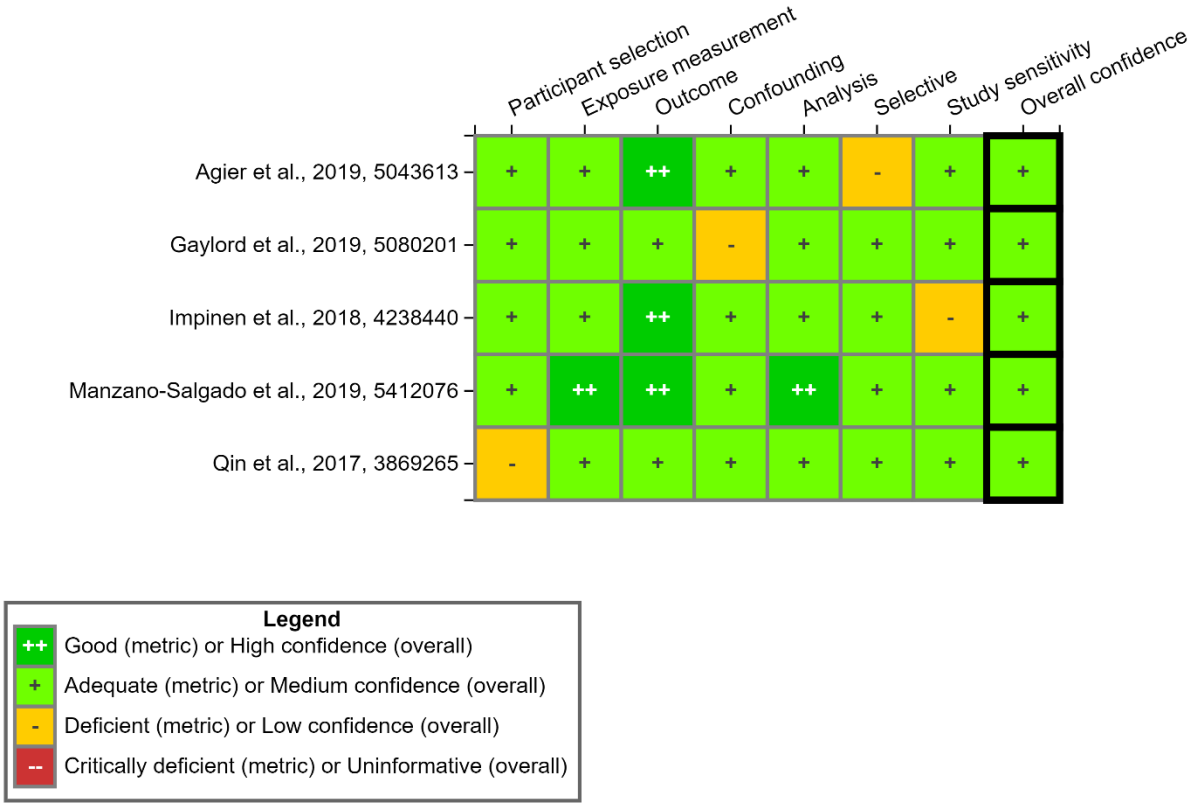


Figure C-37. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Respiratory Effects

Interactive figure and additional study details available on HAWC.

C.7.1.3 Findings From Children and Adolescents

Four studies examined respiratory health effects in children up to 15 years old (Agier et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018; Qin et al., 2017) and one examined adolescents and young adults ages 13–22 years (Gaylord et al., 2019) (Appendix D).

Of the four studies examining FEV1, three reported negative associations (i.e., decrease in FEV1 with higher PFOS levels), while one reported a positive association. Qin et al. (2017) observed significant inverse associations for children ages 10–15 years old with asthma (beta = −0.061, 95% CI: −0.101, −0.021), and in boys with asthma, but not in girls with asthma. There was also a significantly decreasing trend by quartiles of PFOS in children with asthma (p-trend = 0.003). No effects were observed in children without asthma. Results from other studies examining FEV1 were inconsistent and non-significant, with two studies (Gaylord et al., 2019; Manzano-Salgado et al., 2019) observing inverse associations and one study (Agier et al., 2019) reporting a positive association.

For other lung function measures examined there was also limited evidence of associations. Qin et al. (2017) reported a statistically significant association with FVC (beta = −0.055, 95%

CI: −0.1, −0.01) but a non-significant decreasing trend by quartiles of PFOS (p-trend = 0.186). Non-significant associations were observed for $FEF_{25\%–75\%}$ or PEF or for any lung function measures in children without asthma. Impinen et al. (2018) reported a statistically significant association with severe obstructive airways disease at age 2 measured by the Oslo Severity Score (OSS), but only for the lowest severity category (OSS 1–5) (OR per log_2 increase PFOS = 1.71, 95% CI: 1.16, 2.53). The study also reported a non-significant decrease in odds of reduced lung function at birth, as measured by tidal flow volume. Clear patterns were not observed for other lung function measures (i.e., FVC, FVC/FEV_1, lung resistance, total lung capacity, functional residual capacity, and residual volume) in the remaining studies (Gaylord et al., 2019; Manzano-Salgado et al., 2019).

C.7.2 Animal Evidence Study Quality Evaluation and Synthesis

There are five studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and respiratory effects. Study quality evaluations for these five studies are shown in Figure C-38.

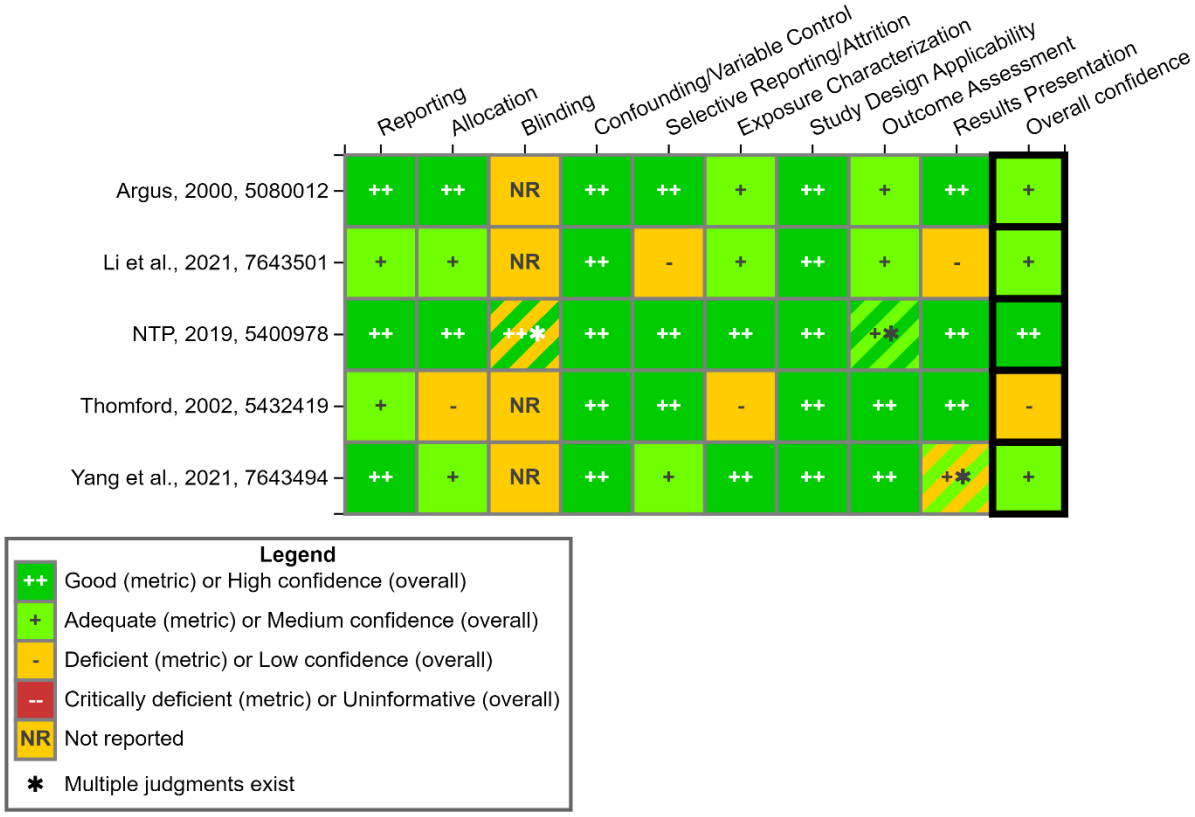


Figure C-38. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Respiratory Effects

Interactive figure and additional study details available on HAWC.

Several studies have reported adverse pulmonary effects resulting from oral PFOS exposure. The available literature primarily focuses on fetal and neonatal outcomes as several groups

hypothesized that the interactions of PFOS with pulmonary surfactants and subsequent reductions in lung function or maturity may play a role in the increased perinatal mortality resulting from gestational PFOS exposure (U.S. EPA, 2016c; Chen et al., 2012b; Ye et al., 2012; Yahia et al., 2008; Grasty et al., 2005; Grasty et al., 2003; Argus Research Laboratories, 2000). There are also several available studies that reported pulmonary effects in adult mammalian models (Li et al., 2021b; Yang et al., 2021; NTP, 2019; Cui et al., 2009; Goldenthal et al., 1979).

Yahia et al. (2008) exposed mouse dams to 0, 1, 10, or 20 mg/kg/day PFOS from GD 0–GD 17 and assessed neonatal and maternal lung histopathology. Initially, a single surviving pup from each dam (n = 5/treatment group) was analyzed at PND 0; all five pups in the 20 mg/kg/day group showed lung atelectasis (i.e., complete or partial lung collapse) which was characterized by alterations in the alveolar epithelium, congestion of alveolar capillary vessels, and reduced alveolar space. Focal or severe atelectasis was also present in some of the pups from the 10 mg/kg/day group (incidence not provided) but not in pups from the control or 1 mg/kg/day groups. No observed histological effects of PFOS exposure were observed on the maternal lung. Yahia et al. (2008) dosed additional dams with 20 mg/kg/day PFOS from GD 0–GD 17 or 10 mg/kg/day PFOS from GD 0–GD 18 to further examine pulmonary effects in fetuses and pups, respectively. Immediately at birth, 27% (4/15) of pups (n = 3 pups/dam) from 3/5 dams dosed with 10 mg/kg/day PFOS showed at least mild lung atelectasis. In contrast, all fetuses in the 20 mg/kg/day group showed normal lung histopathology at GD 18. The authors suggested an increase in the incidence of moderate to severe intracranial blood vessel dilation in fetuses at GD 18 as a cause of the pulmonary effects that were not seen until birth (Yahia et al., 2008).

Chen et al. (2012b) similarly assessed rat pup lung histopathology at PND 0 and PND 21 after gestational exposure to 0 mg/kg/day, 0.1 mg/kg/day, or 2 mg/kg/day PFOS from GD 1–GD 21. With PFOS exposure of 2 mg/kg/day, pups showed marked alveolar hemorrhaging, thickened interalveolar septum, and focal lung consolidation at PND 0 (incidence data not provided). These effects lasted through PND 21, when pups from the 2 mg/kg/day treatment group also showed alveolar hemorrhaging, thickened interalveolar septum, and inflammatory cell infiltration. The 2 mg/kg/day group PND 0 and PND 21 pups also had higher percentages of pulmonary apoptotic cells. There were no pulmonary abnormalities observed in pups from the control or 0.1 mg/kg/day groups.

Zhang et al. (2021) reported that Sprague-Dawley rat pups exposed to 1 or 5 mg/kg/day from GD 12 to GD 18 had higher lung injury scores and that pups in the 5 mg/kg/day group had lower radial alveolar counts on PND 1, 3, 7, and 14 compared with controls.

In an attempt to identify the prenatal window of susceptibility to PFOS in neonatal rats, Grasty et al. (2003) dosed dams with 0 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day PFOS during several 4-day gestational timepoints, including GD 17–GD 20, a period of development they identified in this study as a particularly sensitive window for neonatal mortality. As the last few days of fetal development involve central nervous system and pulmonary maturation, the authors conducted a second exposure of 0 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day PFOS from GD 19–GD 21 and sacrificed fetuses at GD 21 or pups at PND 0 to examine lung histology (Grasty et al., 2003). No histological differences between lung samples of control and treated fetuses sacrificed at GD 21 were observed, though it appeared that PFOS reduced lung expansion and slowed or compromised lung maturation of pups by PND 0; epithelial thickness of lungs of PFOS-treated pups at PND 0 was similar to that of lungs from fetal control animals at GD 21 (incidence data

not provided). Grasty et al. (2005) conducted a follow-up study with the same GD 19–GD 21 exposure paradigm to further explore mechanisms of developmental pulmonary dysfunction and potential methods of therapeutic rescue of delayed lung maturation and effects on pulmonary surfactants seen after gestational PFOS exposure. Grasty et al. (2005) found several morphometric changes in pup lung tissue after 25 mg/kg/day or 50 mg/kg/day PFOS exposure, including increases in the proportion of lung occupied by solid tissue, decreases in the proportion of lung occupied by small airways, and increases in the ratio of solid tissue to small airway space. The authors also note that some lung samples from the 50 mg/kg/day group did not appear to fill fully upon perfusion, potentially indicating a failure of inflation upon birth or atelectasis. Similar to the results of Grasty et al. (2003), the lungs of some PFOS-exposed pups at PND 0 resembled the lungs of control fetuses at GD 21 (incidence of 17% and 50% of pups from the 25 mg/kg/day and 50 mg/kg/day groups, respectively). Co-treatment with the therapeutic agents dexamethasone or retinyl palmitate did not increase neonatal survival, indicating the pulmonary effects of PFOS do not drive neonatal mortality, though the authors did not report histological analyses showing improved pulmonary outcomes in co-treated animals. Ye et al. (2012) did not observe effects on rat fetal lung histopathology following gestational exposure to 5 or 20 mg/kg/day, though the exposure period lasted from GD 12–GD 18 and may have missed the sensitive period of lung development in rats (Grasty et al., 2005; Grasty et al., 2003).

In a rabbit teratology study, Argus (2000) reported a significant increase in the number of fetuses with absent intermediate lung lobes after exposure to 0.1 mg/kg/day PFOS from GD 7–GD 20 (7/172 fetuses compared with 2/175 in controls). However, this increase was not statistically significant when analyzed by litter (4/19 litters compared with 2/20 in controls) and no increase was observed in the higher dose groups of 1 mg/kg/day, 2.5 mg/kg/day, or 3.75 mg/kg/day. Argus (2000) noted that this fetal malformation was likely not related to the test article as varied lung development is frequently observed in New Zealand white rabbits.

Pulmonary effects were observed in adult animals after short-term and subchronic exposures to PFOS. Cui et al. (2009) reported dose-related increases in pulmonary congestion and focal or diffuse thickening of epithelial walls in the lungs of male rats gavaged with 5 mg/kg/day or 20 mg/kg/day PFOS for 28 days (incidence data not provided). Focal or diffuse neutrophil, acidophilia, and lymphocyte cellular infiltration and vasodilatation due to leakage of erythrocytes was also especially apparent in the 20 mg/kg/day dose group (incidence data not provided). In a study with limited sample size (n = 2/sex/treatment), Goldenthal et al. (1979) reported increased moderate diffuse atrophy of the serous alveolar cells in 3/4 rhesus monkeys from the highest dose group (4.5 mg/kg/day) treated with PFOS for 90 days. NTP (2019) did not report nasal, olfactory, or pulmonary histopathological effects in adult male or female rats dosed with up to 5 mg/kg/day PFOS for 28 days. However, female rats dosed with 1.25 mg/kg/day, 2.5 mg/kg/day, or 5 mg/kg/day had significantly increased relative lung weight. The biological significance of this increase is unclear as absolute lung weight was only significantly increased in the 1.25 mg/kg/day group and there were no accompanying histopathological alterations in the lung. Yang et al. (2021) examined the impacts of PFOS exposure on male C57BL/6 mice pulmonary system in a 28-day oral gavage study. Relative lung weights displayed an 1% and 6% increase in 0.25 mg/kg/day and 2.5 mg/kg/day groups, respectively, compared with the control group. The toxicological significance of the increase is unclear due to both low sample size with six animals per group and lack of report on body weight or absolute lung weight. Li et al. (2021b) examined the histopathological effects of PFOS exposure on female BALC/c mice

pulmonary system in a 60-day oral gavage study. Authors reported zero incidence of lesions following respiratory histopathological examination among the female mice gavaged with 0.1 mg/kg/day and 1 mg/kg/day.

Immunological responses in lungs were investigated in Yang et al. (2021). No significant differences in bronchoalveolar lavage fluid (BALF) macrophages, eosinophils, neutrophils, and total cell counts were observed among control or dosed groups. Cytokine IL-4 in BALF displayed significant increases in both the 0.25 mg/kg/day and 2.5 mg/kg/day dose groups. IL-13 in BALF showed a significant increase in the 2.5 mg/kg/day dose group whereas IFN-γ in BALF did not display a significant difference. In the same study, PFOS was found to likely exacerbate asthmatic responses. In the BALF, total cell count and eosinophil numbers were higher in ovalbumin (OVA)-induced mice exposed to 0.25 mg/kg/day or 2.5 mg/kg/day PFOS than to OVA-induced alone. 2.5 mg/kg/day PFOS-treated OVA-induced mice showed a 33% increase in the eosinophil infiltration and 67% increase in mucus production compared with OVA-induced alone mice.

C.7.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse respiratory outcomes is discussed in Sections 3.2.5 and 3.4.1.2 of the 2016 PFOS HESD (U.S. EPA, 2016c). There are three studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to respiratory effects. A summary of these studies is shown in Figure C-39. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to respiratory effects.

Mechanistic Pathway	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	3	3
Inflammation And Immune Response	1	1
Oxidative Stress	1	1
Grand Total	3	3

Figure C-39. Summary of Mechanistic Studies of PFOS and Respiratory Effects

Interactive figure and additional study details available on HAWC.

C.7.4 Evidence Integration

The evidence evaluating associations between PFOS exposure and respiratory effects in humans is slight, with an indication of decreased lung function in infants, children, and adolescents. However, the results across studies are inconsistent, and there are a lack of studies examining respiratory effects in both children and adults. Specifically, no studies were available that assessed respiratory health effects in older adults. While there is some evidence of detrimental

respiratory health effects, particularly in children with asthma, the available epidemiological evidence examining PFOS exposure and respiratory health is limited.

The animal evidence for an association between PFOS exposure and respiratory effects is slight, with an indication that the developing lung may be affected in animal models, but at high doses. Evidence in adults is less consistent with no lesions observed in *medium* or *high* confidence studies (Li et al., 2021b; NTP, 2019), but an exacerbated immune response appears to occur in the lung based on a *medium* confidence study (Yang et al., 2021). Several studies in animal models indicate that PFOS may influence fetal and neonatal lung development which may be consistent with epidemiological assessments of reduced lung function in children, though none of the animal studies provide quantifiable incidence data. Additionally, effects on the pulmonary systems of fetuses and neonates generally occurred at doses above those that result in other adverse developmental effects (see Toxicity Assessment, (U.S. EPA, 2024)), indicating that respiratory toxicity is not likely a highly sensitive health outcome for PFOS exposure.

C.7.4.1 Evidence Integration Judgment

Overall, evidence suggests that PFOS exposure has the potential to cause respiratory effects in humans under relevant exposure circumstances (Table C-12). The conclusion is based on limited evidence of an association between PFOS and detrimental respiratory health effects, particularly in children with asthma, in a small number of epidemiologic studies with median exposure levels from 5.2 ng/mL to 31.5 ng/mL, and on evidence from animal models showing changes in pup lung tissue following exposure to doses as low as 2 mg/kg/day PFOS. However, the limited number of studies and issues with inconsistency across studies raise considerable uncertainty.

Table C-12. Evidence Profile Table for PFOS Respiratory Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.7.1)					⊕⊙⊙ ***Evidence Suggests*** *Primary basis*: Human evidence indicted detrimental respiratory health effects, particularly in children with asthma while animal evidence indicated changes in pup lung tissue following exposure. However, the limited number of studies and issues with imprecision across studies raise considerable uncertainty. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Lung function measures 4 *Medium* confidence studies	Two studies (2/4) observed decreases in forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) in children, with one study reporting significant decreases among asthmatic children. Other studies observed small increases in FEV1/FVC and $FEF_{25\%–75\%}$ at age 4, but the associations were imprecise at age 7.	• *Medium* confidence studies	• *Imprecision* of study findings in children	⊕⊙⊙ *Slight* Several studies of *medium* confidence found evidence for decreases in lung function measures among infants, children, and adolescents, though other *medium* confidence studies did not observe significant effects. Few studies examined obstructive disease effects. Uncertainty remains about respiratory outcomes among adults in occupational settings and in the general population.	
Obstructive disease 1 *Medium* confidence study	One study in infants under 2 years old observed significantly increased odds of low severity obstructive airway disease.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.7.2)					
Histopathology 1 *High* confidence study 3 *Medium* confidence studies	One teratology study in rabbits (1/1) reported a significant increase in the number of fetuses with absent intermediate lung lobes after gestational exposure to the lowest dose of PFOS. This increase was not significant when analyzed by litter and no increase	• *High* and *medium* confidence studies	• *Inconsistency* of findings across species and life stage	⊕⊙⊙ *Slight* Evidence indicates that the developing lung may be affected. Evidence in adults is less convincing as limited findings were observed in adult mice and rats.	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	was observed following exposure to higher doses. Three short-term and subchronic studies in adult male and female mice and rats reported no histopathological effects in the respiratory system after exposure (3/3).				
Organ weight 1 *High* confidence study	One short-term study reported female rats had significantly increased relative lung weight while the absolute weight only increased in one dose group. No change in lung weight was reported in male rats.	• *High* confidence study	• *Limited number* of studies examining outcome		

Notes: $FEF_{25\%–75\%}$ = forced expiratory flow at 25%–75%; FEV1 = forced expiratory volume; FVC = forced vital capacity.

C.8 Musculoskeletal

EPA identified six epidemiological and one animal studies that investigated the association between PFOS and musculoskeletal effects. Of the epidemiological studies, six were classified as *medium* confidence and two as *low* confidence (Section C.8.1). The animal study was classified as *low* confidence (Section C.8.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.8.1 Human Evidence Study Quality Evaluation and Synthesis

C.8.1.1 Introduction

Musculoskeletal health outcomes include bone mineral density, risk of bone fractures, and risk of osteoarthritis. Osteoporosis (characterized by weak, brittle bone) and osteoarthritis disproportionately affect women, older individuals, and certain racial/ethnic groups (Khalil et al., 2016; Uhl et al., 2013).

The 2016 PFOS HESD (U.S. EPA, 2016c) did not previously evaluate musculoskeletal health outcomes in humans.

For this updated review, eight studies (eight publications) examined the association between PFOS exposure and musculoskeletal health outcomes. All studies were in the general population. Different study designs were used, including cross-sectional, prospective cohort, and one clinical trial (Hu et al., 2019). All studies measured PFOS in blood components (i.e., blood, plasma, or serum), and one study (Di Nisio et al., 2019) measured PFOS in semen. Three studies (Khalil et al., 2016; Lin et al., 2014; Uhl et al., 2013) used data from participants in the NHANES, but the study years and outcomes examined in these studies did not overlap. Other studies used data from various cohorts for cross-sectional analyses, including Project Viva (Cluett et al., 2019), the POUNDS Lost clinical trial (Hu et al., 2019), and the ALSPAC (Jeddy et al., 2018). The studies were conducted in different populations, including participants from England, Italy, and the United States. The specific outcomes investigated were osteoporosis; osteoarthritis; bone area, mineral content, mineral density, thickness (e.g., endosteal and periosteal thickness), or circumference; bone stiffness; ultrasound attenuation and speed of sound; lean body mass; height; arm span; bone fracture; and plasma concentrations of β-C-telopeptides of type I collagen (CTX), a marker for bone turnover.

C.8.1.2 Study Quality

Considerations specific to evaluating the quality of studies on the musculoskeletal system relate to the causal pathways for PFOS to alter musculoskeletal development. Expectations for musculoskeletal condition should be interpreted relative to participants' age, pubertal and/or menopause status, thyroid hormone levels, and adiposity (BMI), which could likewise be influenced by PFOS exposure (Cluett et al., 2019; Jeddy et al., 2018; Khalil et al., 2018; Khalil et al., 2016). Ideally, studies would characterize these factors, adjust models for confounding where appropriate, and capture a range of human life stages with prospective measurement of PFOS exposure relative to health outcomes. The outcomes should be well-defined and validated by

biometric testing, a physician diagnosis, or medical records where possible. An exception may be acute traumatic injuries such as fractures, which are less likely to be subject to recall bias.

There are eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and musculoskeletal effects. Study quality evaluations for these eight studies are shown in Figure C-40.

On the basis of the considerations mentioned, six studies were classified as *medium* confidence and two as *low* confidence. The two cross-sectional studies (Di Nisio et al., 2019; Khalil et al., 2018) classified as *low* confidence had deficiencies in participant selection, confounding, and study sensitivity. Participant selection was considered a deficiency mainly due to underreporting about participation rates and participant characteristics. Other deficiencies included potential for residual confounding by SES, small sample sizes and limited ranges of participant exposure to PFOS.

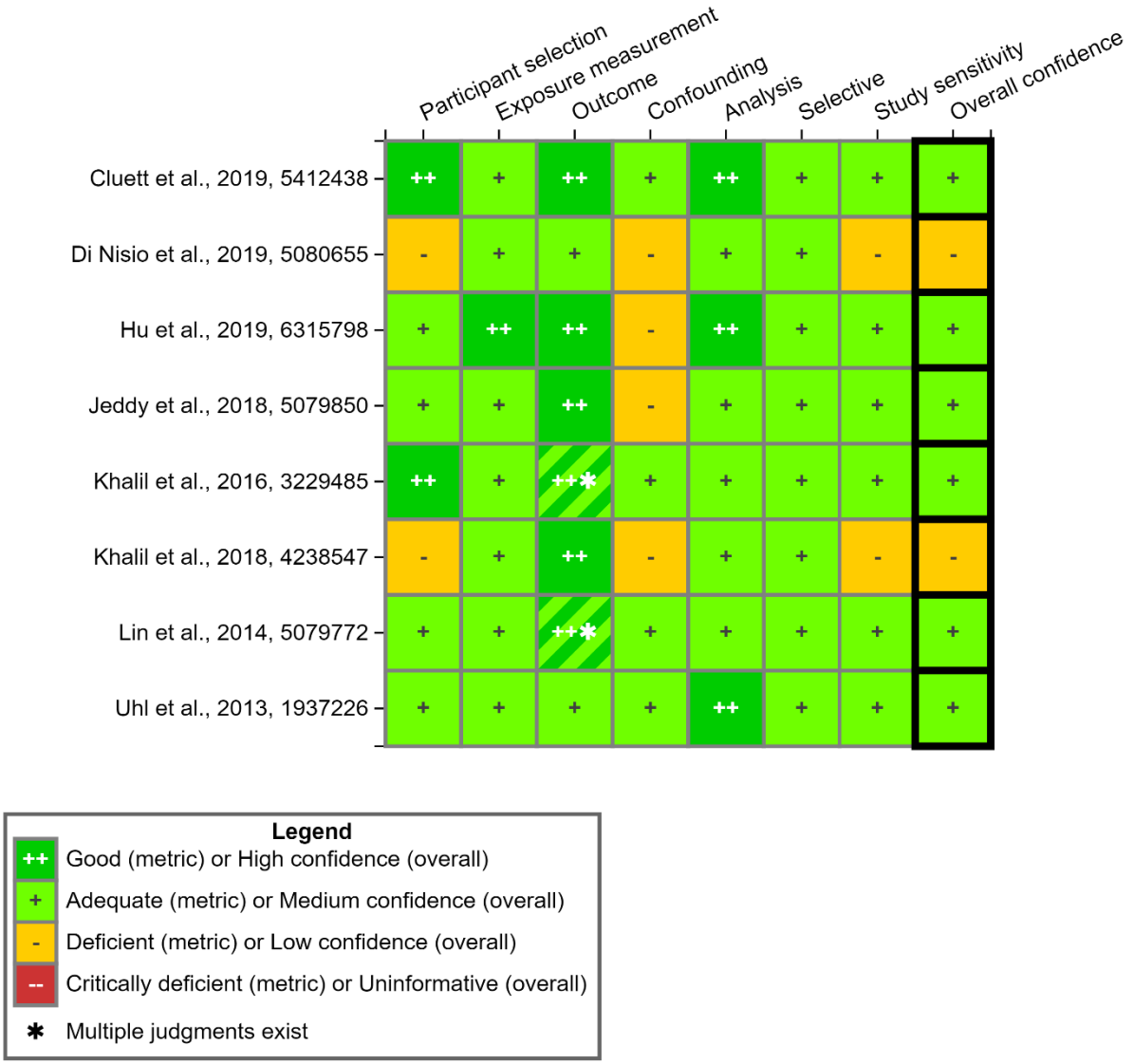


Figure C-40. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

C.8.1.3 Findings From Children and Adolescents

Three studies (Cluett et al., 2019; Jeddy et al., 2018; Khalil et al., 2018) examined musculoskeletal outcomes in children and adolescents, and two observed effects. While the *medium* confidence studies observed few statistically significant associations between PFOS and musculoskeletal health outcomes, the associations consistently supported a harmful, rather than beneficial, direction of effect (Appendix D). Cluett et al. (2019) observed a statistically significant inverse association with areal bone mineral density (aBMD) z-score (a standardized measure of bone mineral amount relative to bone area) in children aged 6–10 years. The sex-stratified results were not statistically significant. Inverse non-significant associations were also observed with a bone mineral density (BMD) in boys and in girls with bone mineral content

(BMC) z-score. Jeddy et al. (2018) identified a statistically significant inverse association between prenatal PFOS exposure and total lean body mass and height in 17-year-old girls. The same study initially showed inverse associations between PFOS exposure and BMC or bone area, but these were not statistically significant after adjusting for participant height.

A *low* confidence study in 8–12-year-old children from a hospital lipids clinic in Dayton, Ohio, (Khalil et al., 2018) observed non-significant inverse associations with bone stiffness index, broadband ultrasound attenuation, or speed of sound.

None of the studies identified in this updated review examined musculoskeletal outcomes in pregnant women and infants.

C.8.1.4 Findings From the General Adult Population

Five studies (Di Nisio et al., 2019; Hu et al., 2019; Khalil et al., 2016; Lin et al., 2014; Uhl et al., 2013) examined musculoskeletal outcomes in adults in the general population and three observed effects (Appendix D).

The four *medium* confidence studies observed a small number of statistically significant associations but a consistently harmful direction of effect. The same outcomes were not examined by multiple studies. Uhl et al. (2013) observed higher odds of osteoarthritis with increased PFOS exposure only in women aged 20–84 from NHANES (2003–2008), who may have differing susceptibility to endocrine disruption. Significant associations were observed only by younger women aged 20–49. In an overlapping NHANES study (Lin et al., 2014), observed decreased total lumbar spine BMD only among younger women not in menopause; no statistically significant association with a history of bone fractures were observed in women aged 20 or older. Khalil et al.(2016) observed a statistically significant inverse association with BMD of the total femur or femoral neck in women aged 12–80 years from NHANES (2009–2010). The same was true for the femoral neck only in males aged 12–80 years. In adults aged 30–70 years from the POUNDS Lost study, Hu et al.(2019) observed small but statistically significant inverse associations with BMD (or two-year change in BMD) in three of the six sites examined: the spine, total hip, and hip intertrochanteric area.

A low confidence study in young men (18–24 years) from the Padova area of northeastern Italy (Di Nisio et al., 2019) did not find evidence of associations between PFOS exposure and arm span.

C.8.2 Animal Evidence Study Quality Evaluation and Synthesis

There is one study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and musculoskeletal effects. Study quality evaluation for this one study is shown in Figure C-41.

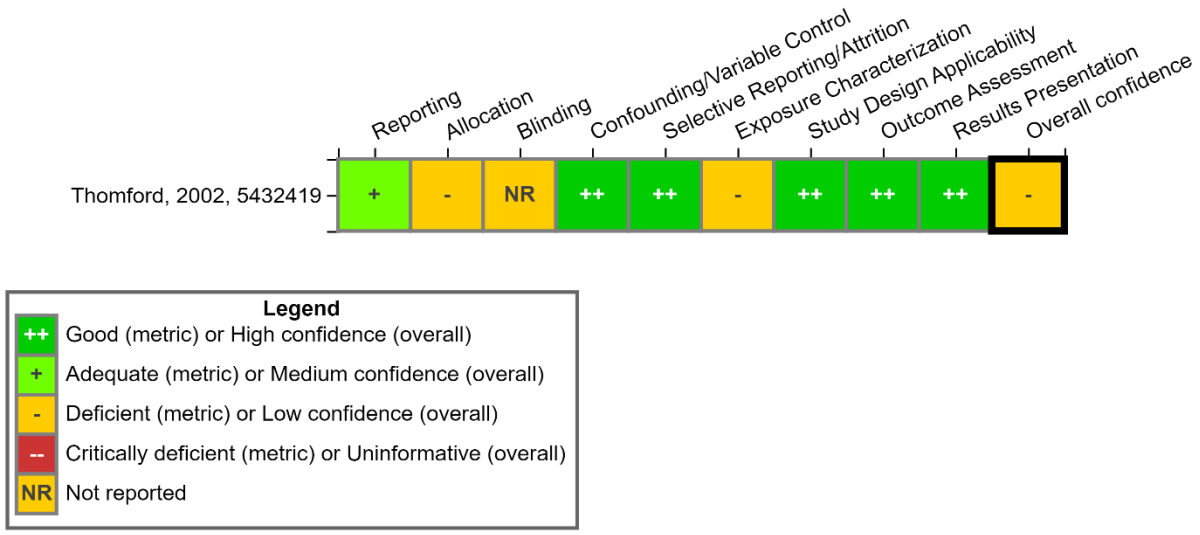


Figure C-41. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

Limited data are available on the effect of PFOS on the musculoskeletal system other than developmental skeletal defects resulting from gestational exposure (see Toxicity Assessment, (U.S. EPA, 2024)). EPA did not identify any publications that reported musculoskeletal effects outside of those associated with developmental toxicity from the 2016 PFOS HESD (U.S. EPA, 2016c) or the recent literature searches that were PECO relevant and determined to be *medium* or *high* confidence rating during study quality evaluation.

C.8.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse musculoskeletal outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There are six studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to musculoskeletal effects. A summary of these studies is shown in Figure C-42. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOS leads to musculoskeletal effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	2	2
Cell Growth, Differentiation, Proliferation, Or Viability	0	4	4
Cell Signaling Or Signal Transduction	1	3	4
Extracellular Matrix Or Molecules	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	1	1
Hormone Function	1	0	1
Other	1	0	1
Grand Total	2	4	6

Figure C-42. Summary of Mechanistic Studies of PFOS and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

C.8.4 Evidence Integration

There is *slight* evidence of an association between PFOS exposure and musculoskeletal effects in humans based on observed effects on BMD and bone health in a limited number of *medium* confidence studies. Limited evidence from individual studies supported possible negative effects of PFOS on skeletal size (height), lean body mass, and connective tissue disorders (osteoarthritis). No musculoskeletal health outcome epidemiologic studies were previously reviewed in the 2016 PFOS HESD (U.S. EPA, 2016c).

Although relatively few studies have investigated musculoskeletal health outcomes related to PFOS exposure, some shared conclusions can be drawn. This review observed evidence of statistically significant associations in about 13% of all tests conducted. The observed associations were primarily between increased PFOS exposure and decreased BMD (inconsistently among various skeletal sites), height and lean body mass in adolescence, and osteoarthritis. These issues with bone density may correspond with the reports of reduced ossification and skeletal deformities in developmental animal models with gestational PFOS exposure (see Toxicity Assessment, (U.S. EPA, 2024)). Arm span measures in adolescents were not associated with PFOS exposure. More severe clinical outcomes, such as fracture, were not observed to be associated with PFOS exposure. No evidence supported beneficial musculoskeletal effects of PFOS exposure. In general, links to musculoskeletal disease were more commonly observed among older women. Some outcomes, such as osteoporosis and osteoarthritis, may be more relevant to examine in females, due to greater prevalence and potentially greater susceptibility to endocrine-disrupting chemicals. Study limitations have somewhat reduced the confidence of most studies; common issues included cross-sectional design or potential for residual confounding.

The animal evidence for an association between PFOS and effects in the musculoskeletal system is considered *indeterminate* based on lack of information in animal models.

C.8.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOS exposure has the potential to cause musculoskeletal effects in humans under relevant exposure circumstances (Table C-13). This conclusion is based primarily on effects on BMD and bone health observed in studies in humans exposed to median PFOS ranging from 6.4 ng/mL to 32.2 ng/mL. Although there is some evidence of negative effects of PFOS exposure on skeletal size (height and arm span) and connective tissue disorders (osteoarthritis, especially in older women), there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-13. Evidence Profile Table for PFOS Musculoskeletal Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.8.1)					⊕⊙⊙ ***Evidence Suggests***
Bone parameters 5 *Medium* confidence studies 1 *Low* confidence study	Decreases in BMC were observed in two studies (2/6), with significant decreases observed among female children. Reductions in BMD were also observed in children and adults (4/6), including site specific BMD measures. Significant decreases in BMD were also observed in analyses stratified by sex. Decreases in other measures of bone health, such as the stiffness index, bone area, and broadband ultrasound attenuation, were observed in children.	• *Medium* confidence studies • *Consistency* of BMD reduction findings across three *medium* studies	• *Imprecision* of findings across exposure groups and studies • *Low* confidence study	⊕⊙⊙ *Slight* Evidence for musculoskeletal effects is based on studies reporting reductions in bone health, bone density, lean body mass, and increased odds of osteoarthritis. Uncertainties remain due to inconsistent or imprecise results, and limited evidence for fractures, size measures, and odds of osteoarthritis or osteoporosis.	*Primary basis*: No animal evidence and human evidence indicated effects on BMD and bone health. Although there is some evidence of negative effects of PFOS exposure on skeletal size (height and arm span) and connective tissue disorders (osteoarthritis, especially in older women), there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Fractures 1 *Medium* confidence study	Findings regarding incidence of fractures in adults ages 20 yr or older were largely imprecise.	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome		
Size measures 1 *Medium* confidence study 1 *Low* confidence study	One study reported significantly decreased height in girls at age 17 (1/2). Findings for arm	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	span were largely imprecise in a study on male high school students.		• *Low* confidence study		
Lean body mass 1 *Medium* confidence study	One study found a significant reduction of total lean body mass in girls at age 17.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Osteoarthritis 1 *Medium* confidence study	Odds of osteoarthritis among adults aged 20–84 and among females aged 20–49 were significantly increased.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Osteoporosis 1 *Medium* confidence study	Findings for osteoporosis in women aged 12–80 were largely imprecise.	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome		

Notes: BMC = bone mineral content; BMD = bone mineral density; yr = years.

C.9 Gastrointestinal

EPA identified four epidemiological and two animal studies that investigated the association between PFOS and gastrointestinal effects. Of the epidemiological studies, three were classified as *medium* confidence and one as *low* confidence (Section C.9.1). Of the animal studies, one was classified as *high* confidence, and one was considered *low* confidence (Section C.9.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.9.1 Human Evidence Study Quality Evaluation and Synthesis

C.9.1.1 Introduction

GI health outcomes were not previously evaluated in the 2016 PFOS HESD, although gastroenteritis frequency was considered as a marker of immune system function. Causation of gastroenteritis cases may be difficult to disentangle, as underlying susceptibility varies, and the infectious agent or irritant is rarely confirmed. Granum et al. (2013) did not observe a statistically significant association between prenatal PFOS exposure and the frequency of gastroenteritis episodes in a child's first three years of life, as they did for PFOA (Granum et al., 2013).

PFOS exposure may affect GI health by altering molecular processes (such as those involved in inflammation), gut mucosa integrity (by acting as surfactants) and intestinal permeability, gut microbiota, and/or systemic susceptibility to infection (Xu et al., 2020d; Steenland et al., 2018). GI outcomes only assessed in the context of immune system health, including ulcerative colitis and Crohn's disease, are discussed (see Toxicity Assessment, (U.S. EPA, 2024)). However, some research suggests an overall immunosuppressive effect of PFOS could reduce the efficiency of routine childhood immunizations (Dalsager et al., 2016) which might include that for rotavirus, a common childhood cause of diarrhea and vomiting. In addition, inflammatory bowel disease (IBD), or the chronic inflammation of the GI tract in response to environmental triggers, can be considered an immune dysregulation response occurring in genetically susceptible individuals (Hammer et al., 2019).

For this updated review, four studies examined the association between PFOS and GI health outcomes. The specific outcomes investigated were diarrhea, vomiting, IBD, and IBD biomarkers (zonulin and calprotectin). PFOS was measured in serum or blood

Dalsager et al. (2016) used data from the ongoing, prospective OCC, a group of pregnant women recruited 2010–2012 and their children living in the Odense area of Denmark. Hammer et al. (2019) examined participants in the Children's Health and the Environment in the Faroes (CHEF) cohort, which enrolled mother-child pairs, the children's fathers and grandparents, and young men from the Faroe Islands hospital system between 1986 and 2009. Xu et al. (2020d) examined child and adult participants from the Ronneby, Sweden exposed to PFAS in drinking water), and unexposed individuals from a nearby town. Timmermann et al. (2020) examined a subset of 4–18-month-old children from a randomized controlled trial of early measles vaccination, conducted in Guinea-Bissau in West Africa from 2012 to 2015.

C.9.1.2 Study Quality

Several considerations were specific to evaluating the quality of the studies of GI symptoms. For example, fever or a stool test might help to confirm that diarrhea and vomiting are attributable to infection, as opposed to a chronic underlying condition or other chemical or dietary irritant. Medical diagnoses are preferred to self-reported symptoms, although knowledge of GI disorders has developed substantially over recent decades and diagnostic indicators continue to rapidly evolve. Causal factors in developing GI conditions have likewise shifted over time, such as changes in emerging contaminants, hygiene, the gut microbiome, activity and stress levels, and dietary trends. These underlying trends may affect cohort studies with extended recruitment or follow-up periods. Reverse causation is possible if GI conditions lead to increased intake of PFOS from food packaging or preparation methods, increased PFOS absorption through the GI tract, or reduced fecal excretion (Xu et al., 2020d). Measuring PFOS and GI outcomes concurrently was considered adequate in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.5 years) (Li et al., 2018), current blood concentrations are expected to correlate well with past exposures.

There are four studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and gastrointestinal effects. Study quality evaluations for these four studies are shown in Figure C-43.

On the basis of the considerations mentioned, one study was considered *medium* confidence (Timmermann et al., 2020) and three as *low* confidence (Xu et al., 2020d; Hammer et al., 2019; Dalsager et al., 2016). The *medium* confidence study (Timmermann et al., 2020) relied on retrospective reporting of GI outcomes, which is subject to recall bias, and did not detail the interview question used. Study sensitivity was also limited by small case numbers and relatively low PFOS exposure levels. However, the concerns were considered relatively minor and likely to minimally impact interpretation of the results.

Concerns in the *low* confidence studies included potential for selection bias, including using unclear recruitment methods and, a convenience sample (Xu et al., 2020d). Another concern was potential for outcome misclassification or underreporting due to inconsistent participation and adherence to the parent reporting mechanism (Dalsager et al., 2016). Another common reason for *low* confidence was a serious risk for residual confounding by SES (Hammer et al., 2019). Exposure misclassification was also a concern in Xu et al. (2020d), due to use of residential history as a proxy. Deficiencies in multiple domains contributed to an overall *low* confidence rating.

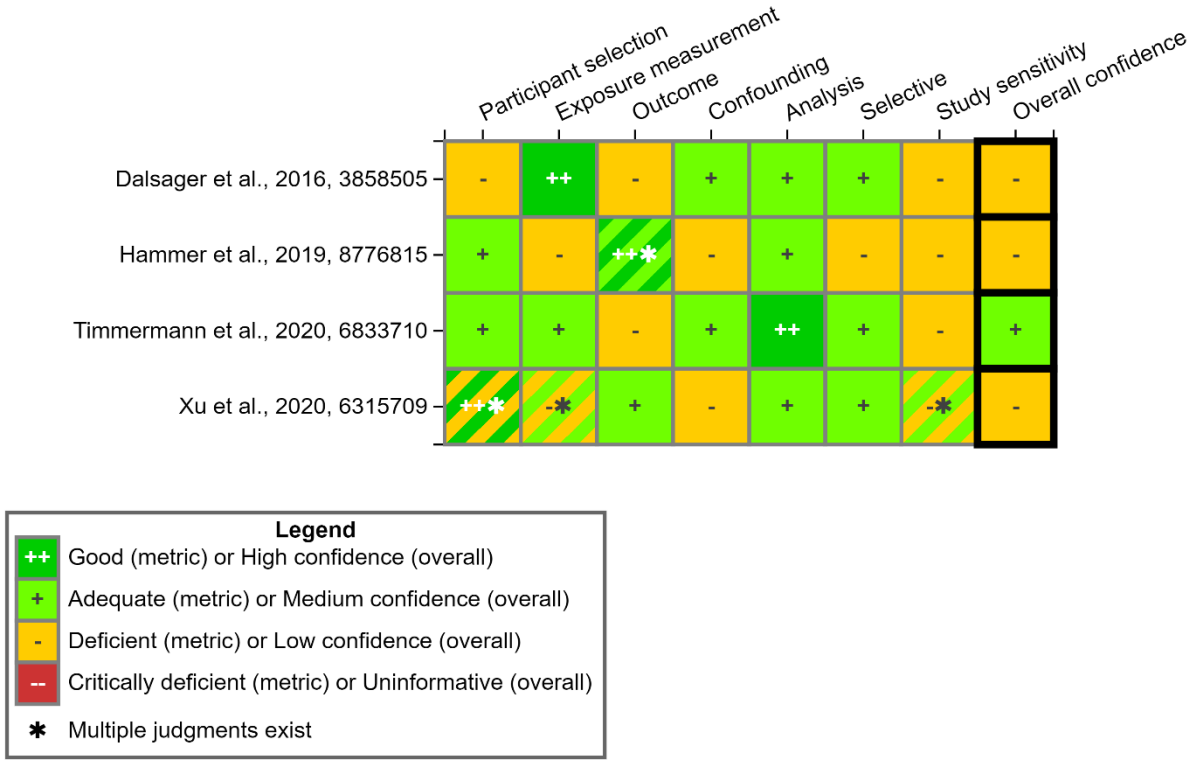


Figure C-43. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

C.9.1.3 Findings

Both studies examining diarrhea observed non-significant increased association with PFOS (Appendix D). Timmermann et al. (2020) observed increased odds of diarrhea in very young children (up to 9 months old) in Guinea-Bissau. Dalsager et al. (2016) observed non-significant increased incidence and inconsistent odds of diarrhea; similar inconsistent associations were observed for vomiting when comparing exposure tertiles to the referent one in 1–4-year-old children in Denmark.

Both studies examining IBD observed no associations with PFOS. Hammer et al. (2019) observed a non-significant decrease in incidence of IBD in Faroese children and adults. Xu et al. (2020d) observed non-significant decreases in levels of IBD biomarkers calprotectin or zonulin in children and adults from Sweden.

C.9.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association

between PFOS and gastrointestinal effects. Study quality evaluations for these two studies are shown in Figure C-44.

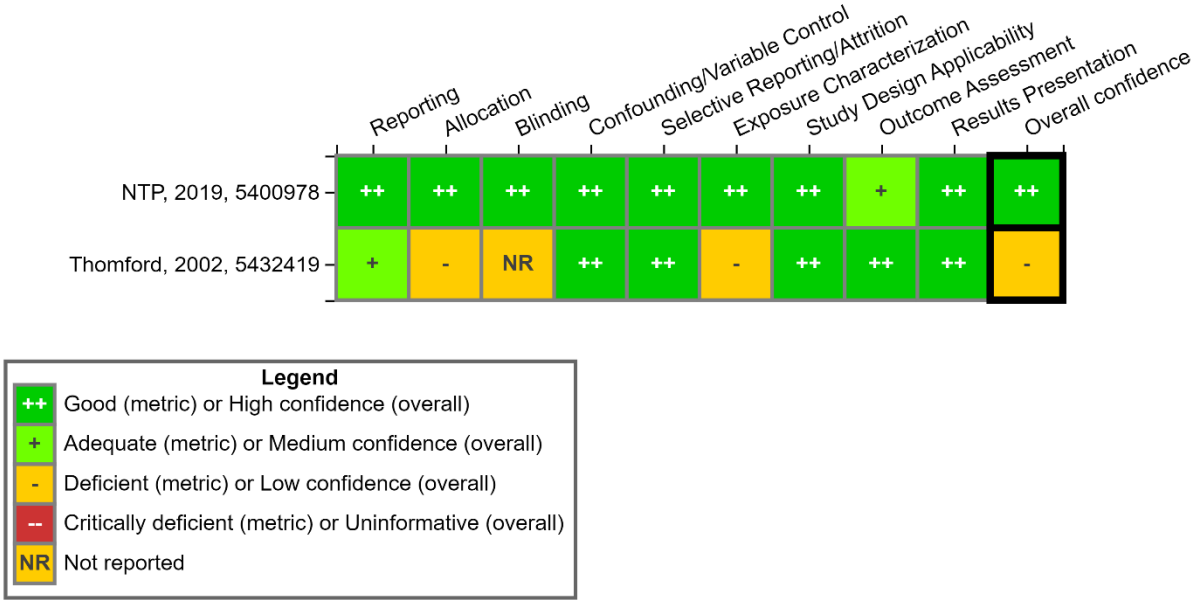


Figure C-44. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

Studies on the GI effects of PFOS exposure are limited. In a study conducted by NTP (2019), male and female Sprague-Dawley rats were orally administered 0 mg/kg/day, 0.312 mg/kg/day, 0.625 mg/kg/day, 1.25 mg/kg/day, 2.5 mg/kg/day, or 5 mg/kg/day PFOS for 28 days. Animals treated at 0 or 5 mg/kg/day showed no effects in the forestomach, glandular stomach, intestines, pancreas, or salivary gland during histopathological examination (NTP, 2019).

The 2016 PFOS HESD identified an acute study in which male and female CD rats were gavaged with a single dose of 0 mg/kg, 100 mg/kg, 215 mg/kg, 464 mg/kg, or 1,000 mg/kg of PFOS suspended in a 20% acetone/80% corn oil mixture. Rats were observed for abnormal signs for 4 hours after exposure and then daily for up to 14 days. All rats died in the 464 mg/kg and 1,000 mg/kg groups, and 3/10 rats died in the 215 mg/kg group. Necropsy results indicated stomach distension and irritation of the glandular mucosa. According to the findings, the acute oral LD_{50} was 233 mg/kg in males, 271 mg/kg in females, and 251 mg/kg combined (Dean et al., 1978).

The 2016 PFOS HESD also identified a sub-acute study in rhesus monkeys in which Goldenthal et al. (1979) exposed two rhesus monkeys/sex/dose to 0 mg/kg/day, 0.5 mg/kg/day, 1.5 mg/kg/day, or 4.5 mg/kg/day of PFOS in distilled water by gavage for 90 days. All monkeys in the 4.5 mg/kg/day group died or were euthanized in extremis by week 7 and exhibited signs of GI tract toxicity (anorexia, emesis, black stool) (Goldenthal et al., 1979).

C.9.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse GI outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There are 10 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to GI effects. A summary of these studies is shown in Figure C-45. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOS leads to GI effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	2	2
Extracellular Matrix Or Molecules	1	0	0	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	0	0	4
Inflammation And Immune Response	1	0	2	2
Other	5	1	1	7
Grand Total	7	1	3	10

Figure C-45. Summary of Mechanistic Studies of PFOS and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

C.9.4 Evidence Integration

The evidence evaluating an association between PFOS exposure and GI effects in humans is *indeterminate* due to the limited number of studies available for evaluation and the methodological shortcomings of those studies. In the 2016 PFOS HESD, GI outcomes in humans were only assessed in the context of immune system health. Evidence is limited due to a paucity of research and the quality of the available studies. The available research has not demonstrated conclusive effects of PFOS on GI effects including vomiting, or diarrhea.

The animal evidence for an association between PFOS exposure and GI effects is *indeterminate* based on the limited data available. The few studies that demonstrated GI effects in animal models appeared to only observe effects in moribund or deceased individuals.

C.9.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOS exposure can cause GI effects in humans under relevant exposure circumstances (Table C-14).

Table C-14. Evidence Profile Table for PFOS Gastrointestinal Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.9.1)					⊙⊙⊙ ***Inadequate Evidence*** *Primary basis:* Evidence in humans and animals are largely non-significant. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Diarrhea and vomiting 1 *Medium* confidence study 1 *Low* confidence study	Two studies observed modest, non-significant positive associations for diarrhea in children under 4 yr of age. One study observed inconsistent non-significant associations with vomiting across exposure tertiles in children ages 1–4 yr. No studies were conducted in adults.	• *Medium* confidence study	• *Low* confidence study • *Inconsistent direction* of effects across exposure levels and endpoints • *Limited number* of studies examining outcome • *Imprecision* of findings • Potential outcome misclassification or underreporting due to inconsistent parental participation	⊙⊙⊙ *Indeterminate* Evidence for gastrointestinal effects is based on one study observing a modest, non-significant association for diarrhea and vomiting in children under 4 yr of age. Considerable uncertainty due to limited number of studies and unexplained inconsistency across exposure levels and endpoints.	
Inflammatory bowel disease 2 *Low* confidence studies	One study in children and adults observed a modest, non-significant negative association for IBD incidence. One community-based study observed no clear associations for IBD biomarkers calprotectin and zonulin.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining outcome • *Imprecision* of findings • Potential for residual confounding by socioeconomic status and decreased study sensitivity		
Evidence From In Vivo Animal Studies (Section C.9.2)					
Histopathology 1 *High* confidence study	No changes in forestomach, glandular stomach, intestines, pancreas, or salivary gland histopathology in one 28-day study in male and female rats.	• *High* confidence study	• *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited to one study reporting no findings of gastrointestinal toxicity.	

Notes: IBD = inflammatory bowel disease; yr = years.

C.10 Dental

EPA identified two epidemiological studies that investigated the association between PFOS and dental effects. No animal studies were identified. The two epidemiological studies were both classified as *medium* confidence (Section C.10.1). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.10.1 Human Evidence Study Quality Evaluation and Synthesis

C.10.1.1 Introduction

PFOS exposure could potentially adversely affect both dentin and bone mineralization, skeletal formation, thyroid hormones that stimulate tooth maturation and enamel sufficiency, and immune responses to cariogenic bacteria (Puttige Ramesh et al., 2019). At a molecular level, PFAS such as PFOS may influence tooth growth and development via activation of peroxisome proliferator-activated receptor alpha, initiation of oxidative stress, altering gene expression in the vascular endothelial growth factor signaling pathway for gastric cells, hemoprotein binding, estrogen disruption, or disruption of carbonic anhydrase (needed for enamel development) (Wiener and Waters, 2019).

For this updated review, two studies examined the association between PFOS exposure and dental caries (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). The dental caries effect was defined as presence of decay or a restoration on any tooth surface or the loss of a tooth following tooth decay, excluding third molars (Puttige Ramesh et al., 2019). Trained dentists performed visual and tactile exams using appropriate tools, but X-rays were not taken. No other dental health outcomes were evaluated.

The two cross-sectional studies used data from the NHANES: Puttige Ramesh et al. (Puttige Ramesh et al., 2019) assessed data from 2,869 12–19-year-old adolescents included in 1999–2012 NHANES and Wiener and Waters (2019) examined data from 639 children ages 3–11 years in the 2013–2014 NHANES cycle. Therefore, no participant overlap is expected between these studies. Exposure to PFOS was assessed via biomarkers in blood.

C.10.1.2 Study Quality

Important considerations specific to evaluating the quality of studies on dental outcomes relate to the difficulty of characterizing risk factors for dental caries, such as diet and oral hygiene practices. Self-reported frequency of brushing, fluoridated product use, and dental visits may be useful indicators. Fluoride levels in local public drinking water supplies are also thought to influence development of dental caries and tap water consumption habits differ among households and individuals (Wiener and Waters, 2019). Measuring PFOS and dental outcomes concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.5 years) (Li et al., 2018), current blood concentrations are expected to correlate well with past exposures.

There are two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association

between PFOS and dental effects. Study quality evaluations for these two studies are shown in Figure C-46.

On the basis of the considerations mentioned, the two included studies were considered *medium* confidence, wherein limitations were not expected to severely affect results interpretation. Limitations included cross-sectional study design, which introduces some concern about whether the exposure preceded the outcome or vice versa (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). Puttige Ramesh et al. (2019) was primarily limited by participant selection, since NHANES data necessarily excluded participants who were unable or unwilling to submit to a dental examination. This could have resulted in selection bias against individuals with the most severe tooth decay. Dental examinations were performed on all NHANES participants aged 2+ who did not have orofacial pain, specific medical conditions, physical limitations, inability to comply, or were uncooperative.

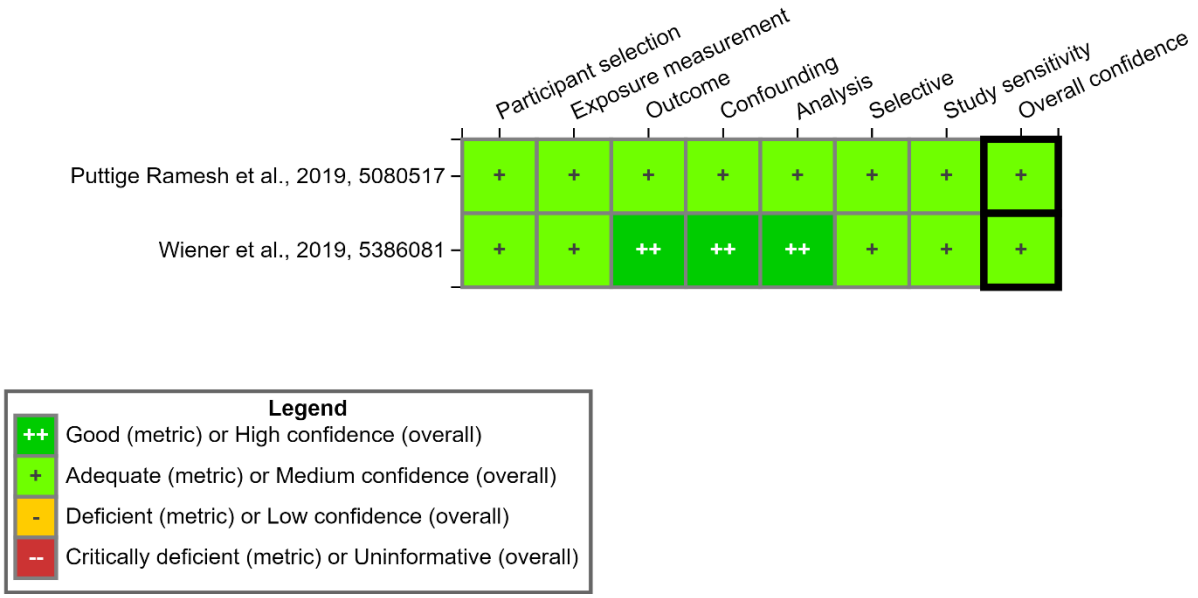


Figure C-46. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Dental Effects

Interactive figure and additional study details available on HAWC.

C.10.1.3 Findings

The two studies observed mixed effects (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). Wiener and Waters (2019) observed borderline significant increased odds of dental caries with increased PFOS exposure in children (OR: 1.41; 95% CI: 0.97, 2.05; p-value = 0.069). The analysis adjusted for age, sex, race/ethnicity, ratio of family-income-to-poverty guidelines, tooth brushing frequency, fluoride in water, percentage of sugar in the diet, and dental visits. Puttige Ramesh et al. (2019) observed increased odds of dental caries only in the third quartile of exposure, but decreased odds in the second and highest quartiles compared with the lowest, and per doubling of PFOS. Analyses did not account for age, but considered gender, race, education level of parent/guardian, family-poverty-to-income ratio, blood lead level, and serum cotinine level (an indicator of exposure to smoking). No studies of dental health outcomes were available for pregnant women, adults, or occupational workers (Appendix D).

C.10.2 Animal Evidence Study Quality Evaluation and Synthesis

In the available literature, there is no reported biological consequence of PFOS exposure on dental outcomes in animals.

C.10.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse dental outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There are no studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to dental effects. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOS may cause dental effects.

C.10.4 Evidence Integration

The evidence evaluating an association between PFOS exposure and dental effects in humans is *indeterminate* based on the limited number of available studies. Dental health outcomes were not previously reviewed in the 2016 PFOS HESD (U.S. EPA, 2016c). The present review was limited by the availability of only two studies. Only one outcome was examined (prevalence of dental caries), and while both studies observed increased odds of dental carries, the associations were non-significant (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). These studies have exposure levels typical in the general population, large sample sizes and low risk of bias.

The animal evidence for an association between PFOS exposure and dental effects is *indeterminate* because there are no available studies in animal models examining the effects of PFOS exposure on dental outcomes.

C.10.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOS exposure can cause dental effects in humans under relevant exposure circumstances (Table C-15).

Table C-15. Evidence Profile Table for PFOS Dental Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.10.1)					⊙⊙⊙ ***Inadequate Evidence***
Dental caries 2 *Medium* confidence studies	Two studies observed non-significant increases and decreases in odds of dental caries. No significant associations observed in studies of children and adolescents from NHANES.	• *Medium* confidence studies	• *Inconsistent direction* of effects across studies and across exposure levels • *Limited number* of studies examining outcome • *Imprecision* of findings	⊙⊙⊙ *Indeterminate* Evidence was limited to two studies that reported non-significant positive associations to dental caries in children and adolescents, but results are imprecise. Uncertainty remains regarding adults and other age groups from the general population.	*Primary basis*: No evidence in animals and evidence in humans is largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.

Notes: NHANES = National Health and Nutrition Examination Survey; N/A = not applicable.

C.11 Ocular

EPA identified one epidemiological and two animal studies that investigated the association between PFOS and ocular effects. The epidemiological study was classified as *medium* confidence (Section C.11.1). Of the animal studies, one was classified as *high* confidence, and one was considered *low* confidence (Section C.11.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.11.1 Human Evidence Study Quality Evaluation and Synthesis

C.11.1.1 Introduction

For this updated review, there is one epidemiological study that investigated the association between PFOS and ocular effects (Zeeshan et al., 2020).

This cross-sectional study conducted in Shenyang, China as part of the Isomers of C8 Health Project in China focused on a high-exposed population, including adults aged 20 years and older, who were randomly selected using multistage, stratified cluster sampling. Median total PFOS serum concentrations among the 1,202 study participants were 24.07 ng/mL. Participants were subject to a complete ophthalmic examination which included ocular history, visual acuity, and anterior and posterior segment examinations. Several ocular conditions, reflecting effects on different segments of the eyes, were assessed, including visual impairment (VI), vitreous disorder, synechia, macular disorder, corneal pannus, anterior chamber depth (ACD)-shallow, retinal disorder, lens opacity, and conjunctival disorder.

C.11.1.2 Study Quality

There is one study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and ocular effects. Study quality evaluation for this one study is shown in Figure C-47.

Zeeshan et al. (2020) was classified as *medium* confidence. The main limitation of this study is the cross-sectional design, which does not allow for establishing temporality. Participants' serum samples were collected at study enrollment only and the utilization of a single exposure measurement may not adequately represent exposure variability; additionally, it is unclear whether exposure occurred at an etiologically relevant time period to reflect changes in ocular function.

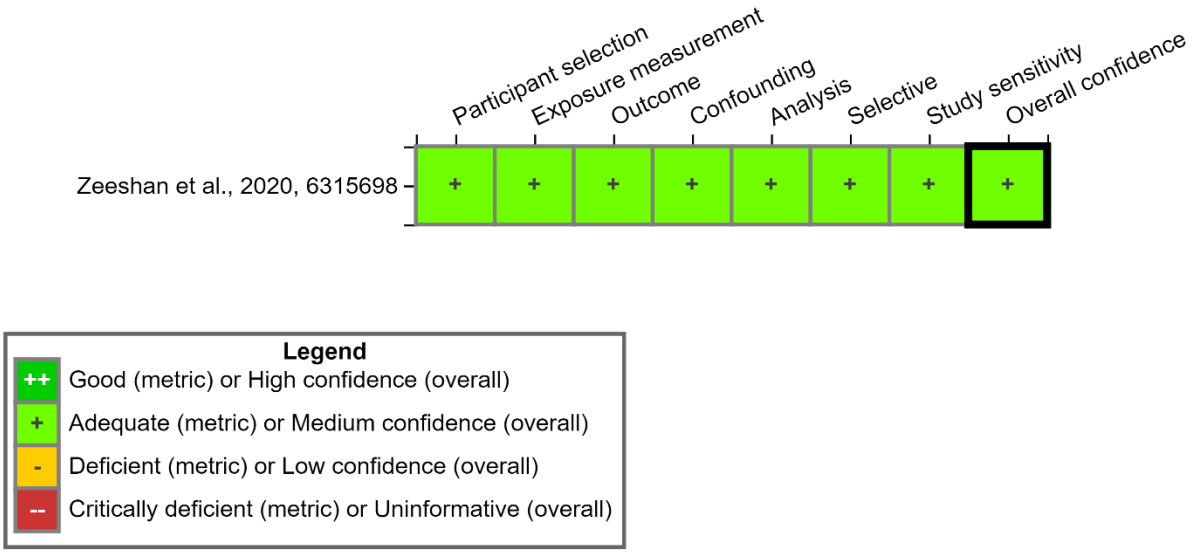


Figure C-47. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Ocular Effects

Interactive figure and additional study details available on HAWC.

C.11.1.3 Findings

Zeeshan et al. (2020) examined the effects of exposure to PFOS in adults aged 22–96 years, who had lived for at least 5 years in in Shenyang, China (Appendix D). Ocular outcomes examined include VI, vitreous disorder, synechia, macular disorder, corneal pannus, and ACD, and combined eye disease (aggregating all ocular conditions examined). A positive statistically significant association between VI and total serum PFOS was observed (OR: 3.11; 95% CI: 2.30, 4.20). When stratified by age, the association between combined eye disease and total serum PFOS was statistically significant for participants aged ≤ 65 years (OR: 1.52; 95%, 1.21, 1.91), but not for the older participants (OR: 0.91; 95% CI: 0.55, 1.51). No other associations were observed.

C.11.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and ocular effects. Study quality evaluations for these two studies are shown in Figure C-48.

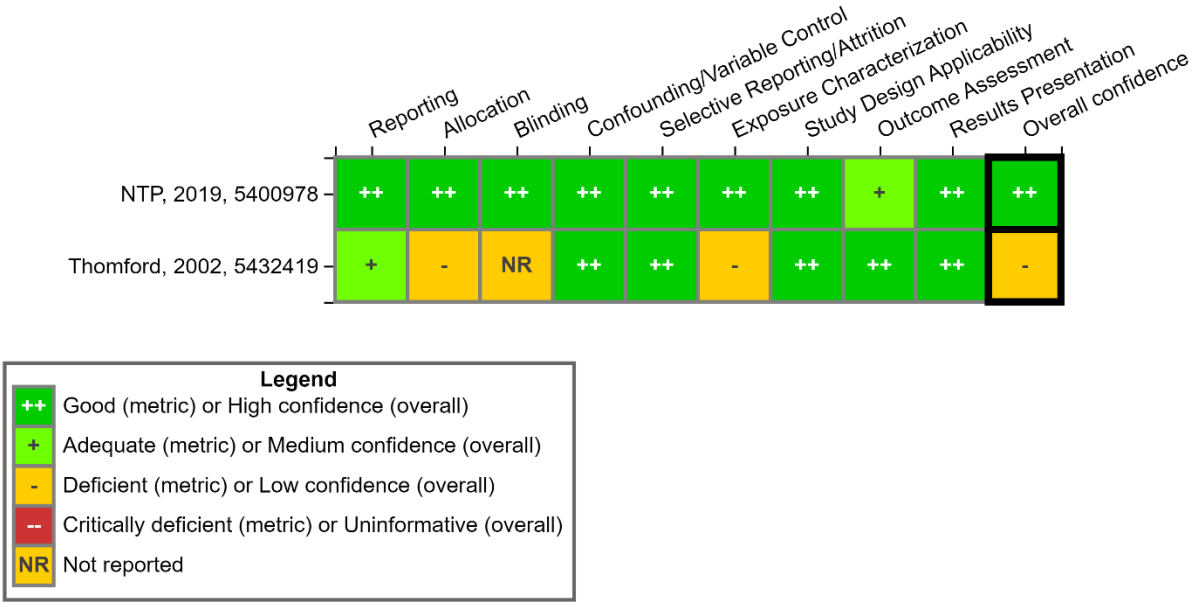


Figure C-48. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Ocular Effects

Interactive figure and additional study details available on HAWC.

An eye irritation study in rabbits suggests that PFOS acts as an ocular irritant (Biesemeier and Harris, 1974); however, in a 28-day oral toxicity study conducted by NTP, no histological abnormalities were noted in male or female Sprague-Dawley rats exposed to 5 mg/kg/day PFOS (NTP, 2019).

C.11.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse ocular outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There is one study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to ocular effects. A summary of these studies is shown in Figure C-49. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOS leads to ocular effects.

Mechanistic Pathway	In Vitro	Grand Total
Atherogenesis And Clot Formation	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	1
Cell Signaling Or Signal Transduction	1	1
Inflammation And Immune Response	1	1
Grand Total	1	1

Figure C-49. Summary of Mechanistic Studies of PFOS and Ocular Effects

Interactive figure and additional study details available on HAWC.

C.11.4 Evidence Integration

The evidence evaluating an association between PFOS exposure and ocular effects in humans is indeterminate due to limited evidence available from epidemiological studies. In the 2016 Health Assessment for PFOS, no epidemiological evidence of an association between PFOS exposure and ocular health effects was examined. One epidemiological study reported an association between PFOS and VI and combined eye disease in humans. However, since only one study was available for review and given its cross-sectional design, existing epidemiological evidence does not allow for a definitive conclusion regarding potential detrimental ocular health effects due to exposure to PFOS.

The association between PFOS and ocular effects is indeterminate due to the limited evidence available in animal models. One available study in an animal model did not report histopathological ocular abnormalities.

C.11.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOS exposure can cause ocular effects in humans under relevant exposure circumstances (Table C-16).

Table C-16. Evidence profile table for PFOS Ocular effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.11.1)					⊙⊙⊙ ***Inadequate Evidence*** *Primary basis*: Evidence in humans is limited and evidence in animals is largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Eye disease 1 *Medium* confidence study	The only study examining eye disease was a cross-sectional study that observed significant positive associations between visual impairment and serum PFOS. The association was also significant for combined eye disease, but only in participants aged ≤65 yr.	• *Medium* confidence study	• *Limited number* of studies examining outcome	*Indeterminate* ⊙⊙⊙ Evidence was limited to one study reporting increases in visual impairment in all ages and increases in combined eye disease in participants aged ≤65 yr.	
Evidence From In Vivo Animal Studies (Section C.11.2)					
Histopathology 1 *High* confidence study	No changes in ocular histopathology were reported in one 28-day study in male and female rats.	• *High* confidence study	• *Limited number* of studies examining outcome	*Indeterminate* ⊙⊙⊙ Evidence was limited to one study reporting no findings of ocular toxicity.	

Notes: yr = years.

C.12 Dermal

EPA identified one epidemiological and two animal studies that investigated the association between PFOS and dermal effects. The epidemiological study was classified as *medium* confidence (Section C.12.1). Of the animal studies, one was classified as *high* confidence, and one was considered *low* confidence (Section C.12.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024)).

C.12.1 Human Evidence Study Quality Evaluation and Synthesis

C.12.1.1 Introduction

For this updated review, one study examined the association between age at the occurrence of acne and PFOS exposure. In the Puberty Cohort, a large sub-cohort of the DNBC in Denmark, Ernst et al. (2019) examined the association between prenatal PFOS exposure and pubertal development. Mother-child pairs were recruited for the DNBC from 1996 to 2002, and eligibility for the Puberty Cohort was determined in 2012. PFAS levels in maternal blood, largely collected during the first trimester of pregnancy, were used to assess prenatal exposure, and age at the occurrence of acne was self-reported by children via bi-annual questionnaire starting in 2012 or at 11 years of age.

C.12.1.2 Study Quality

There is one study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and dermal effects. Study quality evaluation for this one study is shown in Figure C-50.

Ernst et al. (2019) was considered a *medium* confidence study, with no major concerns with the overall quality of the study and any identified concerns were not likely to impact the results. Self-reporting was used to assess the occurrence of acne, a study limitation that could introduce minor bias to the outcome assessment. Additionally, some children were sampled for the Puberty Cohort after the onset of puberty, thus their self-reported outcome information has increased risk of inaccurate recall. However, this was not expected to substantially impact the accuracy of the outcome measures.

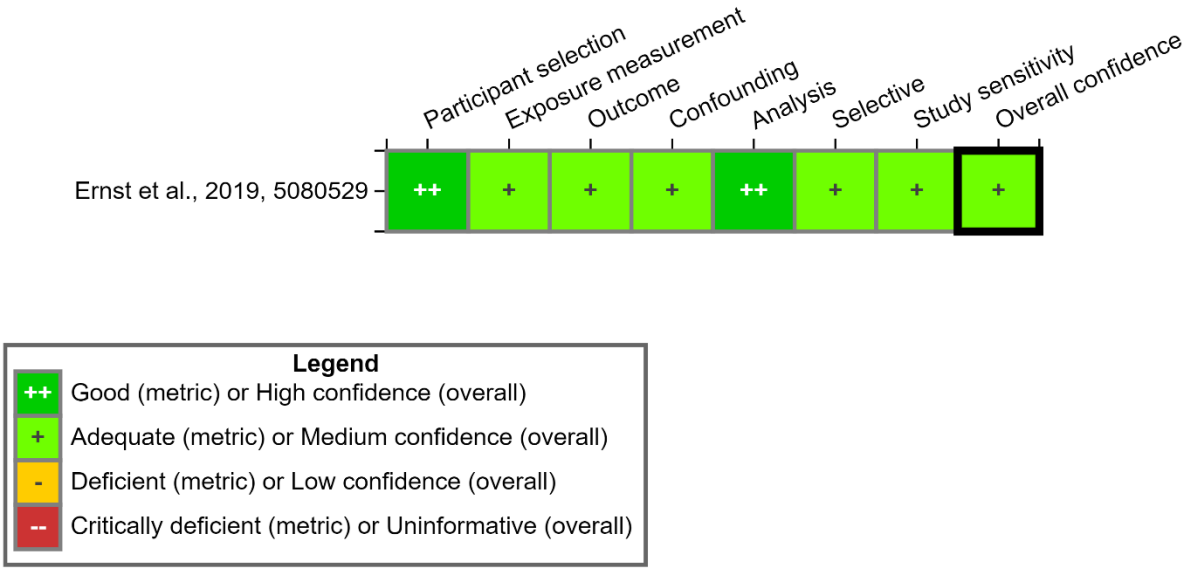


Figure C-50. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Dermal Effects

Interactive figure and additional study details available on HAWC.

C.12.1.3 Findings

Ernst et al. (2019) observed negative non-significant associations between prenatal PFOS exposure and age at the occurrence of acne in both boys and girls. Associations remained negative and non-significant in analyses stratified by tertiles, except for girls in the second tertile of PFOS exposure compared with the lowest (β: 0.09; 95% CI: −4.69, 4.87) (Ernst et al., 2019). Associations in boys were negative and non-significant (Appendix D).

C.12.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016c) that investigated the association between PFOS and dermal effects. Study quality evaluations for these two studies are shown in Figure C-51.

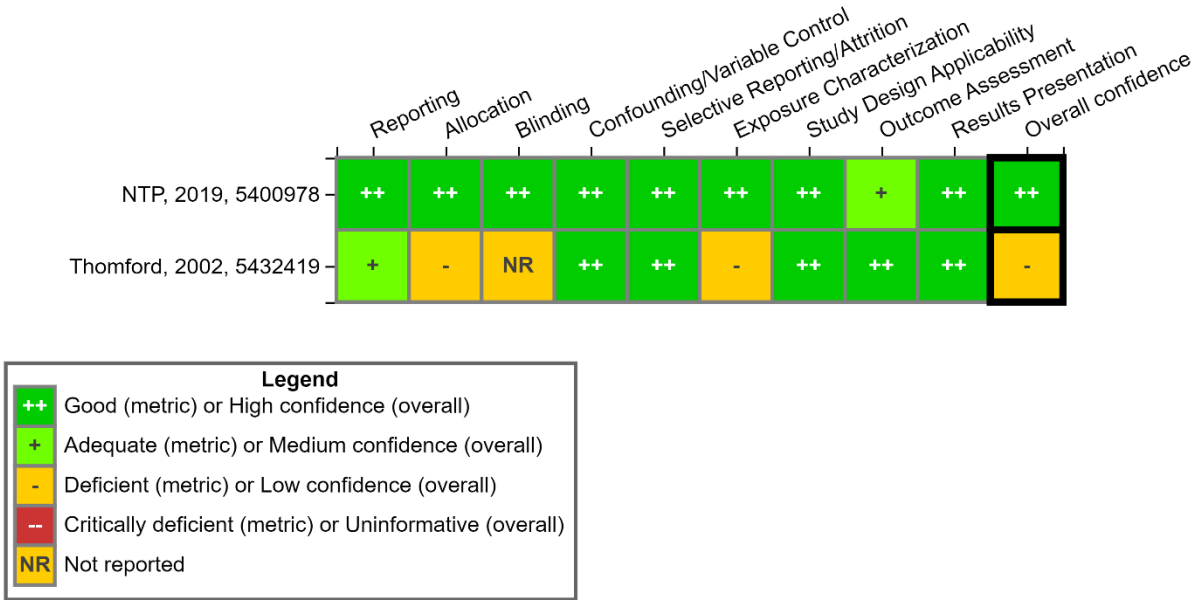


Figure C-51. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Dermal Effects

Interactive figure and additional study details available on HAWC.

There is no evidence in the literature that oral PFOS exposure results in dermal toxicity. In a 28-day oral gavage study in male and female Sprague-Dawley rats with PFOS concentrations up to 5 mg/kg/day, no dermal lesions were observed during histopathological observation (NTP, 2019).

C.12.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse dermal outcomes in the 2016 PFOS HESD (U.S. EPA, 2016c). There are no studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to dermal effects. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOS may cause dermal effects.

C.12.4 Evidence Integration

The evidence evaluating an association between PFOS exposure and dermal effects in humans is *indeterminate* based on the limited number of studies available. In the 2016 PFOS HESD (U.S. EPA, 2016c), the association between oral PFOS exposure and dermal effects was not examined. In this updated review of the epidemiologic literature, one study examined the association between prenatal PFOS exposure and dermal effects during puberty (Ernst et al., 2019) and observed negative non-significant associations in both boys and girls in the study cohort. However, conclusions regarding PFOS exposure and resulting dermal effects are limited by the lack of studies examining the association. Dermal effects beyond acne are not currently represented in the epidemiological literature.

The evidence for potential dermal effects in experimental animals in *indeterminate* and limited to a single *high* confidence study with no dermal lesions observed. In the available literature from animal models, there is no reported biological consequence of oral PFOS exposure on dermal tissue.

C.12.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOS exposure can cause dermal effects in humans under relevant exposure circumstances (Table C-17).

Table C-17. Evidence Profile Table for PFOS Dermal Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section C.12.1)					⊙⊙⊙ ***Inadequate Evidence*** *Primary basis*: Evidence in humans and animals are largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Acne 1 *Medium* confidence study	One study found negative non-significant associations with age of acne onset among adolescent girls and boys.	• *Medium* confidence study	• *Limited number* of studies examining outcome • *Imprecision* of findings	⊙⊙⊙ *Indeterminate* Evidence was limited to one study reporting non-significant associations.	
Evidence From In Vivo Animal Studies (Section C.12.2)					
Histopathology 1 *High* confidence study	No changes in skin histopathology were reported in one 28-day study in male and female rats.	• *High* confidence study	• *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited to one study reporting no findings of dermal toxicity.	

Appendix D. Detailed Information from Epidemiology Studies

D.1 Developmental

Table D-1. Associations Between PFOS Exposure and Developmental Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Ashley-Martin et al. (2017) *High*	Canada, 2008–2011	Cohort	Pregnant women (enrolled if <14 wk gestation, ≥18 yr of age) and their infants at recruitment and from MIREC N = 1,509	Maternal blood Early pregnancy 4.6 (3.2–6.8)	BW (z-score): adequate, inadequate, and excess weight gain	Regression coefficient per log10-unit increase PFOS	BW: 0.05 (−0.18, 0.29) Females: 94.31 (−76.3, 264.92) Males: −11.15 (−174.26, 151.95) Adequate weight gain: −0.03 (−0.49, 0.41) Excess weight gain: 0.25 (−0.11, 0.62) Inadequate weight gain: −0.24 (−0.95, 0.45)
	MIREC = Maternal-Infant Research on Environmental Chemicals **Outcome:** Weight gain adequacy based on Institute of Medicine (IOM) guidelines **Confounding:** Maternal age, pre-pregnancy BMI, parity, household income, smoking, each PFAS.[c]						
Bach et al. (2016) *High*	Denmark, 2008–2013	Cohort	Pregnant women and their infants from the Aarhus Birth Cohort N = 1,507	Maternal serum Early pregnancy 8.3 (6.0–10.8)	BL (cm), BW (g, z-score), gestational length (weeks), HC (cm), PTB	Regression coefficient or OR (PTB) per IQR increase in PFOS and by quartiles	BL: 0 (−0.1, 0.2) Q2: −0.3 (−0.7, 0) Q3: −0.1 (−0.4, 0.3) Q4: −0.1 (−0.5, 0.2) BW (g): −8 (−30, 14) Q2: −66 (−122, −11) Q3: −30 (−86, 26) Q4: −58 (−105, 8) Females: −32 (−71, 7) Q2: −44 (−140, 52) Q3: −55 (−148, 38) Q4: −71 (−174, 31) Males: 26 (−13, 65) Q2: −129 (−239, −19) Q3: 9 (−93, 110) Q4: −37 (−141, 67)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BW (z-score): −0.02 (−0.07, 0.04) Q2: −0.15 (−0.29, −0.02) Q3: −0.06 (−0.19, 0.07) Q4: −0.11 (−0.25, 0.02) Gestational length: 0 (−0.1, 0.1) Q2: −0.1 (−0.4, 0.1) Q3: 0 (−0.2, 0.3) Q4: 0 (−0.3, 0.2) HC: 0 (−0.1, 0.1) Q2: −0.2 (−0.5, 0) Q3: −0.1 (−0.4, 0.1) Q4: −0.1 (−0.3, 0.2) PTB: 0.85 (0.6, 1.21) Q2: 0.96 (0.53, 1.74) Q3: 0.65 (0.34, 1.26) Q4: 0.82 (0.44, 1.53)
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI and educational level, GA.						
Bell et al. (2018) *High*	United States, 2008–2010	Cross-sectional	Singleton and twin infants born in from Upstate KIDS N = 2,071 singletons; 1,040 twins	Blood Later pregnancy Singletons: 1.72 (1.14–2.44) Twins: 1.64 (1.09–2.33)	BL (cm), BW (g), GA (weeks), HC (cm), ponderal index	Regression coefficient per log(PFOS+1) unit increase	BL S: −0.04 (−0.10, 0.1) T: 0.23 (−0.07, 0.53) BW S: −18.32 (−42.41, 5.78) T: 3.91 (−31.07, 38.89) GA S: 0.05 (−0.03, 0.13) T: −0.02 (−0.15, 0.11) HC S: 0.03 (−0.19, 0.24)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T: 0.23 (−0.04, 0.49) Ponderal index S: −0.01 (−0.03, 0.01) T: −0.01 (−0.04, 0.01)
	Comparison: Logarithm base not specified. **Results:** S = Singletons; T = Twins **Confounding:** Maternal age, maternal BMI, maternal education, infertility treatment, parity.						
Bjerregaard-Olesen et al. (2019) *High*	Denmark, 2011–2013	Cohort	Pregnant women and their children from FETOTOX N = 671	Maternal serum Early pregnancy IQR = 4.12	BL (cm), BW (g), HC (cm)	Regression coefficient per IQR increase in serum PFOS	BL: −0.1 (−0.3, 0.2) Females: −0.4 (−0.8, 0) Males: 0.2 (−0.1, 0.5), Interaction p-value = 0.022 BW: −15 (−62, 32) Females: −81 (−147, −14) Males: 38 (−28, 105), Interaction p-value = 0.013 HC: 0 (−0.2, 0.1) Females: −0.1 (−0.4, 0.1) Males: 0 (−0.2, 0.2), Interaction p-value = 0.404
	Confounding: Age at delivery, pre-pregnancy BMI, educational level, smoking, alcohol intake, GA at birth.						
Buck Louis et al. (2018) *High*	United States, 2009–2013	Cohort	Pregnant women (age range 18–40 yr) with singleton pregnancies from the NICHD Fetal Growth Studies N = 2,106	Maternal blood Early pregnancy 5.13 (3.39–7.89)	Umbilical circumference (cm), upper arm length (cm), upper thigh length (cm)	Regression coefficient per SD increase in logPFOS	Umbilical circumference: 0.04 (−0.09, 0.16) Upper arm length: −0.04 (−0.1, 0.1) Upper thigh length: −0.03 (−0.1, 0.04)
	NICHD = National Institute of Child Health and Human Development **Comparison:** Logarithm base not specified. **Confounding:** Maternal age, education, pre-pregnancy BMI, serum cotinine, infant sex, chemical-maternal race/ethnic interaction.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Chu et al. (2020) *High*	China, 2013	Cohort	Pregnant women (aged 18–45 yr) and infants from Guangzhou Birth Cohort Study N = 372	Maternal serum Later pregnancy 1.538 (0.957–2.635) Females: 1.497 (0.920–2.642) Males: 1.558 (0.988–2.628)	BW (g), GA (weeks), LBW, PTB	Regression coefficient (BW, GA) or OR (LBW, PTB) per ln-unit increase in PFOS or by quartiles	BW: −83.28 (−133.2, −33.36) Females: −71.91 (−143.86, 0.05) Males: −71.52 (−142.44, −0.61) p-value for interaction by sex = 0.678 GA: −0.32 (−0.53, −0.11) Females: −0.61 (−0.9, −0.32) Males: 0.004 (−0.31, 0.32) p-value for interaction by sex = 0.003 LBW: 2.43 (1.08, 5.47) Q2: 0.83 (0.11, 6.47) Q3: 1.41 (0.23, 8.82) Q4: 3.7 (0.61, 22.58) p-trend < 0.001 PTB: 2.03 (1.24, 3.32) Q2: 2.22 (0.55, 9.05) Q3: 4.52 (1.21, 16.88) Q4: 4.99 (.134, 18.56) p-trend = 0.003
	Outcome: LBW defined as BW < 2500 g **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, maternal occupation, maternal education, family income, parity for all outcomes; GA for BW and LBW; child sex for BW and GA.						
Costa et al. (2019) *High*	Spain, 2003–2008	Cohort	Pregnant women and their children from INMA study N = 1,230 (Girls = 597, Boys = 633)	Maternal plasma 6.05 (4.52–7.82)	AC, FL, BPD, estimated fetal weight at 12 wk, 20 wk, and 34 wk	Percent change per twofold increase in PFOS	AC 12 wk: 1.4 (−2.1, 4.9) Girls: 2.3 (−2.8, 7.1) Boys: 0.8 (−3.8, 5.4) 20 wk: 2.2 (−1.3, 5.6) Girls: 4.0 (−0.9, 8.8) Boys: 0.5 (−4.1, 5.0) 34 wk: 2.1 (−1.3, 5.5) Girls: 1.2 (−3.6, 5.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Boys: 2.8 (−1.8, 7.2) FL 12 wk: 1.2 (−2.3, 4.8) Girls: 0.3 (−4.7, 4.9) Boys: 2.0 (−2.6, 6.6) 20 wk: −0.6 (−4.1, 2.9) Girls: −1.7 (−6.5, 3.1) Boys: 0.0 (−4.6, 4.7) 34 wk: 1.2 (−4.1, 6.5) Girls: 1.3 (−3.6, 6.1) Boys: 1.7 (−2.9, 6.2) BPD 12 wk: 0.5 (−3.0, 3.9) Girls: 1.6 (−3.3, 6.4) Boys: −0.9 (−8.2, 6.3) 20 wk: 1.3 (−2.3, 4.8) Girls: 1.2 (−3.7, 6.0) Boys: 1.2 (−3.5, 5.9) 34 wk: 0.9 (−2.7, 4.4) Girls: 0.0 (−4.9, 4.7) Boys: 1.2 (−3.5, 5.9) Estimated Fetal Weight 12 wk: 1.9 (−1.7, 5.4) Girls: 1.3 (−3.5, 6.2) Boys: 2.5 (−2.3, 7.1) 20 wk: 2.6 (−0.9, 6.1) Girls: 2.4 (−2.4, 7.2) Boys: 1.0 (−3.7, 5.3) 34 wk: 2.6 (−0.9, 6.1) Girls: 1.8 (−3.2, 6.5) Boys: 3.0 (−1.7, 7.5)

INMA = INfancia y Medio Ambiente (Environment and Childhood) Project

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Cohort, parity, maternal age, country of birth, smoking at week 12, maternal pre-pregnancy BMI, studies, season of last menstrual period.						
Darrow et al. (2013) *High*	United States 2005–2011	Cohort	Pregnant women from the C8HP exposed through drinking water, Ages ≥ 19 LBW, all births N = 1,629 LBW, first prospective birth N = 783 BW, all births N = 1,470 BW, first prospective birth N = 710 PTB, all births N = 1,628 PTB, first prospective birth N = 783	Maternal serum at enrollment 13.9 (9.5–19.7)	LBW, BW (g), PTB	OR (LBW, PTB) and regression coefficient (BW) per ln-unit increase in PFOS, per IQR increase in PFOS, or by quintiles	LBW All births Per ln-unit increase: 1.12 (0.75, 1.67) Per IQR increase: 1.12 (0.87, 1.44) Q2:1.48 (0.71, 3.08) Q3: 1.23 (0.57, 2.65) Q4: 1.31 (0.59, 2.94) Q5:1.33 (0.60, 2.96) p-value for trend = 0.651 First prospective birth Per ln-unit increase: 0.97 (0.61, 1.54) Per IQR increase: 0.93 (0.63, 1.37) Q2: 1.65 (0.52, 5.20) Q3: 0.95 (0.30, 3.01) Q4: 1.17 (0.36, 3.78) Q5: 0.82 (0.25, 2.70) p-value for trend = 0.484 BW All births Per ln-unit increase: −29 (−66, 7) Per IQR increase: −23 (−48, 3) Q2: −25 (−96, 48) Q3: −37 (−109, 35) Q4: −83 (−152, −13) Q5: −54 (−124, 17) p-value for trend = 0.045 First prospective birth Per ln-unit increase: −49 (−90, −8) Per IQR increase: −29 (−58, 0) Q2: −33 (−140, 74) Q3: −115 (−216, −14)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: −149 (−244, −54) Q5: −105 (−196, −13) p-value for trend = 0.006 PTB All births Per ln-unit increase: 1.02 (0.78, 1.35) Per IQR increase: 1.03 (0.83, 1.27) Q2: 1.11 (0.63, 1.94) Q3: 0.76 (0.42, 1.36) Q4: 1.00 (0.56, 1.78) Q5: 1.07 (0.58, 1.95) p-value for trend = 0.976 First prospective births Per ln-unit increase: 1.02 (0.72, 1.45) Per IQR increase: 0.95 (0.73, 1.25) Q2: 1.07 (0.44, 2.59) Q3: 0.63 (0.25, 1.59) Q4: 1.08 (0.47, 2.46) Q5: 0.86 (0.36, 2.04) p-value for trend = 0.818
	C8HP = C8 Health Project **Outcome:** PTB defined as births occurring before 37 wk gestation. LBW defined as those weighing less than 2,500 g. **Results:** Lowest quintile used as reference. **Confounding:** Maternal age, educational level, smoking status, parity, BMI, self-reported diabetes, time between conception and serum management (year strata). Additional confounding for BW: indicator variables for gestational week.						
Eick et al. (2020) *High*	United States 2014–2018	Cohort	Second trimester pregnant women from the CIOB cohort BW (g) N = 461	Maternal serum from the second trimester 1.93 (1.18–3.13)	BW (g, z-score), GA (weeks), PTB	Regression coefficient by tertile PTB: OR by tertile	BW (g) T2: 1.62 (−105.53, 108.77) T3: 14.26 (−101.51, 130.03) BW (z-score) T2: −0.01 (−0.24, 0.22) T3: 0.02 (−0.23, 0.27)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			GA, BW (z-score), PTB N = 506				GA T2: −0.19 (−0.64, 0.26) T3: −0.08 (−0.59, 0.43) PTB T2: 1.21 (0.50, 2.91) T3: 1.87 (0.72, 4.88)
	CIOB = Chemicals in our Bodies **Outcome:** PTB defined as birth at <37 wk gestation. **Results:** Lowest tertile used as reference. **Confounding**: Maternal age, maternal race/ethnicity, pre-pregnancy BMI, maternal education, smoking status, parity, food insecurity.						
Gardener et al. (2021) *High*	United States Recruitment: 2009	Cohort	Pregnant women in third trimester (ages 18–49) and children at birth from the Vanguard Pilot Study of the NCS GA at birth N = 433 BW N = 403	Maternal serum from primarily third trimester 3.9 (2.6–5.9)	GA at birth (weeks), BW (z-score), GA <37 wk	GA at birth and BW: Mean by quartile GA <37 weeks and BW: OR by quartile	GA at birth Mean Q1: 38.92 (38.58, 39.26) Q2: 38.53 (38.19, 38.87) Q3: 38.77 (38.43, 39.09) Q4: 38.77 (38.42, 39.10) p-trend = 0.77 BW Mean Q1: −1.15 (−4.63, 2.32) Q2: 0.56 (−2.72, 3.84) Q3: 1.16 (−2.06, 4.38) Q4: 1.10 (−2.29, 4.46) p-trend = 0.35 OR Q2: 0.93 (0.43, 2.04) Q3: 1.41 (0.66, 3.03) Q4: 0.81 (0.36, 1.82) p-trend = 0.40 GA <37 wk OR Q2: 1.94 (0.66, 5.68) Q3: 1.13 (0.34, 3.73)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: 1.41 (0.46, 4.33) p-trend = 0.82
	NCS = National Children's Study **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, education, race/ethnicity, pre-pregnancy BMI, prenatal smoking, parity, GA at serum collection.						
Govarts et al. (2016) *High*	Belgium, 2008–2009	Cohort	Mother-newborn pairs from FLEHS II N = 213	Cord blood 2.63 µL (1.70–3.90 µL)	BW (g)	Regression coefficient per IQR increase in PFOS	10.82 (−72.4, 94.05), p-value = 0.798
	FLEHS II = Flemish Environmental and Health Study II **Confounding:** GA, child's sex, smoking of the mother during pregnancy, parity, maternal pre-pregnancy BMI.						
Huo et al., 2020, 6835452 *High*	China, 2013–2016	Cohort	Mothers (aged ≥ 20 yr) and their children from the Shanghai Birth Cohort N = 2,849	Maternal blood Later pregnancy 9.33 (6.54–13.65)	GA (weeks), PTB (indicated, non-spontaneous, spontaneous, and overall)	Regression coefficient (GA) per ln-unit increase in PFOS and per tertile	GA: 0.02 (−0.08, 0.12) T1: −0.27 (−0.62, 0.08) T2: 0.26 (−0.43, 0.96) T3: 0.03 (−0.24, 0.29) OR T2: 0.08 (−0.06, 0.21) OR T3: 0.06 (−0.08, 0.19)
						OR (PTB) per ln-unit increase in PFOS and per tertile	PTB, overall: 0.86 (0.63, 1.17) T2: 0.61 (0.4, 0.94) T3: 0.73 (0.48, 1.1) T1 (per ln-unit increase): 2.67 (0.85, 8.29) T2 (per ln-unit increase): 0.63 (0.05, 8.04) T3 (per ln-unit increase): 0.83 (0.33, 2.08) Females: 0.74 (0.45, 1.16) Males: 0.94 (0.62, 1.41) PTB, indicated: 1.13 (0.64, 2.01) T2: 0.79 (0.35, 1.78) T3: 0.99 (0.46, 2.12) PTB, non-spontaneous Females: 1.35 (0.56, 3.26)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Males: 0.98 (0.46, 2.09) PTB, spontaneous: 0.77 (0.53, 1.11) T2: 0.56 (0.34, 0.94) T3: 0.65 (0.4, 1.05) Females: 0.59 (0.33, 1.06) Males: 0.93 (0.57, 1.5)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, parity, parental education levels, pregnancy complicated with chronic disease, infant sex, GA at blood drawing.						
Lauritzen et al. (2017) *High*	Norway and Sweden, 1986–1988	Cohort	Mother-infant pairs from NICHD SGA N = 424 (265 from Norway, 159 from Sweden (78 girls, 81 boys))	Maternal serum Later pregnancy Norway: 9.74 (Range = 0.95–59.6) Sweden: 16.4 (Range = 2.28–55.2)	BL (cm), BW (g), GA (weeks), HC (cm), SGA	Regression coefficient or OR (SGA) per ln-unit increase in PFOS	BL: −0.3 (−0.7, 0.1), p-value = 0.139 NO: 0 (−0.4, 0.4), p-value = 0.987 SE: −1.2 (−2.1, −0.3), p-value = 0.007 BW: −15.1 (−111, 80.7), p-value = 0.757 NO: 74 (−31, 178), p-value = 0.167 SE: −292 (−500, −84), p-value = 0.006 GA: −0.07 (−0.34, 0.2), p-value = 0.601 NO: −0.01 (−0.3, 0.3), p-value = 0.952 SE: −0.4 (−0.9, 0.2), p-value = 0.201 HC: 0.04 (−0.19, 0.27), p-value = 0.748 NO: 0.2 (−0.1, 0.4), p-value = 0.189 SE: −0.4 (−0.9, 0.04), p-value = 0.073

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							SGA: 0.95 (0.62, 1.48), p-value = 0.833 NO: 0.71 (0.42, 1.2), p-value = 0.201 SE: 2.51 (0.93, 6.77), p-value = 0.068
	NICHD SGA = The U.S. National Institute of Child Health and Human Development (NICHD) Scandinavian Successive Small-for-Gestational-Age Births Study **Outcome:** SGA defined as BW below the 10th percentile for GA, sex, and parity. **Results:** NO = Norway; SE = Sweden **Confounding:** Maternal age, height, pre-pregnancy BMI, education, parity, smoking status at conception, interpregnancy interval, offspring sex.						
Lind et al. (2017a) *High*	Denmark 2010–2012	Cohort	Infants prenatally exposed to PFAS from the Odense Child Cohort N = 212 girls, 299 boys	Maternal serum Early pregnancy 8.1 (6.0–11.1)	BW (g), HC (cm), gestational length (days)	Regression coefficient per ln-unit increase in PFOS or by quartiles	BW Males Continuous: −17 (−130, 97) p-trend by quartiles = 0.73 Females Continuous: 92 (−15, 199) p-trend by quartiles = 0.15 HC Males Continuous: −0.2 (−0.6, 0.2) p-trend by quartiles = 0.38 Females Continuous: 0.3 (−0.1, 0.7) p-trend by quartiles = 0.12 Gestational length Males Continuous: −0.5 (−3.4, 2.3) p-trend by quartiles = 0.74 Females Continuous: −1.0 (−4.2, 2.1) p-trend by quartiles = 0.83

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Quartile analysis did not show any statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, weight-for-age z-score, pre-pregnancy BMI, parity, smoking.						
Luo et al. (2021) *High* for BW Medium for birth length and ponderal index	China 2017–2019	Cohort	Mother-newborn pairs N = 224	Maternal blood and cord blood within three days of delivery 5.01 (3.32, 7.62)	BW (g), BL (cm), ponderal index (kg/m^3)	Regression coefficient per ln-unit increase in PFOS	BW −93.34 (−157.92, −28.75), p-value <0.05 BL −0.05 (−0.38, 0.28) Ponderal index −0.67 (−1.08, −0.26), p-value < 0.05
	Confounding: Maternal age, pre-pregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, GA, newborn sex.						
Manzano-Salgado et al. (2017a) *High*	Spain, 2003–2008	Cohort	Mother (aged ≥16 yr)-child pairs from INMA N = 1,202	Maternal plasma Early pregnancy Mean = 6.05 (SD = 2.74)	BL (cm), BW (g), GA (weeks), HC (cm), LBW, LBW at term, PTB, SGA	Regression coefficient per doubling of PFOS or by quartiles LBW, LBW at term, PTB, SGA: OR per log2-unit increase in PFOS	BL: 0.03 (−0.12, 0.17) p-value for sex interaction = 0.98 BW: 0.44 (−32.48, 33.36) p-value for sex interaction = 0.75 GA: −0.06 (−0.19, 0.06) Q2: −0.09 (−0.33, 0.16) Q3: −0.02 (−0.26, 0.23) Q4: −0.31 (−0.55, −0.06); p-value < 0.05 p-value for sex interaction = 0.38 HC: 0 (−0.1, 0.1) p-value for sex interaction = 0.53 LBW: 1.06 (−0.71, 1.58) Females: 0.73 (0.46, 1.19) Males: 1.90 (0.98, 3.68)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-value for sex interaction = 0.01 LBW at term: 0.91 (0.55, 1.50) p-value for sex interaction = 0.15 PTB: 1.10 (0.70, 1.74) p-value for sex interaction = 0.35 SGA: 0.92 (0.70, 1.22) p-value for sex interaction = 0.57 BL, BW, HC: No statistically significant associations by quartiles All outcomes: No statistically significant associations by sex
	INMA = INfancia y Medio Ambiente [Environment and Childhood Project] **Outcome:** SGA defined as newborns weighing below the 10th percentile for GA and sex according to national references. **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, parity, pre-pregnancy BMI, fish intake during pregnancy, type of delivery.						
Minatoya et al. (2017) *High*	Japan 2002–2005	Cohort	Pregnant women and their children from the Sapporo Cohort (Hokkaido Study on Environment and Children's Health) N = 168 (90 girls, 78 boys)	Maternal serum 5.1 (3.7–6.7) Female mean: 5.04 (SD = 2.33) Male mean: 5.85 (SD = 2.63)	BW (g), ponderal index (kg/m2)	Regression coefficient per log10-unit increase in PFOS and LSM by tertiles	BW −29 (−289, 232); p-value = 0.828 Females: −251 (−645, 143) Males: 190 (−162, 543) p-value for sex interaction = 0.201 LSM T1: 3196 (3095, 3298) LSM T2: 3076 (2976, 3176) LSM T3: 3158 (3057, 3258) p-trend = 0.424 Ponderal index −2.25 (−4.01, −0.50); p-value = 0.012 Females: −2.11 (−4.86, 0.64) Males: −2.46 (−4.74, −0.18) p-value for sex interaction = 0.658

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							LSM T1: 28.39 (27.71, 29.06) LSM T2: 26.68 (26.02, 27.34) LSM T3: 27.23 (26.57, 27.90) p-trend = 0.003
	Confounding: Maternal BMI, parity, smoking during pregnancy, blood sampling period, GA.						
Rokoff et al. (2018) *High*	United States 1999–2002	Case-control	Pregnant women and their children from Project Viva N = 1,597	Maternal plasma Mean = 29.1 (SD = 16.5)	BW-for-GA z-score	Regression coefficient per IQR increase in PFOS	−0.03 (−0.07, 0.02)
	Confounding: Maternal age, race/ethnicity, education, pre-pregnancy BMI, and parity, black carbon, prenatal smoking.						
Sagiv et al. (2018) *High*	United States, 1999–2002	Cohort	Pregnant women and infants from Project Viva N = 1,644	Maternal blood Early pregnancy 25.7 (IQR = 16.0)	BW-for-GA (z-score), gestational length (weeks), PTB	Regression coefficient per IQR increase in PFOS and by quartiles PTB: OR per IQR increase in PFOS and by quartiles	BW-for-GA −0.04 (−0.08, 0.01) Q2: −0.09 (−0.22, 0.04) Q3: −0.09 (−0.22, 0.04) Q4: −0.13 (−0.26, 0.00) No statistically significant associations or interactions by sex Gestational length −0.08 (−0.17, 0.02) Q2: −0.20 (−0.47, 0.06) Q3: −0.08 (−0.35, 0.19) Q4: −0.36 (−0.64, −0.09) Females: 0.01 (−0.11, 0.14) Males: −0.19 (−0.33, −0.05) p-value for sex interaction = 0.09 PTB 1.1 (1.0, 1.3) Q2: 2.0 (1.1, 3.7) Q3: 2.0 (1.1, 3.7) Q4: 2.4 (1.3, 4.4)
	Outcome: PTB was defined as <37 wk **Results:** Lowest quartile used as reference.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, paternal education, household income, child's sex, GA at blood draw.						
Shoaff et al. (2018) *High*	United States, 2003–2006; follow-up 4 wk to 2 yr from recruitment	Cohort	Pregnant women (aged ≥18 yr) and their children at birth, 4 wk and 2 yr from the HOME study N = 345	Maternal blood Later pregnancy 14 (9.6–18)	BW (z-score), length-for-age (z-score), rapid weight gain, weight-for-age (z-score), weight-for-length (z-score)	Regression coefficient by tertile (per doubling in PFOS) Rapid weight gain: RR by tertile	BW z-score T2: −0.05 (−0.29, 0.19) T3: −0.12 (−0.36, 0.13) p-value for trend = 0.36 Length-for-age z-score T2: 0.05 (−0.33, 0.44) T3: −0.24 (−0.64, 0.15) p-value for trend = 0.08 Weight-for-age z-score T2:0.01 (−0.31, 0.32) T3: −0.33 (−0.65, −0.01) p-value for trend = 0.07 Weight-for-length z-score T2: −0.16 (−0.41, 0.09) T3: −0.31 (−0.56, −0.05) p-value for trend = 0.66 Rapid weight gain T2: 0.79 (0.55, 1.14) T3: 1.11 (0.81, 1.53)
	HOME = Health Outcomes and Measures of the Environment **Outcome:** Rapid weight gain defined as increase in weight z-score > 0.67 SDs any time between age 4 wk and 2 yr. **Results:** Lowest tertile used as reference **Confounding:** Maternal age at delivery, race, marital status, insurance, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable and fish consumption during pregnancy, prenatal vitamin use.						
Starling et al. (2017) *High*	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 yr) and infants from Healthy Start at birth N = 628	Maternal serum 2.4 (1.5–3.7)	Adiposity (% fat mass), BW (g)	Regression coefficient per ln-unit increase in PFOS and by tertiles	Adiposity: 0.08 (−0.33, 0.49) T2: 0.26 (−0.46, 0.98) T3: −0.41 (−1.15, 0.33) BW: −13.8 (−102.8, 35.2) T2: −33.8 (−102.8, 35.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T3: −71.1 (−142.6, 0.5)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, race/ethnicity, education, gestational weight gain, smoking during pregnancy, gravidity, GA at blood draw, infant sex, and GA at birth.						
Starling et al. (2019) *High*	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 yr) and infants from Healthy Start assessed up to 5 mo N = 415 (202 girls, 213 boys)	Maternal serum 2.2 (1.4–3.4)	Adiposity (%), weight-for-age z-score (WAZ), weight-for-length z-score (WLZ), WAZ and WLZ growth from birth to 5 mo, rapid growth in WAZ or WLZ	Regression coefficient per ln-unit increase in PFOS and by tertiles Rapid growth: OR per ln-unit increase in PFOS	Adiposity at 5 mo −0.13 (−0.83, 0.57) Females: −0.91 (−1.84, 0.02) Female T3: −2.08 (−3.81, −0.35) Males: 0.73 (−0.36, 1.81) Male T2: 1.85 (0.14, 3.47) p-value for sex interaction = 0.05 WAZ at 5 mo: −0.10 (−0.23, 0.02) T3: −0.28 (−0.51, −0.05) Females: −0.26 (−0.43, −0.10) Female T3: −0.56 (−0.87, −0.26) Males: 0.07 (−0.13, 0.27) p-value for sex interaction = 0.10 WLZ at 5 mo: −0.08 (−0.23, 0.06) Females: −0.08 (−0.23, 0.06) Female T3: −0.52 (−0.88, −0.17) Males: 0.06 (−0.17, 0.28) p-value for sex interaction = 0.17 WAZ or WLZ growth from birth to 5 mo, rapid growth: No statistically significant associations
	Outcome: Rapid growth defined as change in WAZ or WLZ >0.67 between birth and 5 mo **Results:** Lowest tertile used as reference **Confounding:** Maternal age, race/ethnicity, pre-pregnancy BMI, any previous pregnancies, any smoking during pregnancy, education, gestational weight gain z-score, infant sex, exclusive breastfeeding to follow-up visit, infant age (days) at follow-up.						
Valvi et al. (2017) *High*	Faroe Islands 1997–2000	Cross-sectional	Pregnant women and their children N = 604 (288 girls, 316 boys)	Maternal serum 27.2 (23.1–33.1)	HC (cm), body length (cm), BW (g)	Regression coefficient per doubling of PFOS	HC 0 (−0.28, 0.27) Girls: 0.48 (0.05, 0.90) Boys: −0.28 (−0.65, 0.09)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-value for sex interaction = 0.01 Body length 0.05 (−0.33, 0.43) Girls: 0.32 (−0.24, 0.89) Boys: −0.18 (−0.60, 0.23) p-value for sex interaction = 0.17 BW −81 (−173, 11) Girls: 5 (−124, 135) Boys: −150 (−282, −17) p-value for sex interaction = 0.08
	Confounding: Maternal age at delivery, education, parity, pre-pregnancy BMI, smoking during pregnancy, child sex.						
Whitworth et al. (2012a) *High*	Norway 2003–2004	Cohort	Pregnant women and their children from MoBa PTB, LGA, SGA N = 901 BW N = 838	Maternal plasma around 17 wk of gestation 13.0 (10.3–16.6)	PTB, BW (z-score), SGA, LGA	OR by quartile BW: Regression coefficient per unit increase in PFOS, or by quartile	PTB Q2: 0.9 (0.3, 2.8) Q3: 0.9 (0.3, 2.7) Q4: 0.3 (0.1, 1.0) p-trend = 0.03 LGA Q2: 0.8 (0.5, 1.6) Q3: 1.0 (0.5, 1.7) Q4: 0.7 (0.3, 1.4) p-trend = 0.33 SGA Q2: 1.2 (0.5, 3.0) Q3: 2.2 (1.0, 5.1) Q4: 1.3 (0.5, 3.4) p-trend = 0.51 BW Per increase: −0.01 (−0.02, 0.01) Q2: −0.08 (−0.29, 0.13) Q3: −0.17 (−0.39, 0.05)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: −0.18 (0.41, 0.05) p-trend = 0.12
	MoBa = Norwegian Mother and Child Cohort Study **Outcome:** PTB defined as GA <37 wk. SGA defined as gender- and gestation age-specific BW less than the 10th percentile. LGA defined as gender- and GA-specific BW greater than the 90th percentile. **Confounding:** Maternal age, pre-pregnancy BMI, parity. Additional confounding for BW: albumin concentration, maternal education, interpregnancy interval, quadratic interpregnancy interval, consumption of lean fish.						
Wikström et al. (2020) *High*	Sweden 2007–2010	Cohort	Infants exposed prenatally to PFAS from the SELMA study N = 1533 (732 girls, 801 boys)	Maternal serum Early pregnancy 5.38 (3.97–7.60)	BW (g), BW-SDS, SGA	Regression coefficient (BW, BW-SDS) and OR (SGA) per ln-unit increase in PFOS or by quartiles	BW Per increase: −46 (−88, −3) Q2: −27 (−89, 35) Q3: −22 (−84, 41) Q4: −80 (−144, −16) Girls Per increase: −85 (−145, −25) Q2: −32 (−115, 52) Q3: −51 (−137, 34) Q4: −142 (−231, −54) Boys Per increase: −13 (−73, 47) Q2: −28 (−118, 63) Q3: 5 (−86, 96) Q4: −28 (−119, 63) BW-SDS Per increase: −0.100 (−0.197, −0.004) Q2: −0.045 −0.185, 0.096) Q3: −0.024 (−0.166, 0.118) Q4: −0.172 (−0.317, −0.027) Girls Per increase: −0.167 (−0.301, −0.034) Q2: −0.044 (−0.232, 0.143) Q3: −0.092 (−0.283, 0.100) Q4: −0.296 (−0.494, −0.098) Boys

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Per increase: −0.027 (−0.166, 0.112) Q2: −0.055 (−0.263, 0.153) Q3: 0.038 (−0.171, 0.246) Q4: −0.066 (−0.276, 0.144) SGA Per increase: 1.19 (0.87, 1.64) Q2: 0.69 (0.43, 1.08) Q3: 0.79 (0.53, 1.18) Q4: 1.56 (1.09, 2.22) Girls Per increase: 1.40 (0.83, 2.35) Q2: 0.89 (0.39, 2.03) Q3: 0.82 (0.36, 2.03) Q4: 2.05 (1.00, 4.21) Boys Per increase: 1.08 (0.72, 1.63) Q2: 1.26 (0.67, 2.37) Q3: 0.86 (0.45, 1.67) Q4: 1.30 (0.7, 2.4)
	SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy **Outcomes:** SGA defined as BW below the 10th percentile for GA and sex. **Results:** Lowest quartile used as reference. **Confounding:** Sex, GA, maternal weight, parity, cotinine levels.						
Wikström et al. (2021) *High*	Sweden, 2007–2010	Nested case-control	Pregnant women from the SELMA study N = 1,527	Serum during first trimester Case: 6.09 (3.99–8.77) Control: 5.45 (4.00–7.68)	Miscarriage	OR per doubling in PFOS	Per doubling: 1.13 (0.82, 1.52)
	SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy **Confounding:** Parity, age, cotinine (tobacco smoke) exposure.						
Xiao et al. (2019) *High*	Denmark 1994–1995	Cohort	Pregnant women and their children N = 171	Maternal blood Later pregnancy	Z-scores for BL, BW, and	Regression coefficient per log2-unit	BL z-score −0.33 (−0.69, 0.03) Girls: −0.23 (−0.75, 0.30)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
				GM = 20.8 µg/g (range: 6.9–47.6 µg/g)	cranial circumference	increase in PFOS	Boys: −0.41 (−0.87, 0.05) BW z-score −0.47 (−0.85, −0.09) Girls: −0.56 (−1.12, 0.00) Boys: −0.40 (−0.89, 0.08) Cranial circumference z-score −0.26 (−0.68, 0.16) Girls: −0.42 (−1.05, 0.21) Boys: −0.15 (−0.68, 0.39)
	Confounding: Child sex, parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury.						
Yao et al. (2021) *High*	China 2010–2013	Cross-sectional	Parents and their children from LWBC N = 369	Maternal and paternal serum within three days of birth Maternal: 4.55 (Range = 0.55–29.85) Paternal: 10.15 (<LOD–43.19)	BW (g)	Regression coefficient per ln-unit increase in PFOS	BW by maternal exposure Model A: −32.28 (−116.2, 51.64) BW by paternal exposure Model A: 0.19 (−74.26, 74.65)
	LWBC = Laizhou Wan Birth Cohort; LOD = limit of detection (0.09 ng/mL) **Confounding:** All models adjusted for characteristics of parent with measured exposure: age, education, BMI (before pregnancy for maternal exposure). Maternal exposure models additionally adjusted for parity. "Adjusted" models additionally adjusted for other parent's exposure and characteristics.						
Yeung et al. (2019) *High*	United States Recruitment 2008–2010, assessment up to age 3	Cohort	Children aged 0–3 from Upstate KIDS study N = 1,954 singletons (S) (930 girls, 1,024 boys) and 902 twins (T)	Blood 1.7 (1.1–2.4)	BMI, BMI z-score, length (cm), length z-score, obesity, weight (g), weight z-score, rapid weight gain, weight-for-	Regression coefficient or OR (rapid weight gain, obesity) per log-SD increase in PFOS or by quartiles	BMI S: −0.11 (−0.17, −0.05); p-value < 0.05 S-girls: −0.16 (−0.24, −0.08); p-value < 0.05 S-boys: −0.06 (−0.15, 0.02) T: −0.06 (−0.16, 0.04) BMI z-score

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					length (WFL) z-score		S: −0.08 (−0.12, −0.04); p-value < 0.05 Q2: 0.03 (−0.09, 0.15) Q3: −0.06 (−0.18, 0.06) Q4: −0.20 (−0.32, −0.09); p-value < 0.05 S-girls: −0.11 (−0.17, −0.05); p-value < 0.05 Q2: 0.07 (−0.10, 0.24) Q3: 0.03 (−0.16, 0.17) Q4: −0.26 (−0.26, −0.10); p-value < 0.05 S-boys: −0.05 (−0.11, 0.01) Q2: −0.01 (−0.16, 0.15) Q3: −0.11 (−0.27, 0.06) Q4: −0.15 (−0.32, 0.02) T: −0.03 (−0.10, 0.05) Q2: 0.11 (−0.09, 0.32) Q3: 0.07 (−0.14, 0.28) Q4: 0.0005 (−0.2, 0.2) Length S: 0.07 (−0.06, 0.19) S-girls: 0.03 (−0.14, 0.20) S-boys: 0.10 (−0.07, 0.27) T: 0.18 (−0.07, 0.42) Length z-score S: 0.03 (−0.03, 0.08) S-girls: 0.008 (−0.07, 0.08) S-boys: 0.05 (−0.03, 0.12) T: 0.07 (−0.04, 0.18) Weight S: −21.99 (−59.52, 15.55)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							S-girls: −51.57 (−102.32, −0.82); p-value < 0.05 S-boys: 6.15 (−48.31, 60.61) T: 62.47 (−13.97, 138.92) Weight z-score S: −0.03 (−0.08, 0.01) S-girls: −0.07 (−0.13, −0.01); p-value < 0.05 S-boys: −0.001 (−0.06, 0.06) T: 0.04 (−0.04, 0.12) WFL z-score S: −0.08 (−0.12, −0.04) S-girls: −0.10 (−0.16, −0.05); p-value < 0.05 S-boys: −0.05 (−0.11, 0.01) T: −0.03 (−0.11, 0.05) Rapid weight gain, obesity: not statistically significant for all children
	Outcome: Rapid weight gain defined as the child's weight gain SD above 0.5 for 4 or 9 mo or about 0.67 for 12 mo. **Comparison:** Logarithm base not specified. **Results:** Lowest quartile used as reference. **Confounding:** Child's age at measurement, age squared, age cubed, sex-age interactions, maternal age, pre-pregnancy BMI category, maternal education, maternal race, private insurance, infertility treatment.						
Andersen et al. (2010) *Medium*	Denmark, 1996–2002	Cohort	Pregnant women and their children followed up at birth, 5 mo, and 12 mo from DNBC	Maternal plasma from first and second trimester 33.4 (6.4, 106.7)	BW (g, z-score), BMI at 5 and 12 mo, height at 5 and 12 mo (cm), weight at 5 and 12 mo (g)	Regression coefficient per unit increase in PFOS	BW z-score: −0.002 (−0.006, 0.002) g: −1 (−3.1, 1.0) Boys z-score: 0.003 (−0.003, 0.008) g: 1.3 (−1.6, 4.2) Girls z-score: −0.006 (−0.011, −0.001), p-value <0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N at birth = 1,114 (552 boys, 562 girls)				g: −3.2 (−6.0, −0.3), p-value <0.05 BMI at 5 mo z-score: −0.001 (−0.006, 0.003) g: −0.002 (−0.10, 0.005) Boys z-score: −0.004 (−0.011, 0.002) g: −0.007 (−0.018, 0.003) Girls z-score: 0.001 (−0.005, 0.007) g: 0.002 (−0.008, 0.012) BMI at 12 mo z-score: −0.007 (−0.011, 0.002), p-value <0.05 g: −0.011 (−0.019, −0.003) Boys z-score: −0.01 (−0.017, −0.003), p-value <0.01 g: −0.017 (−0.028, −0.005), p-value <0.01 Girls z-score: −0.005 (−0.011, 0.002) g: −0.007 (−0.018, 0.003) Height at 5 mo z-score: 0.002 (−0.002, 0.006) g: 0.006 (−0.004, 0.017) Boys z-score: 0.0004 (−0.006, 0.006) g: 0.001 (0.014, 0.016) Girls z-score: 0.004 (−0.001, 0.010) g: 0.011 (−0.004, 0.026) Height at 12 mo

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							z-score: 0.003 (−0.001, 0.008) g: 0.010 (−0.003, 0.023) Boys z-score: 0.003 (−0.004, 0.009) g: 0.008 (−0.011, 0.027) Girls z-score: 0.004 (−0.002, 0.010) g: 0.011 (−0.007, 0.030) Weight at 5 mo z-score: −0.001 (−0.005, 0.003) g: −0.8 (−4.2, 2.6) Boys z-score: −0.004 (−0.009, 0.001) g: −3.7 (−8.7, 1.3) Girls z-score: 0.002 (−0.004, 0.007) g: 1.3 (−3.3, 5.9) Weight at 12 mo z-score:−0.005 (−0.009, 0.001), p-value <0.05 g: −5.8 (−10.4, −1.2), p-value <0.05 Boys z-score: −0.008 (−0.013, −0.002), p-value <0.05 g: −9 (−15.9, −2.2), p-value <0.05 Girls z-score: −0.003 (−0.009, 0.003) g: −3.3 (−9.3, 2.7)
	DNBC = Danish National Birth Cohort **Results:** "Models for weight at 5 or 12 mo included BW, models for length at 5 or 12 mo included birth length, and models for BMI at 5 or 12 mo included birth BMI."; adjusted models were used for all results.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, parity, pre-pregnancy BMI, smoking, socioeconomic status, GA at blood drawing, breastfeeding. Additional confounding for BMI and 5 and 12 mo: birth BMI. Additional confounding height at 5 and 12 mo: birth height. Additional confounding for weight at 5 and 12 mo: BW.						
Apelberg et al. (2007b) *Medium*	United States 2004–2005	Cross-sectional	Pregnant women and their newborns from Baltimore THREE Study, N = 293	Cord blood at birth 5 (3.4–7.9)	BW (g), HC (cm), BL (cm), ponderal index (g/cm^3 × 100), GA (days)	Regression coefficient per ln-unit increase in PFOS, regression coefficient per IQR increase in PFOS	BW Per ln-unit increase: −69 (−149, 10) Per IQR increase: −58 (−125, 9) HC Per ln-unit increase: −0.32 (−0.56, −0.07), p-value <0.05 Per IQR increase: −0.27 (−0.48, −0.06), p-value <0.05 BL Per ln-unit increase: 0.13 (−0.26, 0.52) Per IQR increase: 0.11 (−0.22, 0.44) Ponderal index Per ln-unit increase: −0.074 (−0.123, −0.025), p-value <0.05 Per IQR increase: −0.062 (−0.104, −0.021), p-value <0.05 GA Per ln-unit increase: 1.9 (−1.3, 5) Per IQR increase: 1.0 (−0.7, 2.8)
	Confounding: GA, maternal age, BMI, race, parity, smoking, baby sex, height, net weight gain, diabetes, hypertension. Additional confounding for HC: delivery mode.						
Arbuckle et al. (2020) *Medium*	Canada, 2008–2011	Cohort	Pregnant women (age range = 17–42 yr) and their infants from MIREC	Maternal blood 4.50 µg/L (3.30–6.10 µg/L)	Anoclitoris distance (ACD, mm), anofourchette distance	Regression coefficient per ln-unit increase in PFOS and by quartiles	ACD: 0.07 (−1.03, 1.18) Q2: −0.06 (−1.7, 1.58) Q3: 0.17 (−1.5, 1.85) Q4: −0.05 (−1.68, 1.57)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 205		(AFD, mm), anopenile distance (APD, mm), anoscrotal distance (ASD, mm)		AFD: −0.29 (−1.62, 1.04) Q2: −0.12 (−2.09, 1.85) Q3: 0.89 (−1.12, 2.9) Q4: −0.33 (−2.31, 1.65) APD: 0.13 (−1.13, 1.38) Q2: −0.97 (−2.81, 0.87) Q3: −1.28 (−3.22, 0.66) Q4: 0.22 (−1.68, 2.13) ASD: 1.05 (−0.24, 2.35) Q2: −0.87 (−2.78, 1.04) Q3: 0.33 (−1.67, 2.33) Q4: 0.49 (−1.47, 2.46) No statistically significant trends
	MIREC = Maternal-Infant Research on Environmental Chemicals (MIREC) **Results:** Lowest quartile used as reference. **Confounding:** Household income, education, active smoking status, GA, WLZ, and recruitment site.						
Chang et al. (2022) *Medium*	United States 2014–2018	Cohort	Mother-infant pairs from the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study N = 370	Maternal serum, Early pregnancy, 2.19 (1.45–3.24)	BW (g), SGA	BW: Regression coefficient per doubling in PFOS and by quartiles SGA: Odds ratio per doubling in PFOS and by quartiles	BW Per doubling: −7 (−48, 34) Q2: 78 (−98, 196) Q3: 20 (−98, 138) Q4: −16 (−136, 105) p-trend = 0.48 SGA Per doubling: 1.12 (0.88, 1.42) Q2: 0.92 (0.47, 1.78) Q3: 1.32 (0.69, 2.53) Q4: 1.09 (0.56, 2.13) p-trend = 0.65
	Outcome: SGA defined as a BW below the 10th percentile for GA. **Confounding:** maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex (BW only).						
Chen et al. (2012a)	Taiwan, 2004–2005	Cross-sectional	Mother-infant pairs from TBPS	Cord blood at birth	BW (g), BL (cm), GA	BW: Regression coefficient per	BW

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Medium			N = 429	GM (SD) = 5.94 (1.95)	(weeks), HC (cm), LBW, ponderal index (g/cm^3), PTB, SGA	unit increase in PFOS BW, BL, GA, HC, ponderal index: Regression coefficient per ln-unit increase in PFOS, or by quartile PTB, LBW, SGA: OR per ln-unit increase in PFOS, or by quartile	Per ln-unit increase: −110.2 (−176, −44.5), p-value <0.01 Per unit increase: −11.3 (−17.4, −5.2) Q2: 54 (−44, 152) Q3: 2 (−95, 102) Q4: −92 (−190, 6) p-trend = 0.045 BL Per ln-unit increase: −0.17 (−0.42, 0.09) Q2: 0.08 (−0.39, 0.55) Q3: 0.14 (−0.33, 0.62) Q4: −0.32 (−0.80, 0.15) p-trend = 0.234 GA Per ln-unit increase: −0.37 (−0.6, −0.13), p-value <0.01 Q2: 0.13 (−0.30, 0.57) Q3: −0.65 (−1.07, −0.20) Q4: −0.44 (−0.88, 0.00) p-trend = 0.004 HC Per ln-unit increase: −0.25 (−0.46, −0.05), p-value <0.05 Q2: −0.16 (−0.53, 0.21) Q3: −0.26 (−0.63, 0.12) Q4: −0.42 (−0.80, −0.05) p-trend = 0.025 Ponderal index Per ln-unit increase: −0.01 (−0.05, 0.02)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q2: 0.03 (−0.03, 0.09) Q3: −0.02 (−0.08, 0.04) Q4: −0.03 (−0.09, 0.04) p-trend = 0.232 PTB Per ln-unit increase: 2.45 (1.47, 4.08), p-value <0.001 Q2: 1.0 (0.2, 5.0) Q3: 6.5 (2.0, 24) Q4: 5.5 (1.5, 20) p-trend = 0.0006 LBW Per ln-unit increase: 2.61 (0.85, 8.03) Q2: 0.5 (0.02, 13) Q3: 1.0 (0.06, 18) Q4: 4.5 (0.50, 57) p-trend = 0.062 SGA Per ln-unit increase: 2.27 (1.25, 4.15), p-value <0.01 Q2: 0.8 (0.2, 2.5) Q3: 0.5 (0.1, 2.0) Q4: 1.5 (0.6, 4.5) p-trend = 0.422
	TBPS = Taiwan Birth Panel Study **Outcome:** PTB defined as GA <37 wk. Low BW defined as a BW <2,500 g. SGA defined as a BW below the 10th percentile for GA. **Confounding:** Maternal age, pre-pregnancy BMI, education level, ln-transformed cord blood cotinine levels, type of delivery, parity, infant sex. Additional confounding for BW, BL, HC, ponderal index, low BW, PTB: GA.						
Chen et al. (2017c) *Medium*	Taiwan, 2004–2005	Cohort	Mother-infant pairs from the Taiwan Birth	Cord blood 5.7 (IQR = 5.0)	BMI (z-score, kg/m^2), height (z-score, cm),	Regression coefficient per ln-unit increase in PFOS	BMI Birth: −0.11 (−0.25, 0.02) 0–6 mo: 0.002 (−0.17, 0.18) 6–12 mo: −0.12 (−0.31, 0.08)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			Panel Study (TBPS) N = 429		weight (z-score, kg)		Girls 6–12 mo: −0.33 (−0.59, −0.08); p-value < 0.05 12–24 mo: −0.09 (−0.29, 0.11) Girls 12–24 mo: −0.25 (−0.45, −0.05); p-value < 0.05 24–60 mo: −0.17 (−0.41, 0.06) 60–108 mo: −0.02 (−0.33, 0.28) Girls 60–108 mo: 0.34 (0.007, 0.68); p-value < 0.05 Height Birth: −0.16 (−0.31, −0.02), p-value < 0.05 0–6 mo: −0.04 (−0.23, 0.16) 6–12 mo: −0.02 (−0.23, 0.18) 12–24 mo: 0.04 (−0.17, 0.26) 24–60 mo: 0.09 (−0.12, 0.3) Boys 24–60 mo: 0.18 (0.03, 0.33); *p*-value < 0.05 60–108 mo: 0.06 (−0.19, 0.31) Boys 60–80 mo: 0.19 (0.01, 0.38); p-value < 0.05 Weight Birth: −0.14 (−0.26, −0.01), p-value < 0.05 0–6 mo: −0.008 (−0.17, 0.16) 6–12 mo: −0.13 (−0.32, 0.07) Girls 6–12 mo: −0.25 (−0.47, −0.04); p-value < 0.05 12–24 mo: −0.005 (−0.25, 0.16) Girls 12–24 mo: −0.24 (−0.41, −0.06); p-value < 0.01 24–60 mo: −0.07 (−0.3, 0.16) 60–108 mo: 0.02 (−0.27, 0.31)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BMI, height, and weight: no statistically significant interactions by sex at any age
	Population: Infants were followed up at 4, 6, 13, 24, 60, 84, and 108 mo **Confounding:** Maternal age, pre-pregnancy BMI, education level, ln-cord blood cotinine, infant sex, PTB, postnatal ETS exposure, breastfeeding.						
Chen et al. (2021) *Medium*	China Recruitment: 2013–2015	Cohort	Mother-child pairs from the SBC, Ages ≥ 20, N = 214 95 male children, 119 female children	Maternal plasma from the first trimester 9.70 (6.75–15.35)	BW (g), BL (cm), HC (cm)	Regression coefficient per ln-unit increase in PFOS	BW 2.7 (−84.3, 89.7) BL −0.27 (−0.51, −0.02), p-value <0.05 Males −0.14 (−0.55, 0.26) Females −0.4 (−0.74, −0.06), p-value <0.05 HC −10.6 (−60.7, 39.6)
	SBC = Shanghai Birth Cohort **Confounding:** Maternal age, BMI, educational level, occupation, income, fetal sex, parity, GA, smoking and alcohol.						
Darrow et al. (2014) *Medium*	United States, Recruitment: 2005–2006, Follow-up: 2008–2011	Cohort	Pregnant women with known PFAS exposure (ages ≥20 yr) from C8HP N = 1,438 First pregnancy N = 1,129	Serum collected before pregnancy 15.1 (10.4–21.2)	Primary analysis miscarriage, first pregnancy miscarriage	OR per ln-unit increase in PFOS, OR by quintile	Primary analysis: 1.21 (0.94, 1.55) Q2: 1.34 (0.84, 2.16) Q3: 1.40 (0.88, 2.25) Q4: 1.59 (0.99, 2.54) Q5: 1.41 (0.88, 2.26) First pregnancy: 1.34 (1.02, 1.76) Q2: 1.68 (0.99, 2.84) Q3: 1.93 (1.13, 3.31) Q4: 1.94 (1.14, 3.31) Q5: 1.80 (1.06, 3.06)
	C8HP = C8 Health Project **Outcome:** Primary analysis includes more than one pregnancy for some women (304 miscarriages). First pregnancy is restricted to the first pregnancy conceived per woman after serum measurement (213 miscarriages)						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, educational level, smoking status, BMI, self-reported diabetes, time between conception, serum measurement.						
De Cock et al. (2014) *Medium*	The Netherlands Recruitment: 2011–2013 Follow-up at 1, 2, 4, 6, 9, and 11 mo after birth	Cohort	Mother-child pairs N = 89	Cord blood 1,600.0 ng/L (Range = 570–3,200 ng/L)	BMI (kg/m^2), HC (cm), height (cm), weight (kg)	Regression coefficient for quartiles of PFOS	BMI, HC, height, and weight: no statistically significant associations
	Confounding: BW, GA, maternal height.						
de Cock et al. (2016) *Medium*	The Netherlands, 2011–2013	Cross-sectional	Mother-infant pairs N = 64	Cord blood 1,600 ng/L (Range = 570–3,200 ng/L)	BW (g)	Regression coefficient by tertiles	T2: 254.8 (−99.47, 609.09), p-value = 0.153 T3: 438.4 (55.09, 821.68), p-value = 0.026 Females T2: 143.3 (−361.63, 648.32), p-value = 0.566 T3: 301.1 (−124.87, 727.05), p-value = 0.159 Males T2: 486.9 (−1.21, 975.03), p-value = 0.051 T3: 724.4 (193.83, 1,254.97), p-value = 0.009
	Results: Lowest tertile used as reference. **Confounding:** GA, maternal BMI, maternal height, maternal age at birth, and parity, paternal BMI, paternal height, education, fish intake.						
Fei et al. (2008a) *Medium*	Denmark Recruitment: 1996–2002, Assessment 6–18 mo later	Cohort	Pregnant women and their children at 6 and 18 mo from the DNBC Total N = 1,400 18-mo olds N = 1,380	Maternal plasma during the first trimester 33.3 (26.0–43.2)	Gross motor milestone, language milestone, Apgar score <10	Gross motor milestone: Hazard ratio by quartile Language milestone: OR by quartile Apgar score: OR for Q4 vs. Q1	Gross motor milestone Q2: 0.93 (0.79, 1.08) Q3: 0.85 (0.72, 0.99) Q4: 0.86 (0.73, 1.01) p-trend = 0.041 Language milestone Q2: 1.39 (0.46, 4.25) Q3: 1.58 (0.51, 4.91) Q4: 2.93 (1.00, 8.56)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-trend = 0.039 Apgar score Q4: 1.2 (0.67, 2.14)
	DNBC = Danish National Birth Cohort **Outcome:** Gross motor milestone defined as sitting without support. Language milestone defined as children not using word-like sounds to indicate what they want. **Results:** Lowest quartile used as reference group. **Confounding:** Maternal age, maternal occupational and educational status, parity, pre-pregnancy BMI, smoking and alcohol consumption during pregnancy, gestational weeks at blood drawing, child's sex. Additional confounding for gross motor milestone and language milestone: parity, out-of-home childcare, home density (rooms/person). Additional confounding for language milestone: child's age at interview.						
Fei et al. (2008b) *Medium*	Denmark 1996–2002	Cohort	Pregnant women and their newborns from the DNBC Placental weight N = 1,337 Birth length N = 1,376 HC N = 1,347 Abdominal circumference N = 1,325	Maternal plasma between 4 and 14 wk gestation 33.4 (26.1–43.3)	Placental weight (g), HC (cm), BL (cm), abdominal circumference (cm)	Regression coefficient per unit increase in PFOS Mean difference by quartile	Placental weight Per unit increase: −0.24 (−0.85, 0.37) Q2: −6.6 (−28.8, 15.5) Q3: −13.7 (−36.4, 8.9) Q4: −10.8 (−33.4, 11.8) HC Per unit increase: 0.0 (−0.006, 0.007) Q2: 0.14 (−0.09, 0.36) Q3: 0.09 (−0.14, 0.32) Q4: 0.03 (−0.20, 0.27) BL Per unit increase: −0.002 (−0.011, 0.006) Q2: 0.21 (−0.08, 0.51) Q3: 0.06 (−0.24, 0.36) Q4: 0.05 (−0.25, 0.35) Abdominal circumference Per unit increase: −0.003 (−0.012, 0.005) Q2: 0.24 (−0.07, 0.55)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q3: 0.10 (−0.21, 0.42) Q4: 0.00 (−0.32, 0.32)
	DNBC = Danish National Birth Cohort **Results:** Lowest quartile used as reference group **Confounding:** GA, quadratic GA, infant sex, maternal age, socio-occupational status, parity, cigarette smoking, pre-pregnancy BMI, gestational week at blood drawing.						
Govarts et al. (2018) *Medium*	Belgium, the Netherlands, Norway, and Slovakia 2002–2012	Cohort	Mother-child pairs from FLEHS I and II, HUMIS, LINC, and PCB Cohort N = 657	Cord blood 1,984 ng/L (1,200–3,008 ng/L)	SGA	OR per IQR increase in PFOS	0.823 (0.742, 0.913)
	FLEHS = Flemish Environmental and Health Study; HUMIS = Human Milk Study; LINC = Linking EDCs in Maternal Nutrition to Child Health **Outcome:** SGA defined as newborns weighing below the 10th percentile for the norms defined by GA, country, and infant's sex. **Confounding:** Maternal education, maternal age at delivery, maternal height, maternal pre-pregnancy BMI, smoking during pregnancy, parity, child's sex.						
Gyllenhammar et al. (2018b) *Medium*	Sweden, 1996–2011 and follow-up at 5 yr of age	Cohort and cross-sectional	Mother-infant pairs of singleton births from POPUP study N = 377	Maternal serum Later pregnancy 13 (7.4–19)	BL (SD scores), BW (SD scores), gestational length (days), HC (SD scores), length (SD scores), weight (SD scores)	Regression coefficient per IQR increase in maternal PFOS	BL: 0.1377 (−0.0971, 0.3725) BW: 0.0167 (−0.1878, 0.2225) Gestational length: −2.0342 (−4.1139, 0.0455) HC: 0.0703 (−0.1602, 0.2974) HC, length, and weight: no statistically significant associations by sex
	POPUP = Persistent Organic Pollutants in Uppsala Primiparas **Confounding:** Sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, smoking during pregnancy, total fish consumption.						
Hamm et al. (2010) *Medium*	Canada Recruitment: 2005–2006 Follow-up at delivery: 2006–2007	Cohort	Pregnant women (≥18 yr of age) and their singleton children delivered at or after 22 wk gestation	Maternal serum collected at 15–16 wk gestation GM (SD) = 7.4 (2.0)	BW (g, z-score), SGA, PTB, length of gestation (weeks)	BW: Regression coefficient per ln-unit or per unit increase in PFOS and by tertiles	BW (g per ln-unit): −31.3 (−43.3, 105.9), p-value = 0.03 T2: −13.51 (−136.57, 109.55) T3: 71.25 (−54.97, 197.48) BW (g per unit): 1.5 (−7.6, 10.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 252			SGA, PTB: Relative risk by tertile	BW (z-score per ln-unit): 0.06 (−0.11, 0.23) T2: −0.006 (−0.29, 0.27) T3: 0.16 (−0.13, 0.44)
						Length of gestation: Regression coefficient per ln-unit increase in PFOS and by tertile	SGA: T2: 0.99 (0.27, 3.61) T3: 0.26 (0.10, 0.70) PTB: T2: 1.06 (0.33, 3.45) T3: 1.11 (0.36, 3.38) Length of gestation: Per ln-unit: 0.21 (−0.12, 0.53) T2: 0.13 (−0.42, 0.67) T3: 0.046 (−0.51, 0.60)
	Outcome: SGA defined as BW <10th percentile for GA and infant gender; PTB defined as delivery at 22–36 wk **Results:** Lowest tertile used as reference **Confounding:** Maternal age, maternal race, gravida, maternal weight and height, smoking. Additional confounding for PTB and BW (g): infant gender. Additional confounding for BW (g): GA at birth.						
Hjermitslev et al. (2019) *Medium*	Greenland, Recruitment: 2010–2011, 2013–2015	Cohort	Pregnant women (≥18 yr of age) and their children from ACCEPT N = 256	Maternal serum Early pregnancy, later pregnancy 8.99 (Range = 1.50–61.3)	BW (g), GA at birth (weeks), HC (cm), preterm birth	Regression coefficient per ln-unit increase in PFOS Preterm birth: OR per ln-unit increase in PFOS	BW: −5.47 (−12.6, 1.67) Females: −5.65 (−14.9, 3.55) Males: −1.9 (−14, 10.2) GA: 0.001 (−0.02, 0.03) Females: 0.002 (−0.03, 0.03) Males: −0.006 (−0.05, 0.04) HC: −0.01 (−0.04, 0.01) Females: −0.02 (−0.05, 0.01) Males: 0.005 (−0.04, 0.05) Preterm birth: 0.95 (0.87, 1.05), p-value = 0.321

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							No statistically significant associations
	ACCEPT = Adapting to Climate Change, Environmental Pollution and Dietary Transition **Confounding:** Maternal age, plasma cotinine, alcohol consumption during pregnancy, pre-pregnancy BMI, GA at birth.						
Jensen et al. (2020a) *Medium*	Denmark, 2010–2012 and follow-up at 18 mo of age	Cohort	Pregnant women and infants at 3 and 18 mo of age from Odense Child Cohort N = 593	Maternal serum 8.04 (3.82–15.45)	Ponderal index standard deviation score (SDS)	Regression coefficient per unit increase in PFOS	–0.004 (−0.03, 0.02) Birth: 0.03 (0.01, 0.05), p-value = 0.02 3 mo: −0.005 (−0.03, 0.016) 18 mo: −0.003 (−0.03, 0.02) 3 and 18 mo: no statistically significant associations
	Outcome: Ponderal index (kg/m^3) was calculated as weight (kg) divided by the length cubed (m^3) **Confounding:** Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI^2, education, smoking, sex, visit, adiposity marker at birth.						
Kashino et al. (2020) *Medium*	Japan, 2003–2009	Cohort	Mother-infant pairs from the Hokkaido Study on Environment and Children's Health N = 1,949	Plasma Later pregnancy 3.4 (2.6–4.7)	Birth HC (cm), BL (cm), BW (g)	Regression coefficient per log10-unit increase in PFOS	HC: −0.067 (−0.418, 0.283) Females: 0.001 (−0.531, 0.532) Males: −0.142 (−0.605, 0.321) Length: 0.092 (−0.311, 0.494) Females: 0.25 (−0.321, 0.821) Males: −0.019 (−0.589, 0.551) BW: −35 (−109, 39) Females: −19.9 (−128, 88.2) Males: −46.3 (−148.4, 55.8) HC, BL, and BW: no statistically significant associations overall or stratified by sex
	Confounding: GA, maternal age, pre-pregnancy BMI, parity, infant sex, maternal educational level, plasma cotinine concentration during pregnancy.						
Kishi et al. (2015) *Medium*	Japan, 2002–2005	Cross-section	Pregnant women (aged 28–34 yr) and infants from the Hokkaido	Maternal blood Mean = 5.89 (SD = 0.20)	BW (g)	Regression coefficient by quartiles	Females Q2: −70.1 (−242.5, 102.2) Q3: −39.1 (−216.1, 137.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			Study on Environment and Children's Health Females, N = 165 Males, N = 141				Q4: −186.6 (−363.4, −9.8), p-value < 0.05 p-trend = 0.031 Males Q2: −56.7 (−255.9, 142.4) Q3: 95.9 (−116.5, 308.4) Q4: 30.5 (−169.7, 230.8) p-trend = 0.187
	Results: Lowest quartile used as reference. **Confounding:** GA, maternal age, pre-pregnancy BMI, smoking and drinking during pregnancy, parity, annual household income, blood sampling period.						
Kobayashi et al. (2017) *Medium*	Japan, 2002–2005	Cross-sectional	Pregnant women at 22–35 wk gestation and infants from Hokkaido Study on Environment and Children's Health N = 177	Maternal serum 5.3 (3.9–7.2)	BL (cm), BW (g)	Regression coefficient per ln-unit increase in PFOS	Length: 0.32 (−0.19, 0.82) BW: −56 (−162.8, 50.8) Length and BW: no statistically significant associations
	Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, GA, infant sex, maternal blood sampling period.						
Kobayashi et al. (2022) *Medium*	Japan Recruitment: 2002–2005	Cohort	Mother-child pairs from the Sapporo Cohort of the Hokkaido Birth Cohort N = 372 (198 female children, 174 male children)	Maternal blood in the third trimester 5.2 (3.7–7.2) Females 5.2 (3.4–7.3) Males 5.3 (3.9–7.0)	BW (g), BL (cm)	Regression coefficient per log10-unit increase in PFOS	BW −182.3 (−336.5, −28.2), p-value = 0.021 Females: −292.1 (−504.3, −79.8), p-value = 0.007 Males: 17.7 (−207, 242.5), p-value = 0.876 BL −0.552 (−1.433, 0.328), p-value = 0.218 Females: −1.384 (−2.472, −0.297), p-value = 0.013

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Males: 0.635 (−0.832, 2.102), p-value = 0.394
	Confounding: Maternal age (continuous), pre-pregnancy BMI (continuous), maternal smoking in the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), parity (primiparous, multiparous), educational level, annual household income, cesarean section (yes/no), maternal blood sampling period, GA (continuous), infant sex.						
Kwon et al. (2016) *Medium*	Korea, 2006–2010	Cohort	Pregnant women and infants from EBGRC N = 268	Cord blood 0.64 (0.29–1.09)	BW (g)	Regression coefficient per log-unit increase in PFOS	–49.41 (−95.57, −3.25), p-value = 0.04
	EBGRC = Ewha Birth and Growth Retrospective Cohort **Comparison:** Logarithm base not specified. **Confounding:** Mother's age, pre-pregnancy BMI, past history of alcohol consumption and child's GA, gender, parity.						
Lenters et al. (2016) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cohort	Pregnant women and singleton infants from INUENDO N = 1,250	Maternal serum Later pregnancy GM = 9.357 (2-SD ln-PFOS = 1.600)	BW at term (g)	Regression coefficient per 2-SD increase in ln-PFOS	–114.36 (−206.81, −21.91), p-value = 0.015
	INUENDO = Biopersistent Organochlorines in Diet and Human Fertility **Confounding:** Study population, maternal age, pre-pregnancy BMI, parity.						
Liew et al. (2020) *Medium*	Denmark, 1996–2002	Nested case-control	Females from the Danish National Birth Cohort, N = 438	Plasma Control: 23.35 (18.1, 30.30) Cases: 24.55 (19.5, 32.25)	Miscarriage	OR per doubling of PFOS or by quartiles	1.2 (0.9,1.8) Q2: 1.1 (0.6, 1.9) Q3: 1.3 (0.8, 2.4) Q4: 1.4 (0.8, 2.4)
	Results: Lowest quartile used as the reference group. **Confounding**: Maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, parity.						
Louis et al. (2016) *Medium*	United States, 2005–2009	Cohort	Females from the LIFE Study, Ages 18–40, N = 344	Plasma Pregnant: 12.2 (8.3, 17.8) Infertile: 12.1 (7.1, 17.1)	Pregnancy loss	HR per log-unit increase in PFOS or by tertiles	0.81 (0.65, 1.00) T2: 0.81 (0.50, 1.33) T3: 0.60 (0.35, 1.03)
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI, prior pregnancy loss conditional on previous pregnancy, any alcohol consumption during pregnancy, any cigarette smoking during pregnancy.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Maisonet et al. (2012) *Medium*	Great Britain Recruitment: 1991–1992, followed up until 20 mo of age	Cohort	Pregnant women and their singleton girls assessed at birth, 9, and 20 mo from ALSPAC BW N = 422 BL N = 356 GA N = 444 Ponderal index N = 360 Weight at 20 mo N = 320 (106 upper tertile of BW, 107 middle tertile of BW, 107 lower tertile of BW)	Maternal serum during pregnancy (median 15 wk) 19.6 (Range = 3.8–112.0)	BW (g), BL (cm), GA (weeks), ponderal index (g/cm^3), weight at 20 mo (g)	Regression coefficient by tertile	BW T2: −111.71 (−208.24, −15.17) T3: −140.01 (−238.14, −41.89) p-trend = 0.0053 BL T2: −0.72 (−1.19, −0.25) T3: −0.63 (−1.11, −0.15) p-trend = 0.0103 GA T2: −0.02 (−0.39, 0.35) T3: −0.15 (−0.53, 0.23) p-trend = 0.4352 Ponderal index T2: 0.00 (−0.07, 0.06) T3: 0.05 (−0.01, 0.12) p-trend = 0.1120 Weight at 20 mo T2: 310.64 (27.19, 594.08) T3: 579.82 (301.4, 858.25) p-trend < 0.0001 Upper tertile of BW T2: 333.57 (−301.28, 968.42) T3: 596.22 (−52.98, 1245.42) p-trend = 0.0714 Middle tertile of BW T2: −262.83 (−884.25, 358.60) T3: 165.43 (−439.52, 770.37) p-trend = 0.5886 Lower tertile of BW T2: 602.64 (−150.79, 1356.07) T3: 932.71 (186.90, 1678.52) p-trend = 0.0148

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	ALSPAC = Avon Longitudinal Study of Parents and Children **Results:** Lowest tertile used as reference **Confounding:** BW: maternal smoking during pregnancy, maternal pre-pregnancy BMI, previous live births, and GA; BL additionally adjusted for maternal education. GA: GA when maternal serum sample was obtained. Ponderal index: maternal pre-pregnancy BMI, previous live births, and GA when maternal serum sample was obtained. Weight at 20 mo (all tertiles): height at 20 mo, BW, maternal education, maternal age at delivery, and previous live birth; intratertile analyses adjusted for maternal education, maternal age at delivery, previous live birth, and BW.						
Manzano-Salgado et al. (2017b) *Medium*	Spain, 2003–2008	Cohort	Mother (aged ≥16 yr)-child pairs from INMA assessed at birth and 6 mo N = 1,154 (568 girls, 586 boys)	Maternal blood GM = 5.80 (4.52–7.84)	Weight gain z-score, rapid growth	Regression coefficient or RR per log2-unit increase in PFOS	Weight gain z-score −0.02 (−0.11, 0.07) Girls: −0.09 (−0.21, 0.04) Boys: −0.05 (−0.08, 0.19) p-value for sex interaction = 0.54 Rapid growth 0.92 (0.80, 1.06)
	INMA = INfancia y Medio Ambiente [Environment and Childhood Project] **Outcome:** Rapid growth defined as a z-score >0.67 standard deviation for weight gain from birth until 6 mo. **Confounding:** Maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI), age and sex of child						
Meng et al. (2018) *Medium*	Denmark, 1996–2002	Cohort	Pregnant women and their infants from DNBC N = 3,522 (1,533 girls, 1,969 boys)	Maternal serum Early pregnancy, Later pregnancy 30.1 (22.9–39.0)	BW (g), GA (days), low BW, PTB	Regression coefficient (BW, GA) or OR (LBW, PTB) per doubling of PFOS and by quartiles	BW −45.2 (−76.8., −13.6) Q2: 24.7 (−24.8, 74.1) Q3: −50.1 (−101.1, 0.9) Q4: −48.2 (−99, 2.5) Females: −65.3 (−111.7, −18.9) Males: −24.3 (−67.1, 18.6) p-value for sex interaction = 0.31 GA −1.1 (−1.7, −0.4) Q2: −1.1 (−2.1, −0.1) Q3: −2 (−3.1, −1) Q4: −1.5 (−2.6, −0.5) Females: −1 (−2, 0) Males: −1.1 (−2.0, −0.3) p-value for sex interaction = 0.72

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							LBW 1.3 (0.9, 2.0) Q2: 1.4 (0.7, 2.8) Q3: 1.8 (0.9, 3.6) Q4: 1.2 (0.6, 2.4) PTB 1.5 (1.1, 2.2) Q2: 2.0 (1.1, 3.6) Q3: 3.3 (1.8, 5.8) Q4: 1.9 (1.0, 3.5)
	DNBC = Danish National Birth Cohort **Results:** Lowest quartile used as reference. **Confounding:** Infant sex, infant birth year, gestational week of blood draw, maternal age, parity, socio-occupational status, pre-pregnancy BMI, smoking during pregnancy, alcohol intake during pregnancy, study sample						
Ou et al. (2021) *Medium*	China, 2014–2018	Nested case-control	Pregnant women and their children with (cases) and without (controls) CHD N = 316	Maternal blood and cord blood at delivery Maternal blood Cases: 5.752 (3.655–8.683) Controls: 5.742 (4.156–6.850) Cord blood: Cases: 1.928 (0.823–3.295) Controls: 2.237 (1.505–3.072)	Septal defects, conotruncal defects, total CHD	OR for >75th percentile vs. <75th percentile PFOS	Maternal PFOS Septal defects: 1.92 (0.80, 4.60) Conotruncal defects: 1.65 (0.59, 4.63) Total CHD: 1.61 (0.91, 2.84), p-value <0.10 Cord PFOS Septal defects: 1.15 (0.38, 3.54) Conotruncal defects: 0.63 (0.16, 2.57) Total CHD: 1.03 (0.46, 2.3)
	CHD = Congenital heart defects **Outcome:** Total congenital heart defects included septal defects and conotruncal defects, as well as individual congenital heart defect subtypes with a large number of cases. **Confounding:** Maternal age, parity, infant sex.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Robledo et al. (2015) *Medium*	United States, 2005–2009	Cohort	Couples and their children from the LIFE study N = 234	Serum Early pregnancy Girls: GM = 12.44 (95% CI = 11.50, 13.44) Boys: GM = 21.6 (95% CI = 19.97, 23.39)	BW (g), HC (cm), BL (cm), ponderal index (g/cm^3)	Regression coefficient for mean change per 1-SD increase in ln(maternal PFOS) and in ln(paternal PFOS)	Maternal PFOS Girls: BW: 14.16 (−81.83, 110.15) HC: −0.04 (−0.46, 0.38) BL: 0.30 (−0.26, 0.86) Ponderal Index: −0.03 (−0.10, 0.03) Boys: BW: 37.51 (−73.45, 148.46) HC: 0.07 (−0.45, 0.60) BL: 0.22 (−0.43, 0.86) Ponderal Index: 0.00 (−0.07, 0.08) Paternal PFOS Girls: BW: 38.58 (−59.29, 136.45) HC: 0.29 (−0.14, 0.71) BL: −0.05 (−0.62, 0.52) Ponderal Index: 0.05 (−0.02, 0.11) Boys: BW: 36.85 (−73.14, 146.84) HC: 0.16 (−0.37, 0.68) BL: −0.20 (−0.84, 0.43) Ponderal Index: 0.06 (−0.02, 0.13)
	LIFE = Longitudinal Investigation of Fertility and the Environment **Confounding:** Maternal and paternal serum lipids, serum cotinine, BMI, maternal age, difference in paternal age, infant gender, individual and partner sum of remaining chemical concentrations in each chemical's respective class						
Stein et al. (2009) *Medium*	United States 2005–2006	Cohort	Pregnant women and their infants from the C8HP Birth defects N = 3,996 PTB N = 4,512 Low BW	Maternal serum within 5 yr after pregnancy 13.6 (9.0–17.7)	Birth defects, PTB, LBW	OR per IQR increase in PFOS PTB, LBW: OR by percentile	Birth defects Per IQR increase: 1.1 (0.9, 1.3) PTB Per IQR increase: 1.1 (1.0, 1.3) 50th–75th percentile: 1.1 (0.9, 1.3) 75th–90th percentile: 1.1 (0.9, 1.3) >90th percentile: 1.4 (1.1, 1.7)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 4,561				LBW Per IQR increase: 1.3 (1.1, 1.6) 50th–75th percentile: 1.3 (0.9, 1.8) 75th–90th percentile: 1.6 (1.1, 2.3) >90th percentile: 1.8 (1.2, 2.8)
	C8HP = C8 Health Project **Population:** Includes "women who lived in the same contaminated water district from the approximate start of the pregnancy through the time of enrollment… to ensure that the PFOA level measured at C8 Health Project enrollment would reflect the level at the time of pregnancy." **Outcome:** PTB defined as birth at <37 wk; low BW defined as <5.5 pounds at birth. **Results:** <50th percentile used as reference group. **Confounding:** Maternal age, parity, educational level at interview, smoking status at interview, PFOA in the analysis of PFOS.						
Tian et al. (2019b) *Medium*	China 2012–2014	Cohort	Pregnant women and their sons at birth, 6 mo, and 12 mo from the S-MBCS Birth N = 439 6-mo N = 322 12-mo N = 301	Maternal serum 10.70 (7.61–15.71)	Weight gain z-score (0–6 mo or 6–12 mo), AGDap, AGDas	Regression coefficient per ln-unit increase in PFOS or by quartiles Weight gain z-score: Pearson correlation coefficient	Weight gain z-score 0–6 mo: −0.06 6–12 mo: 0.12; p-value < 0.05 AGDap Quartile analysis showed no other statistically significant associations
	S-MBCS = Shanghai-Minhang Birth Cohort Study; AGDap = anopenile distance; AGDas = anoscrotal distance **Results:** Lowest quartile used as reference. **Confounding:** Maternal age at delivery, GA, maternal education, parity, pre-pregnancy BMI, infant age at physical examination, infant body size						
Toft et al. (2016) *Medium*	Denmark 1980–1996	Case-control	Pregnant women and their sons from the DMBR N = 270 cryptorchidism cases, 75 hypospadias cases, and 300 controls	Amniotic fluid Second exposure tertile: 0.8–1.4	Cryptorchidism, hypospadias	OR per ln-unit increase in PFOS or by tertiles	Cryptorchidism 0.99 (0.75, 1.30) T2: 1.08 (0.71, 1.63) T3: 1.01 (0.66, 1.53) Hypospadias 0.87 (0.57, 1.34) T2: 0.97 (0.51, 1.87) T3: 0.69 (0.35, 1.38)
	DMBR = Danish Medical Birth Registry						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Outcome: Cryptorchidism defined as both a diagnosis of undescended testis and a corrective surgical procedure recorded in the Danish National Patient Registry (DNPR). Hypospadias defined as diagnosis in the DNPR. **Results:** Lowest tertile used as reference **Confounding:** GA of amniocentesis, maternal age, smoking (cotinine groups), and case or control status						
Vesterholm et al. (2014) *Medium*	Denmark and Finland Recruitment 1997–2002, follow-up 3 mo after birth	Nested case-control	Boys with (cases) or without (controls) cryptorchidism N = 215	Cord blood 9.1 (5th–95th percentile: 4.8–16.4)	Cryptorchidism	OR per ln-unit increase in PFOS or by tertiles	Continuous: 0.83 (0.44, 1.58) T2: 0.70 (0.34, 1.46) T3: 0.83 (0.39, 1.78) p-trend = 0.64
	Outcome: Cryptorchidism defined as by Scorer (1964). **Exposure Level:** Denmark cases: 2.4 (5th–95th percentile: 1.4–4.4); controls: 2.70 (5th–95th percentile: 1.4, 4.0); Finland cases: 1.9 (5th–95th percentile: 1.0–3.9); controls: 2.3 (5th–95th percentile: 1.2–4.8) **Results:** Lowest tertile used as reference. **Confounding:** BW, GA, parity						
Wang et al. (2019a) *Medium*	China 2013	Cross-sectional	Pregnant women and their children at birth N = 340 (171 girls, 169 boys)	Cord blood Later pregnancy 0.65 (0.40–1.19)	BL (cm), BW (g), BW z-score, HC (cm), ponderal index (g/cm^3)	Regression coefficient per log10-unit increase in PFOS	BL, BW, HC, ponderal index: no statistically significant associations or interactions by sex BL −0.01 (−0.40, 0.39); p-value = 0.982 Girls: −0.01 (−0.60, 0.58); p-value = 0.968 Boys: −0.17 (−0.71, 0.37); p-value = 0.535 p-value for interaction by sex = 0.557 BW 54.5 (−149.07, 40.06); p-value = 0.259 Girls: −57.3 (−201.38, 86.78); p-value = 0.436 Boys: −61.6 (−184.61, 61.42); p-value = 0.326

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-value for interaction by sex = 0.844 BW z-score −0.15 (−0.41, 0.11); p-value = 0.258 HC 0.02 (−0.26, 0.29); p-value = 0.915 Girls: −0.01 (−0.42, 0.39); p-value = 0.947 Boys: −0.04 (−0.41, 0.32); p-value = 0.821 p-value for interaction by sex = 0.709 Ponderal index −0.04 (−0.09, 0.001); p-value = 0.054 Girls: −0.04 (−0.11, 0.02); p-value = 0.198 Boys: −0.02 (−0.08, 0.03); p-value = 0.427 p-value for interaction by sex = 0.637
	Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, GA, parity, pre-pregnant maternal BMI, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain						
Woods et al. (2017) *Medium*	United States, Recruitment: 2003–2006; outcome assessed at birth	Cohort	Pregnant women and their children at birth from the HOME study N = 272	Maternal serum Later pregnancy 14.4 (10–17.0)	BW (g)	Regression coefficient per log10-unit increase maternal PFOS	–8.7 (−52.8, 34.9)
	HOME = Health Outcomes and Measures of Environment **Confounding:** Maternal race, age at delivery, infant sex, maternal education, tobacco exposure, household annual income, employment, maternal insurance status, marital status, prenatal vitamin use, maternal BMI, GA						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Yang et al. (2022In Press) *Medium*	China 2018–2019	Nested case-control	Infants from the KBCS, N = 768 (403 males, 365 females) PTBs N = 384 (205 males, 179 females) Term births N = 384	Cord blood at birth Term births 0.266 (0.144–0.444) PTBs 0.213 (0.112–0.483)	PTB, GA (weeks)	PTBs: OR per IQR increase in PFOS GA: Regression coefficient per IQR increase in PFOS	PTB 1.44 (1.18, 1.79), p-value <0.01 PFAS-residuals model: 1.71 (1.26, 2.4), p-value <0.001 Males 1.45 (1.10, 2.03) Females 1.40 (1.10, 1.93) p-value for interaction by infant's sex = 0.99 GA PTBs, total −1.26 (−2.46, −0.05), p-value = 0.04 PFAS-residuals model: −2.01 (−3.42, −0.61), p-value = 0.01 PTBs, males −0.41 (−2.2, 1.37) PTBs, females −1.06 (−2.87, 0.74) p-value for interaction by infant's sex = 0.14 Term births −0.16 (−1.81, 1.48), p-value = 0.85
	KBCS = Kashgar Birth Cohort Study **Confounding:** Maternal age, maternal ethnicity, maternal BMI, household income, maternal education level, maternal tobacco smoking during pregnancy, maternal alcohol drinking during pregnancy, parity, living near a factory, periconceptional folic acid intake, gestational diabetes, gestational hypertension. Additional confounding for analyses with both sexes: infant's sex. Additional confounding for PFAS-residuals model: residuals regressed from PFDoA with PFOA, PFDA, PFUdA, PFNA, and PFTrDA.						
Callan et al. (2016) *Low*	Australia 2008–2011	Cross-sectional	Mother-infant pairs enrolled in AMETS, Ages 19–44, N = 98	Maternal blood 1.99 (0.45–8.1)	BW (g), BL (cm), Proportion of optimal BW (POBW), HC (cm),	Regression coefficient per ln-unit increase in PFOS	BW −69 (−231, 94) BL −0.22 (−1, 0.57)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					ponderal index (g/cm3 × 100), proportion of optimal birth length (POBL), proportion of optimal HC (POHC)		POBW 0.48 (−4.2, 5.2) HC −0.39 (−0.98, 0.2) Ponderal Index −0.03 (−0.14, 0.08) POBL −0.12 (−1.4, 1.7) POHC −0.6 (−2.3, 1.1)
	AMETS = Australian Maternal Exposure to Toxic Substances **Confounding:** For BW, BL, HC, and ponderal index: GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy, sex of infant. For POHC: Weight gain during pregnancy, annual household income. For POBL: Weight gain during pregnancy, maternal age, annual household income.						
Cao et al. (2018) *Low*	China, 2013–2015	Cohort	Infants from Zhoukou City, China, N = 337 (183 males, 154 females) Postnatal weight, postnatal length, postnatal HC N = 282 (157 males, 125 females)	Cord blood 1.01 (0.60–1.76)	BW (g), BL (cm), ponderal index (g/cm3), postnatal weight (g), postnatal length (cm), postnatal HC, birth defects	BW, BL, HC and ponderal index: Regression coefficient by tertiles	BW T2: 103.5 (−17.8, 224.8) T3: −17.6 (−141.2, 106) Males T2: 76.2 (−91.1, 243.6) T3: 9.6 (−165.6, 184.8) Females T2: 146.8 (−36.2, 329.9) T3: −6.7 (−184.8, 171.4) BL T2: 0.33 (−0.01, 0.68) T3: 0.07 (−0.27, 0.42) Males T2: 0.4 (−0.05, 0.84) T3: 0.27 (−0.19, 0.74) Females T2: 0.3 (−0.25, 0.86)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T3: −0.04 (−0.58, 0.5) Ponderal index T2: 0.02 (−0.07, 0.1) T3: −0.04 (−0.13, 0.06) Males T2: −0.03 (−0.17, 0.12) T3: −0.06 (−0.21, 0.09) Females T2: −0.03 (−0.17 0.12) T3: −0.06 (−0.21, 0.09) Postnatal weight T2: −138.1 (−573.7, 297.6) T3: −78.3 (−531.6, 374.9) Males T2: −427.6 (−959.2, 104) T3: −321.2 (−894.3, 252) Females T2: 239.6 (−519.6, 998.8) T3: 128 (−620.3, 876.3) Postnatal length T2: 0.08 (−1.78, 1.95) T3: −0.1 (−2.04, 1.84) Males T2: −1.05 (−3.4, 1.29) T3: 0.17 (−2.36, 2.7) Females T2: 1.07 (−2, 4.13) T3: −0.72 (−3.74, 2.31) Postnatal HC T2: 0.17 (−0.76, 1.09) T3: −0.23 (−1.19, 0.73) Males

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T2: 0.27 (−0.92, 1.45) T3: 0.28 (−1, 1.56) Females T2: −0.19 (−1.69, 1.31) T3: −1.22 (−2.7, 0.25) Birth defects T2 OR: 0.84 (0.37, 1.91) T3 OR: 1.27 (0.59, 2.73)
	Comparison: Tertiles were defined as follows: T2 = 0.74–1.52 vs. <0.74. T3 = >1.52 vs. <0.74. **Results:** Lowest tertile used as reference. **Confounding:** Maternal age, household income, parity, infant's gender. Additional confounding for BW, birth defects, ponderal index: smoking of father, drinking of father. Additional confounding for BW, birth defects, ponderal index, postnatal weight, postnatal length, POHC: maternal education. Additional confounding for postnatal weight, postnatal length, and POHC: infant's age.						
Espindola Santos et al. (2021) *Low*	Brazil Recruitment: 2017	Cross-sectional	Mother-child pairs of women enrolled in the PIPA project BW: N = 72 BL: N = 65 Weight-for-length: N = 64 HC: N = 62	Cord blood from newborns 2.06 (1.06–5.21)	BW (z-score), BL (z-score), weight-for-length (z-score), HC (z-score)	Regression coefficient per log10-unit increase in PFOS	BW 0.06 (−0.42, 0.54) BL −0.02 (−0.54, 0.50) Weight-for-length 0.38 (−0.28, 1.04) HC 0.18 (−0.46, 0.82)
	PIPA = Rio Birth Cohort Study **Population:** Mothers were recruited between 29th and 32nd weeks of gestation and were over 16 yr of age. **Exposure:** Year of assessment not reported. **Confounding:** Education, income, race, pre-gestational BMI, smoking active and passive, alcohol consumption, GA, primiparity, age (continuous), and fish consumption.						
Gennings et al. (2020) *Low*	Sweden, Recruitment: 2007–2010, Follow-up at 7 yr	Cohort	Mothers and their children (age 7) from the SELMA study N = 1,312	Prenatal serum Mean (SE) = 0.82 (0.19) log10-ng/mL	BW (g)	Regression coefficient per log10-unit increase in PFOS	BW −70.39 (SE = 16.31), p-value <0.001

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and Allergy **Confounding:** My Nutrition Index (MNI, z-score), sex, maternal smoking status, maternal weight (z-score), premature birth status, maternal education, total energy intake (z-score)						
Gross et al. (2020) *Low*	United States 2012–2014	Nested Case-control	Healthy and overweight 18-mo-old Hispanic children from StEP, N = 98	Newborn blood Mean (SD) = 0.440 (0.364)	BW (z-score), overweight	Regression coefficient (BW) and OR (overweight) for PFOS >mean level vs. PFOS ≤ mean level	BW −0.62 (−0.96, −0.29), p-value <0.00714 Overweight 0.43 (0.17, 1.09)
	StEP = Starting Early Program **Outcome:** Overweight defined as 18-mo weight-for-length z-score ≥ 85th percentile **Confounding:** Maternal age, maternal education, maternal depressive symptoms, pre-pregnancy BMI, GA, parity, intervention status.						

Notes: AC = abdominal circumference; ACCEPT = Adapting to Climate Change, Environmental Pollution and Dietary Transition; ACD = Anoclitoris distance; AFD = anofourchette distance; AGD = anogenital distance; AGDap = anopenile distance; AGDas = anoscrotal distance; ALSPAC = Avon Longitudinal Study of Parents and Children; AMETS = Australian Maternal Exposure to Toxic Substances; ASD = anoscrotal distance; BL = birth length; BMI = body mass index; BPD = biparietal diameter; BW = birth weight; C8HP = C8 Health Project; CHD = congenital heart defects; CIOB = Chemicals in our Bodies; DMBR = Danish Medical Birth Registry; DNBC = Danish National Birth Cohort; DNPR = Danish National Patient Registry; EBGRC = Ewha Birth and Growth Retrospective Cohort FL = femur length; FLEHS = Flemish Environmental and Health Study; FLEHS II = Flemish Environmental and Health Study II; GA = gestational age; GM = geometric mean; HC = head circumference; HOME = Health Outcomes and Measures of Environment; HR = hazard ratio; HUMIS = Human Milk Study; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; IOM = Institute of Medicine; IQR = interquartile range; KBCS = Kashgar Birth Cohort Study; LBW = low birth weight; LGA = large for gestational age; LIFE = Longitudinal Investigation of Fertility and the Environment; LINC = Linking EDCs in Maternal Nutrition to Child Health; LSM = least squares mean; LWBC = Laizhou Wan Birth Cohort; MIREC = Maternal-Infant Research on Environmental Chemicals; MNI = My Nutrition Index mo = months; MoBa = Norwegian Mother and Child Cohort Study; NICHD = National Institute of Child Health and Human Development; NICHD SGA = National Institute of Child Health and Human Development Scandinavian Successive Small-for-Gestational-Age Births Study; NCS = National Children's Study; NO = Norway; OR = odds ratio; PFNA = perfluorononanoic acid; PIPA = Rio Birth Cohort Study; POBL = proportion of optimal birth length; POBW = Proportion of optimal birth weight; POHC = proportion of optimal head circumference; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; PTB = preterm birth; RR = relative risk ratio; S = singletons; SBC = Shanghai Birth Cohort; SD = standard deviation; SDS = standard deviation score; SE = standard error; SE = Sweden; S-MBCS = Shanghai-Minhang Birth Cohort Study; SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and Allergy; SGA = small for gestational age; StEP = Starting Early Program; T = twins; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; TBPS = Taiwan Birth Panel Study; WAZ = weight-for-age z-score; WFL = weight-for-length; WLZ = weight-for-length z-score; wk = weeks; yr = years.

[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

D.2 Reproductive

D.2.1 Male

Table D-2. Associations Between PFOS Exposure and Male Reproductive Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Jensen et al. (2020b) *High*	Denmark 2010–2012	Cohort	Infants from Odense Child Cohort N = 208 boys	Maternal serum 8.33	Levels of FSH (IU/L), testosterone (nmol/L), LH (IU/L), testosterone /LH ratio, DHEAS (nmol/L), DHEA (nmol/L), Androstenedione (nmol/L), 17-OHP (nmol/L)	Regression coefficient (testosterone), or percent change per doubling of PFOS	No statistically significant associations
	Confounding: Age of the child at examination time, maternal parity[c]						
Lind et al. (2017a) *High*	Denmark 2010–2012	Cohort	Infants from Odense Child Cohort N = 649 (296 boys)	Maternal serum Total cohort: 8.1	Penile width (mm), anogenital distance (AGD; scrotal, as; penile, ap) (mm)	Regression coefficient per ln-unit increase in PFOS, or by quartiles	AGDas Continuous: 1.2 (−0.4, 2.7) Q2: 0.9 (−0.9, 2.8) Q3: 0.9 (−0.8, 2.7) Q4: 1.9 (0.04, 3.7) p-trend by quartiles = 0.06 AGDap, penile width: no statistically significant associations AGDap: p-trend by quartiles = 0.55 Penile width: p-trend by quartiles = 0.67

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, WAZ, pre-pregnancy BMI, parity, smoking						
Itoh et al. (2016) *Medium*	Japan 2002–2005	Cohort	Infants from Sapporo Cohort of the Hokkaido Study on Environment and Children's Health N = 83 boys	Maternal serum 5.40	In cord blood, log10-transformed levels of E2 (ng/mL), FSH (mIU/mL), Inhibin B (pg/mL), insulin-like 3 (ng/mL), LH (mIU/mL), progesterone (ng/mL), prolactin (ng/mL), SHBG (not log10-transformed, nmol/L), testosterone (pg/mL) Testosterone/E2 ratio, testosterone/SHBG ratio	Regression coefficient per log10-unit increase in PFOS, LSM by quartiles	E2 0.372 (0.057, 0.687) p-value = 0.021 Q1: 4.34 (3.07, 6.15) Q2: 5.84 (4.34, 8.01) Q3: 8.74 (6.33, 12.05) Q4: 6.39 (4.52, 8.98) p-trend = 0.027 Inhibin B −0.439 (−0.620, 0.257) p-value < 0.001 Q1: 53.4 (42.4, 65.6) Q2: 50.1 (41.2, 60.5) Q3: 39.1 (31.8, 47.6) Q4: 33.3 (26.6, 40.0) p-trend < 0.001 Progesterone −0.344 (−0.678, 0.01) p-value = 0.043 Q1: 238.5 (161.5, 354.9) Q2: 267.6 (192, 375.3) Q3: 241.5 (168.7, 346.2) Q4: 184.7 (126.5, 267.6) p-trend = 0.231 Testosterone/E2 −0.399 (−0.643, −0.156) p-value = 0.002 Q1: 20.3 (15.2, 26.8) Q2: 19.5 (15.2, 24.8) Q3: 14.5 (10.7, 18.6) Q4: 14.5 (10.8, 18.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							p-trend = 0.015 FSH, insulin-like 3, LH, prolactin, SHBG, testosterone, testosterone/SHBG: No statistically significant associations or trends
	Confounding: Age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement, gestational age at birth						
Lopez-Espinosa et al. (2016) *Medium*	United States 2005–2006	Cross-sectional	Male children ages 6–9 yr N = 1,169	Serum 22.4	Total testosterone (ln-ng/dL)	Percent difference between 75th and 25th percentile of ln-unit PFOS or by quartiles	Total testosterone: −5.8 (−9.4, −2.0) Q2: −4.2 (−11.4, 3.6) Q3: −9.2 (−16.1, −1.6) Q4: −11.8 (−18.6, −4.3) p-trend = 0.002
	Results: Results by quartile used lowest quartile as reference. **Confounding:** Age, month and time of sampling						
Goudarzi et al. (2017a) *Medium*	Japan 2002–2005	Cohort	Children from the Hokkaido Study N = 185 (81 males)	Serum Total cohort: 5.20	Levels (log10 ng-mL) of DHEA, androstenedione	Regression coefficient per log10-unit increase in PFOS or by quartiles	Among males DHEA: 0.308 (0.099, 0.755); p-value = 0.011 Androstenedione: −0.011 (−0.312, 0.284); p-value = 0.926
	Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, and blood sampling period						
Ernst et al. (2019) *Medium*	Denmark 1999–2017	Cohort	Children from the Puberty Cohort of the Danish National Birth Cohort N = 565 boys	Maternal blood Sample 1: 31.9 Sample 2: 27.2	Age (months) at axillary hair attainment, voice break, first ejaculation, Tanner stages 2–5 for genital development or pubic hair growth; combined sex-	Regression coefficient per log2-unit increase in first trimester maternal serum PFOS Puberty indicator: mean difference in	No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					specific puberty indicator	age at puberty by tertiles	
	Confounding: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy BMI, and daily number of cigarettes smoked in first trimester						
Tian et al. (2019b) *Medium*	China 2012–2013	Cohort	Male infants at birth, 6 mo, and 12 mo N = 500	Maternal plasma 10.70	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln-unit increase in maternal PFOS or by quartiles	AGDap GEE (Birth, 6 mo, and 12 mo): −0.34 (−1.38, 0.69); p-value = 0.516 Birth: −0.04 (−0.78, 0.69); p-value = 0.925 6 mo.:−1.20 (−3.29, 0.88); p-value = 0.262 12 mo.: 0.69 (−1.83, 3.22); p-value = 0.589 Q2: 1.57 (−1.95, 5.09) Q3: 5.17 (1.53, 8.81); p-value < 0.05 Q4: −0.49 (−4.04, 3.07) AGDas GEE (Birth, 6 mo, and 12 mo): −0.83 (−1.71, 0.06); p-value = 0.067 Birth: −0.65 (−1.27, −0.02); p-value = 0.0429 Q2: 0.17 (−0.79, 1.13) Q3: −0.10 (−1.10, 0.90) Q4: −1.46 (−2.44, −0.49); p-value < 0.05 p-value for trend < 0.05 6 mo.: −2.21 (−4.28, −0.14); p-value = 0.0372 12 mo.: 0.47 (−1.63, 2.58); p-value = 0.6587
	Results: Lowest quartile used as reference.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age at delivery, gestational age, maternal education, parity, pre-pregnancy BMI, infant age at physical examination, and infant body size (birth weight at birth; WLZ at 6 and 12 mo of age)						
Wang et al. (2019a) *Medium*	China 2013	Cross-sectional	Pregnant women and their children N = 340 (169 boys)	Cord blood Total cohort: 0.65 (0.40–1.19)	Levels (log10-ng/mL) of estrone (E1), E2, estriol (E3)	Regression coefficient per log10-unit increase in PFOS	E1: 0.071 (−0.05, 0.18); p-value = 0.247 E2: 0.02 (−0.10, 0.14); p-value = 0.761 E3: 0.36 (0.16, 0.55); p-value < 0.001
	Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, gestational age, parity, pre-pregnant maternal BMI, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain						
Arbuckle et al. (2020) *Medium*	Canada 2008–2011	Cohort	Newborns from the MIREC cohort N = 205 boys	Maternal plasma 4.4	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln-unit increase in maternal PFOS or by quartiles	AGDap Per ln increase: 0.13 (−1.13, 1.38) Q2: −0.97 (−2.81, 0.87) Q3: −1.28 (−3.22, 0.66) Q4: 0.22 (−1.68, 2.13) p-value for trend = 0.908 AGDas Per ln increase: 1.05 (−0.24, 2.35) Q2: −0.87 (−2.78, 1.04) Q3: 0.33 (−1.67, 2.33) Q4: 0.49 (−1.47, 2.46) p-value for trend = 0.3936
	Results: Lowest quartile used as reference. **Confounding:** AGDap: recruitment site, education, active smoking status, gestational age; AGDas: household income, active smoking status, gestational age						
Zhou et al. (2016) *Low*	Taiwan 2009–2010	Cross-sectional	Adolescents ages 13–15 N = 225 (102 boys)	Serum Total: 28.9 Boys: 29.9	Levels (ln-transformed) of E2 (pmol/L), testosterone (nmol/L)	Regression coefficient per unit increase in PFOS	Testosterone, boys: −0.0029 (−0.0055, −0.0003) p-value for interaction by sex = 0.060 E2: No statistically significant associations or interactions
	Confounding: Age, sex, BMI, environmental tobacco smoke exposure, parental education, regular exercise, month of survey						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Zhou et al. (2017c) *Low*	Taiwan 2009–2010	Case-control	Children ages 10–15 with (cases) or without (control) asthma N = 231 cases, 225 controls	Serum Cases: 33.94 Controls: 28.91	Levels of testosterone (ln-nmol/L)	Regression coefficient per unit increase in PFOS	Testosterone Cases: −0.004 (−0.005, −0.003) Controls: −0.002 (−0.008, 0.003)
	Confounding: Age, sex, BMI, parental education, environmental tobacco smoke exposure, physical activity, month of survey						
Di Nisio et al. (2019) *Low*	Italy 2017–2018	Cross-sectional	Male high school students N = 100 (50 unexposed controls, 50 exposed)	Serum Unexposed controls: 0.82 Exposed: 1.11 Semen Unexposed controls: 0.11 Exposed: 0.11	AGD (cm), crown-to-pubis distance (cm), pubis-to-floor distance (cm), crown-to-pubis/pubis-to-floor ratio, penis circumference (cm), penis length (cm), testicular volume (mL), normal morphology (%), semen pH, immotile sperm (%), nonprogressive motility (%), progressive motility (%), total sperm count (10^6), semen volume (mL), sperm concentration (10^6/mL), viability (%), FSH (U/L), testosterone (nmol/L)	Mann-Whitney test (Exposed vs. Controls)	AGD Controls: 4.50 (4.0, 5.2) Exposed: 4.00 (3.5, 5.0) Adjusted p-value for comparison of medians = 0.114 Pubis-to-floor distance Controls: 97.0 (93.0, 101.1) Exposed: 95.0 (90.3, 99.0) Adjusted p-value for comparison of medians = 0.320 Penis circumference Controls: 10.10 (9.9, 11.0) Exposed: 9.50 (9.0, 10.0) Adjusted p-value for comparison of medians < 0.001 Penis length Controls: 10.0 (9.0, 11.0) Exposed: 9.00 (8.0, 10.0) Adjusted p-value for comparison of medians < 0.001 Testicular volume Controls: 16.13 (14.8, 19.0) Exposed: 14.00 (12.6, 16.0)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							Adjusted p-value for comparison of medians < 0.001 Normal morphology Controls: 7.0 (4.0, 12.0) Exposed: 4.0 (2.0, 6.0) Adjusted p-value for comparison of medians < 0.001 Semen pH Controls: 7.60 (7.5, 7.7) Exposed: 7.70 (7.6, 7.7) Adjusted p-value for comparison of medians = 0.042 Testosterone Controls: 18.98 (12.9, 17.9) Exposed: 18.98 (16.3, 21.8) Adjusted p-value for comparison of medians < 0.001 Crown-to-pubis, Crown-to-pubis/pubis-to-floor, sperm motility, sperm count, semen volume, sperm concentration, viability, FSH: No statistically significant associations after adjusting for comparison of medians
	Results: Values for each outcome are reported as median (25^{th}–75^{th} percentile). **Confounding:** Age						
General Population							
Cui et al. (2020) *Medium*	China 2015–2016	Cross-sectional	Adult men N = 651	Serum 9.94	Serum levels (ln-transformed) of E2 (pmol/L), FSH	Percent change per ln-unit increase in serum or semen	SHBG Serum PFOS: −4.94 (−8.71, −1.02); p-value = 0.014

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
				Semen 0.15	(IU/L), LH (IU/L), SHBG (nmol/L), free testosterone, total testosterone (nmol/L); free androgen index, total testosterone/LH ratio	PFOS, or by quartiles	p-trend by quartiles = 0.004 Ages ≤ 30: −3.11 (−6.58, 0.48); p-value = 0.069 Semen PFOS: −5.29 (−8.94, −1.49); p-value = 0.007 p-trend by quartiles = 0.026 Ages ≤ 30: −3.13 (−6.25, −0.10); p-value = 0.009 Total testosterone Serum PFOS: −3.36 (−6.40, −0.22); p-value = 0.036 p-trend by quartiles = 0.022 Ages ≤ 30: −4.25 (−7.77, −0.59); p-value = 0.023 Semen PFOS: −4.20 (−7.13, −1.18); p-value = 0.007 p-trend by quartiles = 0.019 Ages ≤ 30: −4.82 (−7.96, −1.58); p-value = 0.004 Total testosterone/LH, Serum PFOS: −4.53 (−8.99, 0.15); p-value = 0.058 p-trend by quartiles = 0.044 Semen PFOS: −5.00 (−9.32, −0.48); p-value = 0.031 p-trend by quartiles = 0.042 No statistically significant associations by age groups E2, FSH, free androgen, LH, free testosterone: No statistically significant associations or trends
	Confounding: Age, BMI, smoking status, blood sampling time, fasting status						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Petersen et al. (2018) *Medium*	Denmark 2007–2009	Cross-sectional	Faroese men born between 1981 and 1984 N = 263	Serum 19.5	Levels (log-transformed) of E2 (nmol/L), FSH (IU/L), free testosterone (pmol/L), inhibin B (pg/mL), LH, (IU/L), SHBG (nmol/L), testosterone (nmol/L) Ratios of free testosterone/E2, free testosterone/LH, Inhibin B/FSH, testosterone/E2, testosterone/LH Normal morphology (%), motile sperm (logit-%), total sperm count ($(10^6)^{1/3}$) semen volume ($mL^{1/3}$), sperm concentration ($(10^6/mL)^{1/3}$)	Regression coefficient per log-unit increase in PFOS	LH: 0.35 (0.02, 0.68); p-value = 0.04 SHBG: 0.31 (0.02, 0.60); p-value = 0.04 No other statistically significant associations
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI groups, current smoking, time of sampling						
Kvist et al. (2012) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cross-sectional	Male partners of pregnant women from INUENDO N = 359	Serum Mean Greenland: 51.65 Poland: 12.12 Ukraine: 8.20	Y:X-chromosome ratio of sperm	Linear regression adjusted r^2	0.016; p-value = 0.026
	Confounding: Age, abstinence time, alcohol intake and CB-153						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Leter et al. (2014) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cross-sectional	Male partners of pregnant women from INUENDO N = 262	Serum Mean = 27.2	Sperm DNA methylation level (% 5-mC) at LINE-1, Alu, or Sat-alpha; global DNA methylation level (FCM DGML channel no.)	Regression coefficient per ln-unit increase in PFOS	Sat-alpha Total: 1.1 (−3.1, 5.3) Greenland: −1.8 (−8.6, 5.1) Poland: −7.2 (−16, 1.6) Ukraine: 8.2 (0.6, 15.8) Global Total: −21 (−63.2, 21.3) Greenland: −32.1 (−105.6, 41.3) Poland: −108.4 (−191.5, −25.2) Ukraine: 27.2 (−43.1, 97.6) LINE-1, Alu: No statistically significant associations
	Confounding: Site, age (ln-transformed), smoking status						
Pan et al. (2019) *Medium*	China 2015–2016	Cross-sectional	Adult men in Nanjing N = 664	Serum 8.378 Semen 0.097	Sperm normal morphology (%), count ($(10^6)^{1/3}$), concentration ($(10^6/mL)^{1/3}$), progressive motility (%), curvilinear velocity (VCL) (μm/s); straight-line velocity (VSL) (μm/s), DNA fragmentation index (DFI) (ln-%), high DNA stainability (HDS) (ln-%); semen volume (ln-mL)	Regression coefficient per ln-unit increase in serum or serum PFOS, or by quartiles	No statistically significant associations by serum PFOS levels; following results are by semen PFOS Progressive motility: −1.700 (−2.867, −0.532); p-value = 0.03 Q2: −2.30 (−5.27, 0.68) Q3: −1.53 (−4.61, 1.56) Q4: −5.54 (−8.72, −2.36) p-trend = 0.01 VCL: −0.767 (−1.447, −0.087); p-value = 0.1 Q2: −1.60 (−1.50, 2.01) Q3: −2.78 (−2.40, 1.10)[d] Q4: −4.8 (−2.97, −0.72) p-trend = 0.1

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							VSL: −0.773 (−1.337, −0.209); p-value = 0.04 Q2: −1.00 (−2.44, 0.45) Q3: −1.40 (−2.89, 0.09) Q4: −2.06 (−3.60, −0.52) p-trend = 0.1 DFI: 0.087 (0.033, 0.142); p-value = 0.02 Q2: 0.03 (−0.11, 0.17) Q3: 0.08 (−0.07, 0.22) Q4: 0.25 (0.10, 0.40) p-trend = 0.01 Normal morphology, sperm count, sperm concentration, sperm HDS, semen volume: No statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Age, BMI, BMI^2, smoking, alcohol intake, abstinence time						

Notes: 17-OHP = 17-hydroxyprogesterone; AGD = anogenital distance; AGDap = anopenile distance; AGDas = anoscrotal distance; BMI = body mass index; DHEA = dehydroepiandrosterone; DFI = DNA fragmentation index; DNA = deoxyribonucleic acid; E1 = estrone; E2 = estradiol; E3 = estriol; FCM DGML = flow cytometric sperm DNA global methylation assay; FSH = follicle stimulating hormone; GEE = generalized estimating equation; HDS = high DNA stainability; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; LH = luteinizing hormone; LSM = least squares mean; MIREC = Maternal-Infant Research on Environmental Chemicals; mo = months; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SHBG = sex hormone binding globulin; VCL = curvilinear velocity; VSL = straight-line velocity; WAZ = weight-for-age z-score; yr = years.

[a] Exposure levels reported as median in ng/mL unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

[d] Values are reproduced as reported in publication.

D.2.2 Female

Table D-3. Associations Between PFOS Exposure and Female Reproductive Effects in Female Children and Adolescents

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Jensen et al. (2020b) *High*	Denmark, 2010–2012	Cohort	Female infants from the Odense Child Cohort, Age 4 mo, N = 165	Maternal serum 8.07 (5^{th}–95^{th} percentile = 4.21, 15.50)	Levels of 17-OHP (nM), androstenedione (nM), DHEA (nM), DHEAS (nM), FSH (IU/L), LH (IU/L)	Percent change per doubling in PFOS	17-OHP 2.1 (−11.9, 18.2) Androstenedione 0.6 (−14.3, 18.2) DHEA −9.4 (−22.5, 5.9) DHEAS −10.4 (−28.4, 12.2) FSH 0.2 (−12.5, 14.7) LH 9.5 (−12.8, 37.6)
	Confounding: Age of the child at examination time, maternal parity[c]						
Lind et al. (2017a) *High*	Denmark 2010–2012	Cohort	Infants from Odense Child Cohort N = 649 (353 girls)	Maternal serum Total cohort: 8.1	Anogenital distance (AGD) (mm); clitoral (AGDac), fourchette (AGDaf)	Regression coefficient per ln-unit increase in PFOS, or by quartiles	AGDac Continuous: −2.3 (−3.8, −0.7) Q2: −1.0 (−2.6, 0.6) Q3: −1.7 (−3.5, 0) Q4: −2.8 (−4.5, −1.1) p-trend by quartiles < 0.01 AGDaf Continuous: −0.4 (−1.6, 0.8) No statistically significant associations by quartiles, p-trend by quartiles = 0.31
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, WAZ, pre-pregnancy BMI, parity, smoking.						
Yao et al. (2019) *High*	China, 2010–2013	Cross-sectional	Pregnant women (aged > 18 yr)	Cord blood 1.39 (0.92, 2.01)	Testosterone (log10-ng/mL), Estradiol (log10-pg/mL),	Regression coefficient per log10-unit increase in PFOS	Testosterone 0.15 (0.01, 0.29), p-value < 0.05 Estradiol 0.24 (−0.05, 0.07)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
			and female infants N = 171		Testosterone-to-estradiol ratio (log10-transformed)		Testosterone-to-estradiol ratio 0.14 (0.01, 0.27), p-value < 0.05
	Confounding: Maternal age, pre-pregnancy BMI, parity, mode of delivery, passive smoking during pregnancy, gestational age, household income level among male and female infants separately						
Donley et al. (2019) *Medium*	United Kingdom, Recruitment 1991–1992, outcome assessed at adolescence	Nested case-control	Mothers and their daughters from the ALSPAC, N = 446	Maternal serum 19.8 (15.1, 24.9)	AMH (log10-ng/mL)	Regression coefficient per unit increase in PFOS	Complete AMH data: 0.24 (0.00, 0.02) Multiple imputation model: 0.01 (0.00, 0.015)
	Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education						
Ernst et al. (2019) *Medium*	Denmark, Recruitment 1996–2002, outcome assessed 2012–2017	Cohort	Female adolescents from the Danish National Birth Cohort, N = 555	Maternal blood Sample 1 (N = 366): 32.3 (10^{th}–90^{th} percentiles = 19.3, 50.8)	Breast development, pubic hair development, age at attainment of axillary hair (months), age at menarche, age at attainment of combined puberty indicator	Combined puberty indicator: Mean difference by tertiles of PFOS All other outcomes: Regression coefficient per log2-unit increase in PFOS	Combined puberty indicator T2: −3.73 (−6.59, −0.87) T3: −0.17 (−2.83, 2.49) Breast development −3.01 (−7.96, 1.95), p-value = 0.03 Pubic hair development 1.81 (−2.42, 6.04) Axillary hair 0.50 (−2.79, 3.79), p-value = 0.02 Menarche −0.68 (−3.13, 1.77)
	Exposure Levels: [Sample 2] Median = 27.9 ng/mL (10^{th}–90^{th} percentiles = 16.5, 42.2 ng/mL). Samples 1 and 2 combined for analysis. **Outcome**: Age in months at Tanner stage 5 used to measure breast development and pubic hair development. For combined puberty indicator, lowest tertile was used as the reference group. **Confounding**: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy BMI, daily number of cigarettes smoked in first trimester						
Goudarzi et al. (2017a) *Medium*	Japan, 2002–2005	Cohort	Pregnant women and their infants from the Hokkaido Study on the	Maternal serum 5.20 (1.50, 16.20)	Levels of androstenedione (log10-ng/mL), DHEA (log10-ng/mL)	Regression coefficient per log10-unit increase in PFOS	Androstenedione 0.004 (−0.29, 0.30), p-value = 0.059 DHEA 0.24 (−0.02, 0.80)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
			Environment and Children's Health, N = 104				
	Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period						
Itoh et al. (2016) *Medium*	Japan, 2002–2005	Cohort	Female infants from the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health, N = 106	Maternal serum 5.15 (3.45, 7.00)	Cord blood levels of estradiol (log10-ng/mL), testosterone (log10-pg/mL), prolactin (log10-ng/mL), progesterone (log10-ng/mL), SHBG (nmol/L); testosterone-to-SHBG ratio, testosterone-to-estradiol ratio	Regression coefficient per log10-unit increase in PFOS	Estradiol 0.08 (−0.15, 0.31) Testosterone 0.07 (−0.26, 0.40) Prolactin −0.49 (−0.76, −0.22), p-value = 0.001 Progesterone −0.55 (−0.89, −0.21), p-value = 0.002 SHBG −0.18 (−0.42, 0.06) Testosterone/SHBG ratio 0.25 (−0.16, 0.66) Testosterone/estradiol ratio −0.01 (−0.03 0.26)
	Confounding: Maternal age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement, gestational age at birth						
Liu et al. (2020b) *Medium*	China, 2013–2014	Cross-sectional	Female neonates, N = 191	Cord blood 4.15 (2.81, 6.18)	Levels of 17-OHP (ng/mL), progesterone (ng/mL)	Percent change per IQR increase in PFOS	17-OHP −1.27 (−7.52, 5.39) Progesterone −1.68 (−6.93, 3.88)
	Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education status, passive smoking during smoking, parity, gestational weeks, sample-collection time						
Lopez-Espinosa et al. (2016) *Medium*	United States, 2005–2006	Cohort	Females from the C8 Health Project, Ages 6–9,	Serum 20.9 (15.3, 29.4)	Levels of estradiol (ln-pg/mL), total testosterone (ln-ng/dL)	Percent difference for 75th vs. 25th percentiles, or by quartiles	Estradiol 75th vs. 25th percentiles −0.3 (−4.6, 4.2), p-value = 0.048 Q2: 5.2 (−3.7, 14.9)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
			N = 1,123				Q3: 3.7 (−5.2, 13.4) Q4: −1.3 (−9.9, 8.2) Testosterone 75th vs. 25th percentiles –6.6 (−10.1, −2.8) Q2: −1.1 (−8.6, 7.1) Q3: −7.8 (−15.0, −0.1) Q4: −11.1 (−18.2, −3.5)
	Results: Lowest quartile used as the reference group. **Confounding**: Age, month, time of sampling						
Maisonet et al. (2015a) *Medium*	United Kingdom, 1991–1992	Cohort	Female adolescents from ALSPAC, Age 15, N = 72	Maternal serum 19.2 (15.1, 25.0)	Levels of serum total testosterone (nmol/L), SHBG (nmol/L)	Regression coefficient by tertiles of PFOS	Testosterone T2: 0.1 (−0.07, 0.28) T3: 0.18 (0.01, 0.35) SHBG T2: −2.86 (−18.8, 13.09) T3: 3.46 (−12.06, 18.98)
	Results: Lowest tertile used as the reference group. **Confounding**: Maternal education, maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, time of day daughter's blood sample was obtained, daughter's age at menarche, daughter's BMI at 15 yr. SHBG concentration included in testosterone model.						
Tsai et al. (2015) *Medium*	Taiwan, 2006–2008	Cross-sectional	Female adolescents, Ages 12–17, N = 95	Serum, 8.65 (5.37, 13.29)	Levels of serum FSH (ln-mIU/mL), serum SHBG (ln-nmol/L)	Means by quartiles of PFOS	FSH Q1: 1.56 (SE = 0.23) Q2: 1.67 (SE = 0.23) Q3: 1.36 (SE = 0.19) Q4: 1.23 (SE = 0.35) SHBG Q1: 3.58 (SE = 0.29) Q2: 3.36 (SE = 0.29) Q3: 3.49 (SE = 0.24) Q4: 3.41 (SE = 0.44)
	Confounding: Age, BMI, high-fat diet						
Wang et al. (2019a) *Medium*	China, 2013	Cross-sectional	Pregnant women and their children, N = 171	Cord blood 0.65 (0.40, 1.19)	Levels of estrone (log10-ng/mL), β-estradiol (log10-	Regression coefficient per ln-unit increase in PFOS	Estrone 0.15 (0.04, 0.26), p-value = 0.007 β-estradiol

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
					ng/mL), estriol (log10-ng/mL)		−0.17 (−0.31, −0.02), p-value = 0.023 Estriol 0.48 (0.27, 0.70), p-value < 0.001
	Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, gestational age, parity, pre-pregnant maternal BMI, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain						

Notes: 17-OHP = 17-hydroxyprogesterone; AGD = anogenital distance; ALSPAC = Avon Longitudinal Study of Parents and Children; AMH = anti-Mullerian hormone; BMI = body mass index; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle stimulating hormone; LH = luteinizing hormone; mo = months; SHBG = sex hormone binding globulin; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; WAZ = weight-for-age z-score; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

Table D-4. Associations Between PFOS Exposure and Female Reproductive Health Effects in Pregnant Women

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Huo et al. (2020) *High*	China, 2013–2016	Cohort	Females from the Shanghai Birth Cohort Study, Ages > 20, N = 3,220	Plasma 9.36 (6.57, 13.69)	Gestational hypertension, Preeclampsia/Eclampsia	OR per ln-unit increase in PFOS	Gestational hypertension 0.91 (0.57, 1.43) Preeclampsia/Eclampsia: 1.24 (0.82, 1.90)
	Confounding: Maternal age, pre-pregnancy BMI, parity, parental educational levels, gestational age of blood drawn, fetal sex[c]						
Mitro et al. (2020) *High*	United States, Recruitment 1999–2002, outcome assessed 3-yr postpartum	Cohort	Females from Project Viva, N = 812	Plasma 24.7 (18.1, 33.9)	Sex hormone binding globulin (nmol/L)	Percent difference per log2-unit increase in PFOS	Sex hormone binding globulin: −0.6 (−7.6, 6.9) Ages ≤ 35: −0.8 (−11.9, 11.7) Ages ≥ 35: −1.5 (−10.0, 7.8)
	Confounding: Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Borghese et al. (2020) *Medium*	Canada, 2008–2011	Cohort	Females from the MIREC study, Ages > 18, N = 1,739	Plasma GM = 4.56 (95% CI: 4.44, 4.69)	DBP (mmHg), SBP (mmHg), preeclampsia, gestational hypertension	Regression coefficient (DBP, SBP), OR (preeclampsia, gestational hypertension) per log2-unit increase in PFOS or by tertiles	DBP Trimester 1 to delivery: 0.47 (0.10, 0.85) Trimester 1: 0.46 (0.01, 0.90) Trimester 2: 0.33 (−0.10, 0.76) Trimester 3: 0.66 (0.18, 1.14) SBP Delivery: 1.19 (0.28, 2.1) Preeclampsia 1.25 (0.84, 1.82) T2: 1.72 (0.77, 3.82) T3: 1.55 (0.68, 3.49) Gestational hypertension 1.15 (0.91, 1.45) T2: 1.43 (0.90, 2.29) T3: 1.38 (0.84, 2.23)
	Results: Lowest tertile used as the reference group. **Confounding**: Maternal age, education, smoking status, pre-pregnancy BMI, parity						
Huang et al. (2019b) *Medium*	China, 2011–2012	Cross-sectional	Females from mother-infant pairs, N = 687	Plasma 2.38 (1.81, 3.23)	Gestational hypertension, preeclampsia	OR per increase in standardized PFOS	Gestational hypertension 0.87 (0.57, 1.34) Preeclampsia 0.83 (0.52, 1.32)
	Comparison: Standardized PFOS calculated by subtracting PFOS concentration from mean PFOS concentration and dividing by the SD. **Confounding**: Age, pre-pregnancy BMI, parity, education level						
Lyngsø et al. (2014) *Medium*	Greenland, 2002–2004	Cross-sectional	Pregnant women from the INUENDO cohort, N = 1,623	Serum, 8.0 (10th–90th percentile = 3.6, 25.6)	Menstrual cycle length (long), irregularity	OR per log-unit increase in PFOS or by tertiles	Length 1.1 (0.8, 1.6) T2: 1.3 (0.8, 2.2) T3: 1.2 (0.6, 2.5) Irregularity 1.2 (0.9, 1.8) T2: 1.1 (0.6, 2.1) T3: 1.7 (0.8, 3.5)
	Results: Lowest tertile used as the reference group. **Comparison:** Logarithm base not specified. **Confounding**: Age at menarche, age at pregnancy, parity, pre-pregnancy BMI, smoking, country						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Romano et al. (2016) *Medium*	United States, 2003–2006	Cohort	Females from the HOME study, Ages > 18, N = 336	Serum 13.9 (9.6, 18.2)	Breastfeeding termination (by 3 mo postpartum), Breastfeeding termination (by 6 mo postpartum)	RR by quartiles of PFOS	Termination at 3 mo Q2: 1.08 (0.79, 1.46) Q3: 1.39 (1.04, 1.88) Q4: 1.32 (0.97, 1.79) Termination at 6 mo Q2: 1.17 (0.93, 1.48) Q3: 1.16 (0.91, 1.48) Q4: 1.25 (0.98, 1.58)
	Results: Lowest quartile used as the reference group. **Confounding**: Maternal age at delivery, household income, total weeks of prior breastfeeding, gestational week at blood draw, marital status, race, parity, maternal serum cotinine during pregnancy, alcohol use during pregnancy						
Rylander et al. (2020) *Medium*	Sweden, 1989	Case-control	Females with or without pre-eclampsia, Ages 15–44, N = 876	Serum Primiparous cases: 12.9 (Minimum, maximum = 2.15, 50.0)	Preeclampsia	OR by quartiles of PFOS	Q2: 0.81 (0.5, 1.32) Q3: 1.23 (0.78, 1.93) Q4: 0.96 (0.60, 1.53)
	Exposure Levels: [Multiparous cases] Median = 10.9 ng/mL (Minimum, maximum = 1.49, 66.6 ng/mL); [Primiparous controls] Median = 12.4 ng/mL (Minimum, maximum = 0.52, 54.5 ng/mL); [Multiparous controls] Median = 9.36 ng/mL (Minimum, maximum = 1.13, 47.0 ng/mL) **Results:** Lowest quartile used as the reference group. **Confounding**: Maternal age, BMI in early pregnancy, maternal smoking in early pregnancy, parity						
Timmermann et al. (2017b) *Medium*	Denmark, 1997–2000, 2007–2009	Cohort	Pregnant and postpartum females, N = 987	Serum 19.47 (8.67, 28.22)	Total breastfeeding duration (months), Exclusive breastfeeding duration (months)	Regression coefficient per doubling of PFOS	Total breastfeeding duration −1.4 (−2.1, −0.6) Exclusive breastfeeding duration −0.3 (−0.6, −0.1)
	Confounding: Cohort, maternal age, pre-pregnancy BMI, pregnancy alcohol intake, pregnancy smoking, education, employment, parity						
Toft et al. (2016) *Medium*	Denmark 1980–1996	Case-control	Pregnant females and their male infants, N = 545	Amniotic fluid Tertile 2: (Range: 0.8, 1.4)	Amniotic fluid levels of 17-OHP (ln-nmol/L), androstenedione (ln-nmol/L), DHEAS (ln-nmol/L), progesterone (ln-nmol/L), testosterone (ln-nmol/L)	Percent difference in median level per 1% increase in PFOS or by tertiles	17-OHP 0.15 (0.11, 0.20) T2: 7 (−1, 13) T3: 18 (11, 26) p-value for trend < 0.001 Androstenedione 0.15 (0.10, 0.21) T2: 8 (0, 17)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							T3: 17 (8, 25) p-value for trend = 0.001 DHEAS 0.07 (−0.03, 0.16) T2: 5 (−10, 20) T3: 2 (−14, 17) p-value for trend = 0.93 Progesterone 0.21 (0.14, 0.29) T2: 11 (0, 23) T3: 22 (11, 34) p-value for trend = 0.001 Testosterone 0.16 (0.09, 0.23) T2: 9 (−2, 20) T3: 18 (7, 29) p-value for trend = 0.002
	Results: Lowest tertile used as the reference group. **Confounding:** Gestational age of amniocentesis, maternal age, smoking (cotinine groups), case or control status.						
Wikström et al. (2019) *Medium*	Sweden, 2007–2010	Cohort	Females from the SELMA study, Ages 28–35, N = 1,773	Serum 5.39 (3.95, 7.61)	Preeclampsia	OR per log2 increase in PFOS or by quartiles	1.53 (1.07, 2.20) Q4: 2.68 (1.17, 6.12)
	Results: Lowest quartile used as the reference group **Confounding**: Parity, women's age, body weight, smoke exposure						

Notes: 17-OHP = 17-hydroxyprogesterone; BMI = body mass index; DBP = diastolic blood pressure; DHEAS = dehydroepiandrosterone sulfate; GM = geometric mean; HOME = Health Outcomes and Measures of the Environment; HR = hazard ratio; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; LIFE = Longitudinal Investigation of Fertility and the Environment Study; MIREC = Maternal-Infant Research on Environmental Chemicals; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = relative risk ratio; SBP = systolic blood pressure; SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

Table D-5. Associations Between PFOS Exposure and Female Reproductive Health Effects in Non-Pregnant Adult Women

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Ding et al. (2020) *High*	United States, 1999–2017	Cohort	Pre-menopausal women from the Study of Women's Health Across the Nation, Ages 42–52, N = 1,120	Serum Sm-PFOS: 7.2 (4.6, 10.8) n-PFOS: 17.1 (12.2, 24.5)	Natural menopause	HR per doubling increase in PFOS or by tertiles	Sm-PFOS: 1.08 (0.99, 1.19) T2: 1.11 (0.90, 1.37) T3: 1.27 (1.01, 1.59) p-value for trend = 0.03 n-PFOS: 1.11 (0.99, 1.23) T2: 1.06 (0.86, 1.31) T3: 1.26 (1.02, 1.57) p-value for trend = 0.03
	Results: Lowest tertile used as the reference group. **Confounding**: Age at baseline, race/ethnicity, study site, education, parity, BMI at baseline, physical activity, smoking status, prior hormone use at baseline[c]						
Crawford et al. (2017) *Medium*	United States, 2008–2009	Cohort	Females from the Time to Conceive Study, Ages 30–44, N = 99	Serum 9.29 (8.31, 10.38)	Cycle-specific time to pregnancy, day-specific time to pregnancy, AMH (ln-ng/mL)	Time to pregnancy outcomes: Fecundability ratio per ln-unit increase in PFOS AMH: Regression coefficient per ln-unit increase in PFOS	Cycle-specific time to pregnancy 0.89 (0.49, 1.60) Day-specific time to pregnancy 0.99 (0.28, 2.32) AMH 0.07
	Confounding: Age, mean cycle length (added for cycle-specific time to pregnancy model)						
Kim et al. (2020b) *Medium*	Australia, 2006–2011	Cross-sectional	Females undergoing fertility treatment, Ages 23–42, N = 97	Follicular fluid Mean = 4.8 (Minimum, Maximum = 0.7, 22.4)	Fertilization rate	Regression coefficient per unit increase in PFOS	2.28 (−0.56, 5.11)
	Confounding: Age						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Lum et al. (2017) *Medium*	United States, 2005–2009	Cohort	Females from the LIFE study, Ages 18–40, N = 483	Serum Women with ≤ 24-day cycle: 12.3 (9.7, 17.0) Women with 25 to 31-day cycle: 12.6 (8.2, 17.6) Women with ≥ 32-day cycle: 11.5 (7.3, 16.9)	Day-specific probability of pregnancy	Regression coefficient by tertiles of PFOS	All women: T2: 1.0 (0.7, 1.5) T3: 0.9 (0.6, 1.3)
	Results: Lowest tertile used as the reference group **Confounding**: Couple intercourse pattern, female menstrual cycle length, age, BMI, active smoking at enrollment						
Tsai et al. (2015) *Medium*	Taiwan, 2006–2008	Cross-sectional	Females, Ages 18–30, N = 265	Serum, 8.65 (5.37, 13.29)	Levels of FSH in serum (ln-mIU/mL), SHBG in serum (ln-nmol/L)	Means by quartiles of PFOS	FSH Q1: 1.71 (SE = 0.25) Q2: 1.66 (SE = 0.23) Q3: 1.71 (SE = 0.25) Q4: 1.69 (SE = 0.25) SHBG Q1: 3.90 (SE = 0.21) Q2: 3.82 (SE = 0.20) Q3: 3.89 (SE = 0.22) Q4: 3.80 (SE = 0.21)
	Confounding: Age, BMI, high-fat diet						
Wang et al. (2017) *Medium*	China, 2014–2015	Case-control	Females of reproductive age, N = 335	Plasma, Cases: 6.40 (4.02, 11.42) Controls: 6.60 (3.92, 13.54)	Endometriosis-related infertility	OR by tertiles of PFOS	T2: 1.11 (0.61, 1.99) T3: 0.66 (0.36, 1.21)
	Confounding: Age, BMI, household income, and education						

Notes: AMH = anti-Mullerian hormone; BMI = body mass index; FSH = follicle stimulating hormone; LIFE = Longitudinal Investigation of Fertility and the Environment; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SHBG = sex hormone binding globulin; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.3 Hepatic

Table D-6. Associations Between PFOS Exposure and Hepatic Effects in Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Adults			
Omoike et al. (2020) *Medium*	United States 2005–2012	Cross-sectional	Adults from NHANES, Age ≥ 20, N = 6,652	Serum 11.40 (20th–80th percentile = 5.80–23.18)	Levels of iron in serum, bilirubin, and albumin	Percent change per one percent increase in PFOS	Iron concentration in serum 0.05 (0.03, 0.07), p-value < 0.05 Bilirubin 0.03 (0.02, 0.05), p-value < 0.05 Albumin 0.02 (0.02, 0.03), p-value < 0.05
	Confounding: Age, sex, race, education, poverty-income ratio, serum cotinine, BMI						
Jain (2019) *Medium*	United States 2003–2014	Cross-sectional	Adults from NHANES, Ages > 20, N = 108−3,562	Serum	Levels of ALT (log10-IU/L), AST (log10-IU/L)	Regression coefficient per log10-unit increase in PFOS	ALT, Non-obese, GF-1: −0.008 GF-2: 0.011 GF-3A: −0.013 GF-3B/4: −0.088, p-value < 0.01 Obese, GF-1: 0.048, p-value < 0.01 GF-2: 0.005 GF-3A: 0.038 GF-3B/4: 0.0696, p-value < 0.01 AST Non-obese, GF-1: −0.013 GF-2: 0.007 GF-3A : −0.015 GF-3B/4: −0.004 Obese,

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							GF-1: 0.011 GF-2: −0.013 GF-3A : 0.041, p-value = 0.01 GF-3B/4: 0.023
	Confounding: Gender, race/ethnicity, smoking status, age, log10(BMI), diabetes status, hypertension status, fasting time, poverty-income ratio, survey year, alcohol consumption[c]						
Liu et al. (2018a) *Medium*	United States, 2004–2007	Controlled trial	Overweight and Obese patients from the POUNDS Lost, Age 30–70 study, N = 150	Plasma Males 27.2 (19.9–45.2) Females 22.3 (14.3–34.9)	Hepatic fat mass	Partial Spearman correlation coefficient among baseline PFOS (ng/ml) and hepatic fat mass	Hepatic fat mass: 0.11
	Confounding: age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups						
Liu et al. (2018b) *Medium*	United States, 2013–2014	Cross-sectional	Adults from NHANES, Age > 18, N = 1871	Serum GM = 5.28 (SE = 1.02)	Levels of albumin (g/dL)	Regression coefficient per ln-unit increase in PFOS	Albumin 0.04, SE = 0.01, p-value < 0.005
	Confounding: age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Salihovic et al. (2018) *Medium*	Sweden 2001–2014	Cohort	Elderly adults in Sweden, Ages 70 N = 1002 Ages 75 N = 817 Age 80 N = 603	Plasma Age 70 13.2 (9.95, 17.8) Age 75 12.6 (7.97, 19.2) Age 80 0.57 (5.36, 11.5)	Levels of ALT (µkat/L)	Regression coefficient per ln-unit increase in PFOS	0.03 (0.02, 0.04), p-value < 0.0016
	Confounding: Sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, smoking						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Nian et al. (2019) *Medium*	China 2015–2016	Cross-sectional	Adults in high exposure area in China, Ages 22–96, N = 1,605	Serum 24.22 (14.62−37.19)	Levels of ALT (ln-U/L), AST (ln-U/L)	Percent change per 2.71-fold increase in PFOS	ALT 4.1 (0.6, 7.7), p-value < 0.05 AST 2.0 (−0.3, 4.3)
	Confounding: Age, sex, career, income, education, drink, smoke, giblet, seafood consumption, exercise, BMI						
Yamaguchi et al. (2013) *Medium*	Japan 2008–2010	Cross-sectional	Participants from the "Survey on the Accumulation of Dioxins and Other Chemical Compounds" project from urban, agricultural and fishing areas, Ages 15–76, N = 590	Blood 5.8 (3.7–8.8)	Levels of GGT (IU/L), AST (IU/L), ALT (IU/L)	Spearman rank correlation	GGT 0.06, p-value = 0.120 AST 0.11, p-value = 0.010 ALT 0.12, p-value = 0.004
	Confounding: Age, sex, BMI, regional block, smoking habits, frequency of alcohol intake						
Gallo et al. (2012) *Medium*	United States 2005–2006	Cross-sectional	Adults from the C8 Health Project, Ages ≥ 18 yr, N = 46, 452	Serum 20.3 (13.7–29.4)	Levels of ALT (ln-IU/L), GGT (ln-IU/L), Direct bilirubin (ln-mg/dL), ALT (IU/L, elevated)	ALT, GGT, direct bilirubin: Regression coefficient per ln-unit increase in PFOS Elevated ALT: OR per ln-unit increase in PFOS, or by deciles	ALT 0.02 (0.014, 0.026), p-value < 0.001 Direct bilirubin 0.029 (0.024, 0.034), p-value < 0.001 ALT, elevated (OR): Decile 2: 1.01 (0.87, 1.16) Decile 3: 1.06 (0.91, 1.22) Decile 4: 1.11 (0.96, 1.28) Decile 5: 1.19 (1.04, 1.37) Decile 6: 1.19 (1.04, 1.37) Decile 7: 1.20 (1.04, 1.38) Decile 8: 1.24 (1.08, 1.43)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Decile 9: 1.18 (1.02, 1.36) Decile 10: 1.25 (1.08, 1.44) p-trend < 0.001 Per ln-unit increase: 1.13 (1.07, 1.18), p-value < 0.001 GGT: No statistically significant associations
	Results: Lowest decile used as the reference group **Confounding:** Age, sex, alcohol consumption, socioeconomic status, fasting status, month of blood sample collection, smoking status, BMI, physical activity, insulin resistance. Additional confounding for ALT, GGT, and direct bilirubin analyses: Race. Additional confounding for OR analyses: increased serum iron.						
Lin et al. (2010) *Medium*	United States 1999–2000, 2003–2004	Cross-sectional	Adults from NHANES, Ages ≥ 18 yr, N = 2,216	Serum 23.50 (15.50–33.80)	Levels of bilirubin (μM), GGT (log-U/I), ALT (U/I)	Regression coefficient per log-unit increase in PFOS	Bilirubin Separate analysis: −0.30 (SE = 0.24), p-value = 0.223 Composite analysis: −1.06 (SE = 0.27), p-value = 0.001 GGT Separate analysis: 0.01 (SE = 0.03), p-value = 0.808 Composite analysis: −0.06 (SE = 0.03), p-value = 0.025 ALT Separate analysis: 1.01 (SE = 0.53), p-value = 0.066 Composite analysis: −0.19 (SE = 0.63), p-value = 0.769
	Comparison: Logarithm base not specified. **Confounding:** Age, gender, race/ethnicity, smoking status, drinking status, education level, BMI, HOMA-IR, metabolic syndrome, iron saturation status. Additional confounding for composite analyses: PFHxS exposure, PFNA exposure, PFOA exposure.						
van den Dungen et al. (2017) *Low*	The Netherlands 2015	Cross-sectional	Men with habitual eel consumption, Ages 40−70,	Serum 40 ng/g wet weight (15−93)	Levels of ALT, AST	Standardized regression coefficient per unit increase in PFOS	ALT 0.01 (−0.32, 0.34) AST

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 37				0.19 (−0.17, 0.55)
	Confounding: Age, waist-to-hip ratio						
Olsen et al. (2003a) *Medium*	United States, Belgium 1994–2000	Cross-sectional	Current and former workers at two fluorochemical production plants Male N = 421, Female N = 97, Regression analysis N = 174	Serum Antwerp Mean (SD) = 0.96 ppm (0.97); Decatur = 1.40 ppm (1.15)	Levels of ALT (IU/L), ALP (IU/L), AST (IU/L), GGT (IU/L)	Comparison of mean outcome by PFOS quartile	Males Elevated ($p < 0.05$) ALT for employees in Q4 compared with Q1 Elevated ($p < 0.05$) ALP for employees in Q3 and Q4 compared with Q1 No significant differences in mean AST or GGT by PFOS exposure quartile Females Elevated ($p < 0.05$) ALP for employees in Q4 compared with Q1 and Q2, and in Q3 compared with Q2 Elevated ($p < 0.05$) GGT for employees in Q4 compared with Q1 No significant differences in mean ALT or AST by PFOS exposure quartile
	Confounding: Sex						
Olsen et al. (2001) *Medium*	United States, Belgium 1994–2000	Cohort	Male 3M fluorochemical plant workers in Antwerp, Belgium and Decatur, Alabama	Antwerp (2000) Mean (SD): 1.16 ppm (1.07); Decatur (2000): 1.67 ppm (1.39)	Levels of ALT (ln-IU/L), ALP (ln-IU/L), AST (ln-IU/L), GGT (ln-IU/L)	Regression coefficient per unit increase in PFOS	ALT 0.010 (SE = 0.016), p-value = 0.54 PFOS × Years of observation interaction p-value < 0.001 AST 0.010 (SE = 0.011), p-value = 0.39

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 175				PFOS × Years of observation interaction p-value = 0.79 ALP 0.002 (SE = 0.009), p-value = 0.87 PFOS × Years of observation interaction p-value = 0.47 GGT –0.004 (SE = 0.020), p-value < 0.001 PFOS × Years of observation interaction p-value = 0.42
	Confounding: Years of observation, PFOS × Years of observation, age, BMI, drinks/day, cigarettes/day, location, entry period, baseline years worked, triglycerides						
Olsen et al. (2012a) *Low*	United States 2008–2010	Cohort	3M fluorochemical plant employees and contractors, N = 179	Serum Mean change from baseline, Employees: −101.3 ng/mL; Contractors: 1	Levels of ALT (IU/L), AST (IU/L)	Regression coefficient per unit increase in PFOS	ALT −0.045 (SD = 0.015), p-value = 0.005 AST −0.007 (SD = 0.009)
	Confounding: Sex, age at baseline, BMI at baseline, alcohol consumption at baseline						
Rantakokko et al. (2015) *Medium*	Finland 2005–2011	Cross-sectional	Morbidly obese adults undergoing bariatric surgery, N = 160	Serum 3.2 (5th–95th percentile: 0.89, 10.3)	Lobular inflammation	OR per log-unit increase in PFOS by level of lobular inflammation	< 2 foci: 0.52 (0.13, 2.09) 2–4 foci: 0.14 (0.01, 1.66)
	Comparison: Logarithm base not specified. **Results:** No foci used as the reference group. Foci measured per 200x field. **Confounding**: Age, sex, BMI, serum lipids, fasting insulin						
				Children and Adolescents			
Gleason et al. (2015) *Medium*	United States 2007–2010	Cross-sectional	Adolescents from NHANES, Ages ≥ 12,	Serum 11.3 (7.0–18.0)	Levels of ALT (ln-U/L), GGT (ln-U/L), AST	Regression coefficient per ln-	ALT (0.013) (−0.009, 0.034)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 4,333		(ln-U/L), ALP (ln-U/L)	unit increase in PFOS	GGT 0.036 (0.001, 0.071) AST 0.004 (−0.010, 0.018) ALP −0.010 (−0.027, 0.007)
	Confounding: Age, gender, race/ethnicity, BMI group, smoking, alcohol consumption "if statistically significant associated with both the exposure and outcome in univariate analysis."						
Mora et al. (2018) *Medium*	United States 1999–2010	Cohort	Children from Project Viva, N, prenatal exposure = 508, N, mid-childhood exposure = 630	Plasma Prenatal exposure: 24.6 (17.9−34.0) Mid-childhood exposure: 6.2 (4.2−9.7)	Levels of ALT (U/L)	Regression coefficient per IQR increase in PFOS	Prenatal exposure: −0.4 (−1.1, 0.2) Mid-childhood exposure: −0.3 (−0.9, 0.2)
	Confounding: Maternal education, prenatal smoking, gestational age at blood draw, and child's sex, race/ethnicity, age at lipids/ALT measurements						
Attanasio (2019) *Medium*	United States 2013–2016	Cross-sectional	Adolescents from NHANES, Ages 12−19, N, boys = 354 N, girls = 305	Serum Boys: GM = 3.68 (SE = 0.12) Girls: GM = 2.76 (SE = 0.14)	Levels of ALT (ln-IU/L), AST (ln-IU/L)	Regression coefficient per ln-unit increase in PFOS or by quartiles	ALT Boys, (−0.09, 0.10) Q2: −0.05 (−0.21, 0.11) Q3: 0.07 (−0.05, 0.18) Q4: −0.01 (−0.14, 0.13) Girls, 0.09 (−0.01, 0.18) Q2: −0.02 (−0.17, 0.14) Q3: 0.01 (−0.11, 0.13) Q4: 0.11 (−0.02, 0.24) AST Boys, −0.02 (−0.11, 0.06) Q2: −0.02 (−0.11,0.08) Q3: 0.01 (−0.07, 0.10)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Q4: −0.01 (−0.12, 0.10) Girls, 0.07 (0.00, 0.013) Q2: 0.03 (−0.08, 0.14) Q3: 0.05 (−0.04, 0.13) Q4: 0.12 (0.03, 0.21), p-value = 0.01
	Results: Lowest quartile used as the reference group. **Confounding**: Age, race/ethnicity, body weight status, education, poverty-income ratio, exposure to smoking						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children, Ages 8−12, N = 48	Serum 2.79 (IQR = 2.10)	Levels of ALT (U/L), AST (U/L)	Regression coefficient per unit increase in PFOS	ALT 0.16 (−1.84, 2.15) AST −0.28 (−1.22, 0.65)
	Confounding: Age, sex, race						
Children and Adolescents – Other Hepatic Outcomes							
Jin et al. (2020) *Medium*	United States 2007–2015	Cross-sectional	Children and adolescents diagnosed with nonalcoholic fatty liver disease, Ages 7–19, N = 74	Plasma 3.59 (2.35–6.81)	Ballooning, Grade of steatosis, Liver fibrosis, Lobular inflammation, Nonalcoholic steatohepatitis, Portal inflammation	OR per IQR increase in PFOS	Ballooning Few balloon cells: 1.11 (0.52, 2.37) Many cells/prominent ballooning: 1.12 (0.26, 4.95) Grade of steatosis 34%–66% steatosis: 1.37 (0.54, 3.51) > 66% steatosis: 0.88 (0.39, 1.97) Liver fibrosis Mild (stage 1): 1.71 (0.73, 4.03) Significant (stages 2–4): 1.51 (0.53, 4.35) Lobular inflammation < 2 foci: 0.50 (0.21, 1.22) 2–4 foci: 2.92 (0.92, 9.23)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Nonalcoholic steatohepatitis 3.32 (1.40, 7.87), p-value < 0.05 Portal inflammation Mild: 1.85 (0.82, 4.21) Moderate-to-severe: 2.26 (0.75, 6.79)
	Results: For ballooning, none was used as the reference group. For grade of steatosis < 5%–33% was used as the reference group. For liver fibrosis, none was used as the reference group. For lobular inflammation, no foci used as the reference group. Foci measured per 200x field. For portal inflammation, none was used as the reference group. **Confounding**: Age, sex, ethnicity, and BMI z-score						

Notes: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; GF = glomerular filtration; GGT = γ-glutamyltransferase; GM = geometric mean; HDL = high-density lipoprotein; HOMA-IR = homeostasis model assessment of insulin resistance; HR = hazard ratio; IQR = interquartile range; LDL = low-density lipoprotein; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; POUNDS = Preventing Overweight Using Novel Dietary Strategies; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SD = standard deviation; SE = standard error; SMR = standardized mortality ratio; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.4 Immune

Table D-7. Associations Between PFOS Exposure and Vaccine Response in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Children				
Grandjean et al. (2012) *Medium*	Faroe Islands, Denmark Recruitment 1997–2000, Follow-up through 2008	Cohort	Children followed from birth to age 7 Birth and infancy: N = 587 Prebooster (mean age 5.0) examination: N = 532	Maternal serum (prenatal) Geometric mean = 27.3 (23.2–33.1) Child serum (5 yr)	Antibody concentrations (log-IU/mL) for tetanus and diphtheria	Percent change per doubling in age 5 and maternal PFOS	Child serum Anti-diphtheria, prebooster, age 5 −16 (−34.9, 8.3) Anti-diphtheria, postbooster, age 5 −15.5 (−31.5, 4.3) Anti-diphtheria, age 7

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Postbooster (mean age 5.2) examination: N = 456 Age 7 (mean age 7.5) examination: N = 464	Geometric mean = 16.7 (13.5–21.1)			−27.6 (−45.8, −3.3) Anti-diphtheria, age 7 adjusted for age 5 Ab −20.6 (−38.2, 2.1) Maternal serum Anti-diphtheria, prebooster, age 5 −38.6 (−54.7, −16.9) Anti-diphtheria, postbooster, age 5 −20.6 (−37.5, 0.9) Anti-diphtheria, age 7 −19.7 (−41.8, 10.7) Anti-diphtheria, age 7 adjusted for age 5 Ab −10 (−32.6, 20) Child serum Anti-tetanus, prebooster, age 5 −11.9 (−30, 10.9) Anti-tetanus, postbooster, age 5 −28.5 (−45.5, 6.1) Anti-tetanus, age 7 −23.8 (−44.3, 4.2) Anti-tetanus, age 7 adjusted for age 5 Ab −11.4 (−30.5, 12.8) Maternal serum Anti-tetanus, prebooster, age 5 −10.1 (−31.9, 18.7) Anti-tetanus, postbooster, age 5

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							−2.3 (−28.6, 33.6) Anti-tetanus, age 7 35.3 (−3.9, 90.6) Anti-tetanus, age 7 adjusted for age 5 Ab 33.1 (1.5, 74.6)
	Confounding: Age, sex. Additional confounding for postbooster analyses: time since vaccination, booster type. Additional confounding for year 7 analyses: booster type. Additional confounding for year 7 analyses adjusted for age 5 Ab: booster type, child's specific antibody concentration at age 5 yr						
Granum et al. (2013) *Medium*	Norway 1999–2008	Cohort	Mother-infant pairs from MoBa at 3-yr follow-up N = 56	Maternal serum with three days of delivery 5.5 (3.8–7.1)	Levels (OD) of rubella anti-vaccine antibodies	Regression coefficient per unit increase PFOS	Rubella antibody –0.08 (−0.14, −0.02) p-value = 0.007
	Confounding: maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-yr follow-up.						
Mogensen et al. (2015a) *Medium*	Faroe Islands, Denmark 2002–2007	Cohort	Children aged 5–7 yr N = 443 at age 7	Serum 15.5 (12.8–19.2)	Antibody concentrations (log2-IU/mL) for diphtheria or tetanus	Percent change per doubling of PFOS	Anti-diphtheria, age 7 −30.3 (−47.3, −7.8) Anti-tetanus, age 7 −9.1 (−32.8, 23)
	Confounding: Age, sex, booster type[c]						
Stein et al. (2016b) *Medium*	United States, 1999–2000, 2003–2004, 2005–2006	Cross-sectional	Children aged 12–19 years, NHANES N = 1,190 (All) N = 1,152 (Seropositive)	Serum GM = 20.8 (95% CI: 19.1, 22.7)	Antibody concentrations for measles, mumps, and rubella	Percent change per doubling serum PFOS	Measles antibodies All –3.5 (–18.3, 14.0) Seropositive –2.9 (–17.3, 13.9) Mumps antibodies All –7.4 (–12.8, –1.7) Seropositive –5.9 (–9.9, –1.6) Rubella antibodies All –8.4 (–17.9, 2.1) Seropositive

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							–13.3 (–19.9, –6.2)
	Confounding: Age, sex, race/ethnicity, survey year.						
Grandjean et al. (2017a) *Medium*	Faroe Islands, Denmark Enrollment: 1997–2000	Cohort and cross-sectional	Children followed up at 7 yr and 13 yr N = 505 (13 yr) N = 427 (7 yr)	Serum 13 yr: 6.7 (5.2–8.5) 7 yr: 15.3 (12.4–19.0)	Levels of diphtheria antibody (log2-IU/mL), tetanus antibody (log2-IU/mL)	Percent change per doubling of PFOS	Diphtheria antibody Age 7: −23.8 (−43.2, 2.3) p-value = 0.07 Age 13: −8.6 (−27.7, 15.6) p-value = 0.454 Tetanus antibody Age 7: 30 (−16.1, 101.4) p-value = 0.24 Age 13: 22.2 (−12.4, 70.3) p-value = 0.237
	Confounding: Sex, age at antibody assessment, booster type at age 5						
Grandjean et al. (2017b) *Medium*	Faroe Islands, Denmark 1997–2000 and 2007–2009 (year of birth)	Cross-sectional	Infants 2 wk after expected term date, followed up at 18 mo and 5 yr All: N = 490, 18 mo: N = 275, 5 yr: N = 349	Serum 18 mo: 7.1 (4.5–10.0) 5 yr: 4.7 (3.5–6.3)	Levels of tetanus antibody (IU/mL), diphtheria antibody (IU/mL)	Percent change per doubling of PFOS	2007–2009 cohort Tetanus antibody Birth: −10.84 (−28.34, 10.94) p-value = 0.3 18 mo:−7.027 (−21.63, 10.3) p-value = 0.4 5 yr: −9.076 (−28.1, 14.98) p-value = 0.43 Diphtheria antibody: Birth: −14 (−31.59, 8.11) p-value = 0.20 18 mo: 17.55 (−0.84, 39.34) p-value = 0.062 5 yr: 17.17 (−8.66, 50.31) p-value = 0.21 Combined cohort

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Tetanus antibody Birth: −10.55 (−24.63, 6.16 p-value = 0.2 18 mo: −7.08 (−21.29, 9.70) p-value = 0.39 5 yr: −10.52 (−24, 5.35) p-value = 0.18 Diphtheria antibody Birth: −24.47 (−36.90, −9.60) p-value = 0.002 18 mo: 15.07 (−2.49, 35.79) p-value = 0.096 5 yr: −1.34 (−17.05, 17.34) p-value = 0.88
	Confounding: Age, sex						
Abraham et al. (2020) *Medium*	Berlin, Germany Enrollment: 1997–1999	Cross-sectional	Children, 1 yr old All: N = 101, formula fed: N = 21, breastfed: N = 80	Plasma Formula fed: mean = 6.8 (range = 2.8–19.3) Breastfed: mean = 15.2 (range = 1.9–34.8)	Levels of Hib antibody, tetanus antibody IgG, tetanus antibody IgG1, diphtheria antibody	Spearman correlation coefficient	Hib antibody: −0.05 Tetanus antibody IgG: −0.02 Tetanus antibody IgG1: −0.07 Diphtheria antibody: −0.02
	Confounding: Time since last vaccination						
Timmermann et al. (2020) *Medium*	Guinea-Bissau 2012–2015	Cohort	Infants enrolled at 4–7 mo old (inclusion), followed up at 9 mo and 2 yr	Maternal blood 0.77 (0.53–1.02)	Measles antibody concentration (mIU/mL)	Percent difference per doubling of PFOS	Inclusion (no measles vaccination): −13 (−26, 4) 9-mo visit

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Inclusion: N = 236 9-mo Unvaccinated controls: N = 100 Intervention: N = 133 2-yr Unvaccinated controls: N = 100 Intervention: N = 91				Control (no measles vaccination): −27 (−44, −4) Intervention (1 measles vaccination): −21 (−37, −2) 2-yr visit Control (1 measles vaccination): −6 (−25, 18) Intervention (2 measles vaccinations): −3 (−20, 17)
	Confounding: Weight and age at inclusion, maternal education, breastfeeding without solids, maternal measles antibody concentration, sex, and time from vaccination to blood sampling						
Timmerman et al. (2021) *Medium*	Greenland Recruitment: 1999–2005, Examination: 2012–2015	Cohort and cross-sectional	Vaccinated children ages 7–12 yr and their mothers at pregnancy Maternal serum N = 57 Child serum N = 169	Maternal serum from pregnancy 19.16 (15.20–24.06) Child serum 8.68 (6.52–12.23)	Levels (IU/mL) of diphtheria and tetanus antibody	Percent difference per unit increase in PFOS OR per log10-unit increase in PFOS	Diphtheria antibody Child serum Percent difference: 9 (−16, −2) OR: 1.14 (1.04, 1.26) Maternal serum Percent difference: 1 (−4, 6) Tetanus antibody Child serum Percent difference: −3 (−8, 3) Maternal serum Percent difference: 2 (−3, 6)
	Confounding: Area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq). Additional confounding for percent difference analyses: duration of being breastfed (<6 mo, 12 mo, >1 yr). Additional confounding for child serum analyses: time since vaccine booster (only children with known vaccination date were included).						
Zeng et al. (2019b)	China 2013	Cohort	Infants from Guangzhou Birth	Cord blood 3.17 (1.88–4.94)	HFMD antibody titers (CA16 or	Percent change or OR (below	CA16

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low			Cohort Study at birth and 3 mo Birth N = 194 (91 girls, 103 boys) 3-mo N = 180 (89 girls, 91 boys)		EV71) in serum of cord blood or at 3 mo	clinical protection) per doubling of PFOS	Cord blood: −20.6 (−30.0, −9.9) Girls: −14.0 (−27.5, 1.9) Boys: −24.7 (−37.6, −9.1) 3 mo: −6.9 (−13.9, 0.7) Girls: −2.8 (−10.9, 6.2) Boys: −12.2 (−23.7, 1.1) CA16 below clinical protection Cord blood: 1.75 (1.16, 2.63); p-value = 0.007 Girls: 1/43 (0.80, 2.56) Boys: 1.98 (1.03, 3.81) p-value for interaction by sex = 0.311 3 mo: 1.71 (1.12, 2.60); p-value = 0.013 Girls: 0.97 (0.88, 1.08) Boys: 2.29 (1.20, 4.36) p-value for interaction by sex = 0.318 EV71 Cord blood: −23.6 (−33.9, −11.8) Girls: −23.5 (−37.9, −5.8) Boys: −23.4 (−37.2, −6.6) 3 mo: −10.6 (−16.9, −3.9) Girls: −8.6 (−17.1, 0.9) Boys: −12.2 (−21.3, −1.9) EV71 below clinical protection Cord blood: 1.66 (1.12, 2.45); p-value = 0.011 Girls: 1.48 (0.92, 2.37)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Boys: 2.01 (1.03, 3.90) p-value for interaction by sex = 0.265 3 mo: 2.25 (1.44, 3.51); p-value < 0.05 Girls: 2.05 (1.11, 3.79) Boys: 2.35 (1.19, 4.65) p-value for interaction by sex = 0.579
	Outcome: Clinical protection threshold defined as titers ≥ 1:8 in modified cytopathogenic effect assay. **Confounding:** Sex, age, parental education, parental occupation, family income, parity, and birth weight						
			Adults and Adolescents				
Looker et al. (2014) *Medium*	United States Baseline: 2005–2006, Follow-up: 2010	Cohort	Adults near water districts of Ohio and West Virginia with contaminated drinking water N = 403	Serum GM (95% CI) = 8.32 (7.65–9.05)	Influenza antibodies (titer ratio and titer rise, log10-transformed): A/H1N1, A/H3N2, type B	Regression coefficient per log10-unit increase, or by quartiles	Influenza type B titer rise Per log10-unit: 0.5 (−0.11, 0.21), p-value = 0.56 Q2: 0.02 (−0.13, 0.18), p-value = 0.76 Q3: −0.03 (−0.19, 0.14), p-value = 0.73 Q4: 0.04 (−0.14, 0.21), p-value = 0.68 Influenza type B titer ratio Per log10-unit: 0.05 (−0.09, 0.18), p-value = 0.52 Q2: 0.004 (−0.14, 0.14), p-value = 0.96 Q3: −0.02 (−0.16, 0.12), p-value = 0.78 Q4: 0.03 (−0.12, 0.18), p-value = 0.71 Influenza A/H3N2 titer rise Per log10-unit: 0.09 (−0.13, 0.32), p-value = 0.42

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q2: 0.03 (−0.19, 0.26), p-value = 0.78 Q3: 0.18 (−0.06, 0.41), p-value = 0.14 Q4: −0.04 (−0.28, 0.21), p-value = 0.77 Influenza A/H3N2 titer ratio Per log10-unit: −0.005 (−0.20, 0.19), p-value = 0.96 Q2: −0.06 (−0.26, 0.14), p-value = 0.56 Q3: 0.02 (−0.18, 0.23), p-value = 0.84 Q4: −0.03 (−0.24, 0.19), p-value = 0.82 Influenza A/H1N1 titer rise Per log10-unit: 0.15 (−0.02, 0.32), p-value = 0.08 Q2: −0.04 (−0.21, 0.14), p-value = 0.68 Q3: 0.13 (−0.04, 0.31), p-value = 0.14 Q4: 0.10 (−0.09, 0.29), p-value = 0.30 Influenza A/ H1N1 titer ratio Per log10-unit: 0.10 (−0.11, 0.3), p-value = 0.36 Q2: −0.07 (−0.28, 0.13), p-value = 0.47

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q3: 0.03 (−0.18, 0.24), p-value = 0.78 Q4: 0.03 (−0.19, 0.26, p-value = 0.77
	Results: Lowest quartile used as reference group **Confounding:** Age (cubic spline), gender, mobility, and history of previous influenza vaccination						
Pilkerton et al. (2018) *Medium* for youth *Low* for adult	United States 1999–2000	Cross-sectional	Adults and adolescents 12 yr and older Youths: N = 1,012 Adults: N = 542 women, 613 men	Serum Women: mean = 22.1, SE = 0.9 Men: mean = 28.1 SE = 1.3	Rubella IgA titers (log-IU)	Regression coefficient by quartiles or per quartile increase	Adolescents: Per quartile increase: F-value = 1.44, p-value = 0.251 Adults: Per quartile increase: F-value = 3.44, p-value = 0.030 Women Q2: 0.05 (−0.34, 0.43) p-value = 0.81 Q3: 0.04 (−0.51, 0.6) p-value = 0.87 Q4: −0.17 (−1.13, 0.8) p-value = 0.73 Men Q2: −0.20 (−0.62, 0.23) p-value = 0.35 Q3: −0.32 (−0.69, 0.05) p-value = 0.08 Q4: 0.01 (−0.54, 0.56) p-value = 0.97
	Outcome: Logarithm base not reported **Results:** Lowest quartile used as reference group **Confounding:** Women: age, ethnicity, BMI, educational level, number of live births; men: age, ethnicity, BMI, educational level						
Bulka et al. (2021) *Medium*	Unites States 1999–2000, 2003–2016	Cross-sectional	NHANES adolescents and adults aged 12–49 yr	Serum 12–19 yr: GM (SE) = 7.54 (0.26)	Persistent infections of cytomegalovirus,	Persistent infections: Prevalence	Cytomegalovirus 12–19 yr: 0.92 (0.77, 1.09), p-value = 0.36

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			12–19 yr: N = 3,189 20–49 yr: N = 5,589	20–49 yr: GM (SE) = 8.67 (0.24)	Epstein-Barr virus, hepatitis C, hepatitis E, herpes simplex virus 1, herpes simplex virus 2, Toxoplasma gondii, and Toxocara species; pathogen burden	ratio per doubling in PFOS Pathogen burden: Relative difference per log2-unit increase in PFOS	20–49 yr: 0.99 (0.92, 1.05), p-value = 0.70 Epstein-Barr virus 12–19 yr: 1.01 (0.96, 1.05), p-value = 0.74 Hepatitis C virus 20–49 yr: 0.96 (0.71, 1.29), p-value = 0.77 Hepatitis E virus 20–49 yr: 1.00 (0.83, 1.20), p-value = 0.99 Herpes simplex virus 1 12–19 yr: 1.05 (0.99, 1.11), p-value = 0.13 20–49 yr: 1.04 (1.01, 1.06), p-value < 0.01 Herpes simplex virus 2 20–49 yr: 1.04 (0.99, 1.09), p-value = 0.1 Toxoplasma gondii 12–19 yr: 1.15 (0.90, 1.48), p-value = 0.27 20–49 yr: 1.1 (0.97, 1.26), p-value = 0.15 Toxocara species 12–19 yr: 1.12 (0.66, 1.91), p-value = 0.68 20–49 yr: 1.57 (1.26, 1.96), p-value < 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Pathogen burden 12–19 yr: 1.30 (1.25, 1.36) 20–49 yr:1.10 (1.07, 1.12)
	Outcome: Pathogen burden defined as the sum of pathogens for which an individual was seropositive (including any pathogens with a seroprevalence < 1.0%) **Confounding:** Age, race/ethnicity, sex, ratio of family income to the federal poverty threshold, educational attainment, serum cotinine concentrations, and BMI						
Lopez-Espinosa et al. (2021) *Medium*	United States 2005–2006, 2010	Cohort and cross-sectional	Adults from C8HP 2005–2006: N = 42,782 2010: N = 526	Serum 2005–2006: 19.7 (13.3–28.4) 2010: 9.60 (6.10–14.9)	Levels (ln-cells/µL or percentage of white blood cells/lymphocytes) of white blood cells, neutrophils, monocytes, eosinophils, lymphocytes, CD3+ T cells, CD3+CD4+ T-helper cells, CD3+CD4+CD8+ double positive T cells, CD3+CD8+ T-cytotoxic cells, CD3-CD16+CD56+ natural killer cells, CD3-CD19+ B cells; CD4+/CD8+ ratio	Counts: Precent difference per IQR increase in PFOS Percentages: Difference per IQR increase in PFOS	White blood cells, total 2005–2006: −0.55 (−0.84, −0.26) 2010: 0.55 (−1.35, 2.49) Likelihood ratio test p-value < 0.001 for the comparison between the two time periods
	Outcome: All cell types reported as cell counts; eosinophils, lymphocytes, monocytes, and neutrophils additionally reported as percentage of white blood cells; CD3+ T cells, CD3+CD4+ T-helper cells, CD3+CD4+CD8+ double positive T cells, CD3+CD8+ T-cytotoxic cells, CD3-CD16+CD56+ natural killer cells, and CD3-CD19+ B cells additionally reported as percentage of lymphocytes **Confounding:** Gender, age, smoking, month of sampling, alcohol intake, and educational level						
Shih et al. (2021) *Medium*	Faroe Islands, Denmark	Cohort and cross-sectional	Faroe Island residents at birth, 7, 14, 22, and 28 yr	Cord blood at birth 5.96 (IQR = 3.09)	Levels (IU/mL) of hepatitis A antibody, hepatitis	Percent change per doubling of PFOS	Hepatitis Type B Cord blood: −23.24 (−46.77, 10.69)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Recruitment: 1986–1987, Follow-up through 2015		N = 399	Serum 7 yr: 31.89 (IQR = 13.37) 14 yr: 31.29 (IQR = 9.62) 22 yr: 12.55 (IQR = 7.24) 28 yr: 6.85 (IQR = 5.29)	B antibody, diphtheria antibody, tetanus antibody; Hepatitis A antibody signal-to-cutoff ratio		7-yr serum: −4.65 (−45.87, 67.87) 14-yr serum: 22.17 (−34.09, 126.46) 22-yr serum: 15.26 (−22.88, 72.26) 28-yr serum: 6.12 (−23.36, 46.93) Hepatitis Type A Cord blood: 0.11 (−0.36, 0.59) 7-yr serum: 0.21 (−0.54, 0.96) 14-yr serum: −0.14 (−1.01, 0.74) 22-yr serum: −0.1 (−0.63, 0.44) 28-yr serum: −0.23 (−0.66, 0.21) Diphtheria Cord blood: 28.26 (−5.7, 74.44) 7-yr serum: 5.04 (−36.45, 73.59) 14-yr serum: −3.5 (−42.87, 63.01) 22-yr serum: 5.29 (−21.69, 41.56) 28-yr serum: 6.91 (−14.26, 33.31) Tetanus Cord blood: 2 (−20.24, 30.44)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							7-yr serum: 8.91 (−25.85, 59.95) 14-yr serum: −19.44 (−48.36, 54.7) 22-yr serum: −9.1 (−28.42, 15.44) 28-yr serum: −2.1 (−17.77, 16.56)
	Confounding: Sex						
Stein et al. (2016a) *Low*	United States 2010	Cohort	Adults enrolled at 18–49 yr, followed up at day 30 Total population: N = 75, low baseline Ab: N = 29	Serum GM = 5.22 (95% CI: 4.52–6.02)	Anti-A-H1N1 antibody response measured by HAI or by IHC	RR by tertiles	HAI anti-A-H1N1 antibody Total population T2: 2.6 (0.4, 15.1) T3: 1.3 (0.2, 7.3) p-value for trend = 0.81 Low baseline Ab T2: 6.7 (1.2, 37.9) T3: 1.6 (0.3, 9.7) p-value for trend = 0.81 IHC anti-A-H1N1 antibody Total population T2: 2.6 (0.9, 7.4) T3: 2.4 (0.9, 6.6) p-value for trend = 0.12 Low baseline Ab T2: 4.5 (1, 20.3) T3: 3.1 (1, 10.2) p-value for trend = 0.13
	Results: Lowest tertile used as the reference group. **Confounding:** Age, sex, and race/ethnicity						
Zeng et al. (2020) *Low*	China 2015–2016	Cross-sectional	Adults from the Isomers of C8 Health Project N = 605	Serum 10.7 (6.82–16.2)	Hepatitis B surface antibody (HBsAb) (log-mIU/mL) or surface antigen	Regression coefficient or OR (HBsAb seronegative)	HBsAb concentration Linear: −0.51 (−0.84, −0.18); p-value = 0.002

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					(HBsAg) (mIU-mL); HBsAb seronegative (<10 mIU/mL)	per log10-unit increase in linear or branched PFOS	Branched: −0.31 (−0.7, 0.07); p-value = 0.114 HBsAb seronegative Linear: 1.96 (1.37, 2.81); p-value < 0.001 Branched: 1.64 (1.05, 2.56); p-value = 0.03 HBsAg concentration Linear: 0.74 (−0.02, 1.49); p-value = 0.056 Branched: 1.08 (0.06, 2.09); p-value = 0.037
	Confounding: Age, gender, BMI, career, income, alcohol drinking, smoking, regular exercise; education for HBsAb concentration alone						
Zhang et al. (2023c) *Medium*	United States 2003–2004, 2009–2010	Cross-sectional	Children and adolescents aged 12–19 from NHANES N = 819	Serum Mean 12.44 (7.35–21.90)	Levels of rubella antibody, mumps antibody, measles antibody	Percent change per 2.7-fold increase in serum PFOA	Rubella levels –8.16 (−13.67, −2.31) p-value < 0.05 Mumps levels –2.12 (−8.11, 4.25) Measles levels –2.38 (−11.94, 8.21)
	Confounding: Age, sex, race, income-poverty ratio, BMI, serum cotinine concentrations, survey cycle, and dietary intake of milk.						

Notes: Ab = antibody; BMI = body mass index; C8HP = C8 Health Project; CI = confidence interval; GM = geometric mean; HAI = hemagglutinin inhibition; HBsAb = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; HFMD = hand, foot, and mouth disease; ICH = immunohistochemistry; IQR = interquartile range; mo = months; MoBa = Norwegian Mother and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; OD = optical density; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SE = standard error; T2 = tertile 2; T3 = tertile 3; wk = weeks; yr = year(s).

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-8. Associations Between PFOS Exposure and Infectious Disease in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Children			
Fei et al. (2010) *Medium*	Denmark, Recruitment: 1996–2003; Follow-up: 2008	Cross-sectional and cohort	Mother-infant pairs with follow-up to 11 yr (DNBC) N = 1,400	Maternal plasma Mean (range) = 35.3 (6.4–106.7)	Infectious disease hospitalizations	IRR by quartiles or per quartile increase in PFOS	Girls Q2: 1.14 (0.73, 1.79) Q3: 1.61 (1.05, 2.47) Q4: 1.59 (1.02, 2.49) Per quartile increase: 1.18 (1.03, 1.36) Boys Q2: 0.8 (0.57, 1.13) Q3: 0.61 (0.42, 0.89) Q4: 0.77 (0.54, 1.12) Per quartile increase: 0.90 (0.80, 1.02) All children Q2: 0.93 (0.71, 1.21) Q3: 0.90 (0.68, 1.18) Q4: 1.0 (0.76, 1.32) Per quartile increase: 1.0 (0.91, 1.09) Results stratified by age not statistically significant
	Results: Lowest quartile used as reference group **Confounding:** Parity, maternal age, pre-pregnancy BMI, breastfeeding, smoking during pregnancy, socio-occupational status, home density, child's age, sibling age difference, gestational age at blood drawing, birth year, and birth season						
Gourdazi et al. (2017b) *Medium*	Hokkaido, Japan 2003–2009	Cohort	Children, early pregnancy followed up at 4 yr N = ,1558 (793 boys, 765 girls)	Maternal blood 4.93 (3.67–6.65)	Infectious diseases, total (including Otitis media, Pneumonia, RS virus, Varicella)	OR by quartiles	Girls Q2: 1.42 (0.91, 2.23) Q3: 1.32 (0.86, 2.06) Q4: 1.71 (1.08, 2.72) p-value for trend = 0.036 Boys

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q2: 1.45 (0.95, 2.22) Q3: 1.25 (0.83, 1.91) Q4: 1.59 (1.03, 2.46) p-value for trend = 0.071 All Q2: 1.44 (1.06, 1.96) Q3: 1.28 (0.95, 1.73) Q4: 1.61 (1.18, 2.21) p-value for trend = 0.008
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, number of elder siblings, child sex, breastfeeding period, and smoking during pregnancy[c]						
Manzano-Salgado et al. (2019) *Medium*	Spain, 2003–2008	Cohort	Children ages 1.5, 4, or 7 yr Age 1.5: N = 1,188 Age 4: N = 1,184 Age 7: N = 1,071	Maternal blood 6.06 (4.25–7.82)	LRTI	OR or RR per log2-unit increase in PFOS	OR 1.5 yr: 0.99 (0.83, 1.18) 4 yr: 0.95 (0.79, 1.16) 7 yr: 0.83 (0.57, 1.2) RR, 1.5–7 yr All: 0.96 (0.85, 1.09) Boys: 0.97 (0.81, 1.15) Girls: 0.94 (0.77, 1.14)
	Confounding: OR assessment: Age-at-follow-up of the child; RR assessment: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth						
Ait Bamai et al. (2020) *Medium*	Hokkaido, Japan Enrollment: 2003–2012	Cohort	Children, early pregnancy followed up at 7 yr N = 2,689	Maternal blood 5.12 (3.75–7.02)	Chicken pox, RSV, otitis media, pneumonia, wheeze, eczema	OR or RR per ln-unit increase in PFOS	Pneumonia: OR: 1.14 (0.93, 1.38); p-value = 0.21 Otitis media: OR: 1 (0.83, 1.2); p-value = 0.989 Chicken pox: OR: 1.1 (0.91, 1.32); p-value = 0.348 RSV: OR: 0.72 (0.56, 0.91); p-value = 0.007

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Wheeze: RR: 0.93 (0.82, 1.06); p-value = 0.255 Eczema: RR: 0.86 (0.76, 0.98); p-value = 0.02
	Confounding: Sex, maternal age, parity, maternal smoking during pregnancy, BMI pre-pregnancy, annual household income during pregnancy, duration nursing, and presence of siblings						
Huang et al. (2020) *Medium*	China Recruitment: 2011–2013, Follow-up at 5 yr	Cohort	Children ages 1–5 yr N = 344 (182 boys, 162 girls)	Cord blood 2.44 (1.74–3.22)	Respiratory tract infections (total and recurrent)	Recurrent respiratory tract infections: OR for > 75th percentile vs. ≤ 75th percentile PFOS	Total respiratory tract infections –0.64 (−4.38, 3.1), p-value = 0.738 Recurrent respiratory tract infections 0.91 (0.51, 1.65), p-value = 0.762 Results stratified by age and sex not statistically significant
	Confounding: Infant sex, maternal age, maternal education level, birth weight						
Grandjean et al. (2020) *Medium*	Denmark 2020	Cross-sectional	Adults, ages 30–70 yr, with known SARS-CoV-2 infection N = 323	Plasma 4.86 (2.85–8.29)	COVID-19 severity	OR per unit increase in PFOS	Covid-19 severity 0.97 (0.92, 1.02) Covid-19 severity (hospitalization vs. no hospitalization) 0.96 (0.84, 1.10) Covid-19 severity (intensive care unit and/or deceased vs. hospitalization) 1.08 (0.94, 1.24)
	Confounding: Age, sex, kidney disease, other chronic disease, national origin, place of testing, and days between blood sampling and diagnosis						
Dalsager et al. (2021) *Medium*	Denmark Recruitment: 2010–2012,	Cohort	Pregnant women and their children from the OCC,	Maternal serum 7.52 (0.49–27.5)	Hospitalization from infection (any infection, upper	Hazard ratio per log2-unit increase in PFOS	Any infection 1.23 (1.05, 1.44) Boys: 1.36 (1.10, 1.67) Girls: 1.04 (0.85, 1.28)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up until 2015		followed up to 4 yr N = 1,472		respiratory tract, lower respiratory tract, gastrointestinal, other)		Upper respiratory infection 1.25 (0.97, 1.61) Lower respiratory infection 1.54 (1.11, 2.15) Gastrointestinal infection 0.77 (0.46, 1.29) Other infection 1.17 (0.98, 1.40)
	Confounding: Maternal age, parity, maternal educational level, child sex, child age						
Ji et al. (2021) *Medium*	China 2020	Case-control	Adults N = 160	Urine Controls: 42.4 (25.5–61.3) ng/g creatinine Cases: 67.6 (41.0–96.5) ng/g creatinine	COVID-19 infection	OR per log2-SD change in PFOS	COVID-19 1.94 (1.39, 2.96)
	Confounding: Age, gender, BMI, diabetes, cardiovascular diseases, and urine albumin-to-creatinine ratio						
Wang et al. (2022b) *Medium*	China Recruitment: 2010–2013, Follow-up after 1 yr	Cohort	Pregnant women and their children at 1 yr from LWBC N = 235	Maternal serum at delivery 4.58 (3.31–6.14)	Common cold, bronchitis/pneumonia, diarrhea	OR per log10-unit increase in PFOS IRR per log10-unit increase in PFOS	Common cold OR: 1.86 (0.53, 6.50), p-value = 0.334 IRR: 1.24 (0.76, 2.02), p-value = 0.382 Bronchitis/pneumonia OR: 1.54 (0.30, 7.78), p-value = 0.602 IRR: 0.76 (0.23, 2.46), p-value = 0.644 Diarrhea

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							OR: 2.6 (0.67, 10.09), p-value = 0.167 IRR: 1.89 (1.08, 3.32), p-value = 0.027
	Confounding: Maternal age, pre-pregnancy BMI, smoking during pregnancy, maternal education level, and parity						
Dalsager et al. (2016) *Low*	Odense, Denmark 2010–2012	Cohort	Children, pregnancy followed up at 1–4 yr N = 346	Maternal serum 8.07 (range = 2.36–25.10)	Fever, cough, nasal discharge, diarrhea, vomiting	OR (of proportion of days with symptoms) by tertiles	Fever T2: 1.41 (0.81, 2.44) T3: 2.35 (1.34, 4.11); p-value < 0.05 Cough T2: 1.16 (0.67, 2.01) T3: 1.03 (0.59, 1.79) Nasal discharge T2: 1.11 (0.65, 1.93) T3: 1.07 (0.62, 1.85) Diarrhea T2: 0.89 (0.51, 1.56) T3: 1.04 (0.59, 1.82) Vomiting T2: 1.47 (0.86, 2.54) T3: 0.78 (0.45, 1.35)
	Results: Lowest tertile used as reference group. **Confounding:** Maternal age, maternal educational level, parity, and child age.						
Impinen et al. (2018) *Low*	Oslo, Norway Recruited 1992–1993, followed up for 10 yr	Cohort, Nested case-control	Infants followed up at 2 and 10 yr of age N = 641	Cord blood 5.2 (4.0–6.6)	Common cold episodes from 0 to 2 yr, LRTI episodes from 0 to 10 yr	Regression coefficient per log2-unit increase in PFOS	Common cold 0–2 yr −0.03 (−0.08, 0.01) p-value = 0.173 LRTI 0–10 yr 0.5 (0.42, 0.57) p-value < 0.0001
	Confounding: Child sex						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Impinen et al. (2019) *Low*	Oslo, Norway Enrollment: 1999–2008	Cohort	Pregnant women and their infants followed up at 3 and 7 yr 0–3 yr: N = 1,207 6–7 yr: N = 921	Maternal blood 12.87 (9.92–16.63)	Common cold, bronchitis/pneumonia, throat infection with strep, pseudocroup, ear infection, diarrhea/gastric flu, urinary tract infection	OR per IQR increase in PFOS	Common cold 0–3 yr: 0.94 (0.92, 0.97); p-value < 0.05 Bronchitis/pneumonia 0–3 yr: 1.20 (1.07, 1.34); p-value < 0.05 6–7 yr: 0.77 (0.50, 1.19) Throat infection with strep 0–3 yr: 0.90 (0.78, 1.04) Other throat infections 0–3 yr: 0.90 (0.81, 1.01) Pseudocroup 0–3 yr: 1.07 (0.96, 1.20) Ear infection 0–3 yr: 0.88 (0.82, 0.94); p-value < 0.05 6–7 yr: 1.13 (0.92, 1.40) Diarrhea/gastric flu 0–3 yr: 0.98 (0.93, 1.03) 6–7 yr: 1.12 (1.01, 1.24) Urinary tract infection 0–3 yr: 0.78 (0.70, 0.87); p-value < 0.05 6–7 yr: 0.91 (0.63, 1.31)
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy						
Kvalem et al. (2020) *Low*	Norway Enrollment: 1992–1993	Cohort and cross-sectional	Children, 10 yr, all: 378, boys: 193, girls: 185	Serum All: 19.4 (IQR: 9.23)	Common cold, LRTI	Colds: OR (reference: 1–2 colds)	Colds, 10–16 yr 3–5 colds All: 1.26 (0.34, 4.55) p-value = 0.73

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up: 2002–2009		Children, 10–16 yr, all: 375, boys: 191, girls: 184 Children, 16 yr, all: 330, boys: 170, girls: 160	Boys: 21.7 (IQR: 8.86) Girls: 17.52 (IQR: 8.02)		LRTI: RR per IQR increase in PFOS	Boys: 2.54 (0.38, 17.3) p-value = 0.34 Girls: 0.86 (0.16, 4.75) p-value = 0.86 > 5 colds All: 1.16 (0.33, 4.07)12.54 p-value = 0.82 Boys: 1.99 (0.3, 13.2) p-value = 0.48 Girls: 1.07 (0.21, 5.45) p-value = 0.93 LTRI 10–16 yr All: 1.34 (1.17, 1.55) p-value < 0.001 Boys: 1.33 (1.26, 1.39) p-value < 0.001 Girls: 1.23 (0.91, 1.66) p-value = 0.17 16 yr All: 0.82 (0.4, 1.69) p-value = 0.6 Boys: 0.62 (0.22, 1.78) p-value = 0.38 Girls: 1.11 (0.41, 3) p-value = 0.84
	Confounding: Puberty status at 16 yr, mother's education, physical activity level at 16 yr						

Notes: BMI = body mass index; CI = confidence interval; DNBC = Danish National Birth Cohort; IQR = interquartile range; IRR = incidence rate ratio; LRTI = lower respiratory tract infection; LWBC = Laizhou Wan Birth Cohort; OCC = Odense Child Cohort; OR = odds ratio; RR = risk ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RSV = respiratory syncytial virus; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SD = standard deviation; SE = standard error; T2 = tertile 2; T3 = tertile 3; yr = year(s).

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-9. Associations Between PFOS Exposure and Asthma in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Dong et al. (2013) *Medium*	Taiwan, 2009–2010	Case-control and cross-sectional	Children from GBCA with (cases) or without (controls) asthma, ages 10–15 yr, N = 231 (cases), N = 225 (controls)	Serum Cases: 33.9 (19.6–61.1) Controls: 28.9 (14.1–43.0)	Asthma, Asthma Control Test score, asthma severity score, IgE in serum (IU/mL), AEC (10^6/L), ECP in serum (µg/L)	Asthma: OR by quartiles of PFOS Asthma Control Test score, asthma severity score, IgE, AEC, ECP: mean values by quartiles	Asthma Q2: 1.96 (1.11, 3.47) Q3: 1.32 (0.75, 2.32) Q4: 2.63 (1.48, 4.69) p-trend = 0.003 IgE Q1:517.9 (336.7, 699.2) Q2: 686.2 (501.3, 871.1) Q3: 658.1 (475.2, 841.1) Q4: 877.3 (695.2, 1,059.5), p-value < 0.05 p-trend = 0.008 AEC Q1: 329.4 (255.8, 403.0) Q2: 368.6 (293.9, 443.3) Q3: 431.3 (358.1, 504.6) Q4: 453.4 (379.4, 527.3) p-trend = 0.009 ECP Q1: 25.9 (10.4, 41.3) Q2: 37.4 (21.9, 52.8) Q3: 43.5 (27.5, 59.4) Q4: 62.4 (46.3, 78.4), p-value < 0.05 p-trend = 0.001 Asthma severity score Q1: 3.33 (2.36, 4.31) Q2: 4.18 (3.19, 5.17) Q3: 4.49 (3.52, 5.45) Q4: 4.57 (3.61, 5.54) p-trend = 0.045

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Asthma Control Test score: trends across quartiles not statistically significant
	Results: Lowest quartile used as reference group **Confounding:** age, sex, BMI, parental education, ETS exposure, and month of survey						
Humblet et al. (2014) *Medium*	Unites States, 1999–2008	Cross-sectional	Adolescents, ages 12–19 yr old from NHANES N = 1,877	Serum Never asthma 16.8 (10.8–26.2) Ever asthma 17.0 (10.8–25.8) No current asthma 16.8 (10.8–26.2) Current asthma 16.7 (10.3–25.3) No wheezing 16.8 (10.8–26.2) Wheezing 17.2 (10.9–25.4)	Asthma, wheeze	OR per doubling in PFOS or per unit increase in PFOS	Ever asthma Per doubling: 0.88 (0.74, 1.04), p-value = 0.13 Per unit increase: 0.99 (0.98, 1.0), p-value = 0.07 Current asthma Per doubling: 0.88 (0.72, 1.09), p-value = 0.24 Per unit increase: 0.99 (0.98, 1.01), p-value = 0.34 Wheeze Per doubling: 0.83 (0.67, 1.02), p-value = 0.08 Per unit increase: 0.99 (0.98, 1.01), p-value = 0.37
	Exposure: No wheezing defined as no wheezing in the past 12 mo. Wheezing defined as history of wheezing in the past 12 mo. **Confounding:** Sex, smoking, age, race/ethnicity, survey cycle, poverty-income ratio, health insurance						
Smit et al. (2015) *Medium*	Ukraine and Greenland, Exposure: 2002–2004, Outcome: 2010–2012	Cohort	Mother-child pairs with follow-up when the children were 5–9 yr of age, N = 1,024	Maternal blood Ukraine: GM = 4.88 (P5–P95: 2.34–9.94) Greenland: GM = 20.6 (P5–P95: 10.2–49.6)	Asthma	OR per SD increase in PFOS	Asthma ever (combined): 0.86 (0.67, 1.10) Ukraine: 0.75 (0.39, 1.42) Greenland: 0.88 (0.67, 1.15)
	Confounding: Maternal allergy, smoking during pregnancy, education level, maternal age, child sex, child age at follow-up, gestational age at blood sample, parity, breastfeeding, and birthweight[c]						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Stein et al. (2016b) *Medium*	United States, 1999–2000, 2003–2004, 2005–2006	Cross-sectional	Children aged 12–19 years, NHANES N = 638	Serum GM = 20.8 (95% CI: 19.1, 22.7)	Asthma and wheeze	OR [per IQR(lnPFOS) increase (0.76 ln-ng/mL)]	Asthma 1.20 (0.88, 1.63) Wheeze 0.76 (0.45, 1.29)
	Confounding: Age, sex, race/ethnicity, survey year; for Wheeze: age, gender, race, weight status, serum cotinine.						
Impinen et al. (2018) *Medium*	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 and 10 yr of age, N = 641	Cord blood 5.2 (4.0–6.6)	Asthma	OR per log2-unit increase in PFOS	Current asthma (10 yr): 1.14 (0.84, 1.54); p-value = 0.392 Asthma ever (10 yr): 1.32 (0.89,1.97); p-value = 0.167
	Confounding: Sex						
Beck et al. (2019) *Medium*	Denmark, Enrollment: 2010–2012	Cohort	Children, early pregnancy to 5 yr N = 970 (507 boys, 363 girls)	Maternal blood 7.73 (5.68–10.44)	Wheeze, self-reported asthma, doctor-diagnosed asthma	OR per doubling in maternal serum PFOS	Wheeze All: 1.01 (0.79, 1.30) Boys: 1.02 (0.74, 1.39) Girls: 1.01 (0.67, 1.52) Self-reported asthma All: 1.22 (0.65, 2.28) Boys: 2.39 (0.92, 6.21) Girls: 0.67 (0.29, 1.53) Doctor-diagnosed asthma All: 0.83 (0.52, 1.31) Boys: 0.74 (0.46, 1.20) Girls: 1.60 (0.46, 5.59)
	Confounding: Parity, maternal education level, maternal pre-pregnancy BMI, asthma predisposition, child sex						
Gaylord et al. (2019) *Medium*	New York City, NY 2014–2016	Case-control	Children with (cases) or without (controls) asthma aged 13–22, N = 118 (cases),	Serum Cases: 3.72 (Range: 1.01–14.2) Controls: 2.75 (Range: 0.60–27.8)	Asthma	OR per log-unit increase in PFOS	0.89 (0.45, 1.76)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 169 (controls)				
	Comparison: Logarithm base not specified. **Confounding**: Sex, race/ ethnicity, age, BMI, tobacco smoke exposure						
Impinen et al. (2019) *Medium*	Oslo, Norway, Enrollment: 1999–2008	Cohort	Pregnant women and their infants (followed to age 7), N = 921	Maternal blood 12.87 (9.92–16.63)	Asthma	OR per IQR increase in PFOS	Current asthma: Total: 1.11 (0.72, 1.69); p-value = 0.643 Boys: 1.17 (0.64, 2.15); p-value = 0.616 Girls: 1.03 (0.56,1.91); p-value = 0.927 Ever asthma: Total: 0.93 (0.68, 1.26); p-value = 0.631 Boys: 0.94 (0.63, 1.40); p-value = 0.744 Girls: 0.92 (0.57, 1.49); p-value = 0.745
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy						
Manzano-Salgado et al. (2019) *Medium*	Spain, 2003–2008	Cohort	Children, 4 yr, N = 1,184 7 yr, N = 1,068	Maternal blood 6.06 (4.52–7.82)	Asthma	OR or RR per log2-unit increase in maternal PFOS	4-yr follow-up: OR = 0.72 (0.45, 1.13) 7-yr follow-up: OR = 0.84 (0.57, 1.25) 4 and 7 yr Girls: RR = 0.68 (0.38. 1.22) Boys: RR = 0.91 (0.58, 1.41)
	Confounding: OR assessment: Age at follow-up of the child; RR assessment: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth						
Zeng et al. (2019a) *Medium*	Shanghai, China, 2012–2015	Cohort	Enrolled in pregnancy, follow-up at 5 yr	Cord blood Boys: 2.49 (1.81–3.51) Girls: 2.38 (1.73–3.13)	Asthma	OR per log10-unit increase in PFOS	All: 1.49 (0.29, 7.54), p-value = 0.63 Boys: 4.69 (0.51,42.77), p-value = 0.17

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 358 (187 boys, 171 girls)				Girls: 0.17 (0.01, 4.15), p-value = 0.27
	Confounding: Child weight at age 5, gestational age, breastfeeding during the first 6 mo, maternal education, maternal pre-pregnancy BMI, and annual household income						
Jackson-Browne et al. (2020) *Medium*	NHANES, United States, 2013–2014	Cross-sectional	Children, ages 3–11 yr, N = 607	Serum GM = 3.7 (2.6–5.5)	Asthma	OR per ln-SD increase in PFOS	1.2 (0.8, 1.7) By age: 3–5 yr: 1.7 (1.0, 3.0) 6–11 yr: 1.1 (0.7, 1.6) p-value for interaction by age = 0.03 By sex: Females: 1.1 (0.7, 1.7) Males: 1.2 (0.8, 2.0) p-value for interaction by sex = 0.82 By race/ethnicity: White, non-Hispanic: 1.4 (0.8, 2.6) Black, non-Hispanic: 1.3 (0.8, 2.2) Hispanic: 1.3 (0.8, 2.0) Other: 1.1 (0.7, 1.7) p-value for interaction by race = 0.35
	Confounding: Sex, age, race/ethnicity, serum cotinine, poverty to income ratio						
Kvalem et al. (2020) *Medium*	Norway Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, 10 yr N = 378 (193 boys, 185 girls) Children, 10–16 yr N = 375 (191 boys, 184 girls)	All: 19.4 (IQR: 9.23) Girls: 17.52 (IQR: 8.02) Boys: 21.7 (IQR: 8.86)	Asthma	RR per IQR increase in PFOS	10 yr All: 1.01 (0.86, 1.19) Boys: 1.06 (0.89, 1.26) Girls: 0.76 (0.52, 1.12) 10–16 yr All: 0.94 (0.74, 1.20) Boys: 0.96 (0.71, 1.31) Girls: 0.85 (0.54, 1.31)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Children, 16 yr N = 375 (191 boys, 184 girls)				16 yr All: 1.00 (0.79, 1.27) Boys: 1.01 (0.76, 1.36) Girls: 0.91 (0.60, 1.38)
	Confounding: 10 yr: Age at follow-up, physical activity, mothers' education; 16 yr: BMI at 16 yr, puberty status at 16 yr, mothers' education, physical activity level at 16 yr						
Huang et al. (2020) *Medium*	China Recruitment: 2011–2013, Follow-up at 5 yr	Cohort	Children ages 1–5 yr N = 344 (182 boys, 162 girls)	Cord blood 2.44 (1.74–3.22)	IgG (ng/mL), IgE (ng/mL)	Regression coefficient per log10-unit increase in PFOS	IgG –0.01 (−0.06, 0.04), p-value = 0.643 IgE –0.04 (−0.35, 0.27), p-value = 0.805 Results stratified by age and sex not statistically significant
	Confounding: Infant sex, maternal age, maternal education level, birth weight						
Xu et al. (2020a) *Medium*	United States 2007–2012	Cross-sectional	Adults from NHANES, ages 20–79 yr N = 3,630	Serum Mean (SD) = 13.33 (12.92) µg/L	Fractional exhaled nitric oxide (ppb)	Percent change per doubling of PFOS, or by tertile	Fractional exhaled nitric oxide 2.03 (0.11, 4.00), p-value < 0.05 T2: 1.80 (−1.53, 5.25) T3: 5.02 (1.40, 8.77), p-value < 0.01 p-trend < 0.006
	Results: Lowest tertile used as reference group **Confounding:** Age, sex, race/ethnicity, BMI, annual family income, education level, serum cotinine, recent respiratory symptom, and smoking status						
Zhou et al. (2017b) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158	Serum Case boys: 36.9 (22.6–67.8) Case girls: 28.2 (13.9–46.0) Control boys: 29.9 (13.0–43.8) Control girls: 28.8 (14.8–42.6)	Asthma	Asthma: Comparison of PFOS distributions (Wilcoxon rank-sum test)	Asthma: Increased PFOS among asthmatics, p-value = 0.002

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Case girls: 73 Control boys: 102 Control girls: 123				
	Confounding: Cases and controls were matched on age and sex						
Zhu et al. (2016) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123	Serum Case boys: 36.94 Case girls: 28.16 Control boys: 26.24 Control girls: 30.12	Asthma	OR for highest vs. lowest quartiles of PFOS	Boys: 4.24 (1.81, 9.42); p-value for trend = 0.001 Girls: No statistically significant associations or trends
	Confounding: Age, BMI, parental education, environmental tobacco smoke, parental asthma, month of survey						
Zhou et al. (2017c) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123	Serum Cases: 33.94 (19.59–61.10) Controls: 28.91 (14.06–42.02)	Asthma	OR per unit increase in PFOS	Females with high testosterone: 0.58 (0.36, 0.93) Females with low testosterone: 1.32 (0.88, 1.99) p-value for interaction by low/high testosterone = 0.010 Males with high testosterone: 1.04 (0.87, 1.25) Males with low testosterone: 2.54 (1.40, 4.60) p-value for interaction by low/high testosterone = 0.005

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Sexes evenly divided into high/low hormone classifications				Females with high estradiol: 1.25 (0.84, 1.86) Females with low estradiol: 0.65 (0.42, 0.99) p-value for interaction by low/high estradiol = 0.026 Males with high estradiol: 1.25 (0.90, 1.72) Males with low estradiol: 1.06 (0.87, 1.30) p-value for interaction by low/high estradiol = 0.407
	Confounding: Age, sex, BMI, parental education, environmental tobacco smoke exposure, physical activity, month of survey						
Timmermann et al. (2017a) *Low*	Faroe Islands, recruitment: 1997–2000	Cohort	Pregnant women and infants, follow-up at ages 5, 7, and 13 yr, N = 559	Maternal serum Prenatal/At birth: 27.4 (23.3–33.3) Age 5/7: 16.8 (13.5–21.1) Age 13: 6.7 (5.2–8.5)	Asthma	OR per doubling of maternal PFOS	Asthma (age 5): Total: 1.21 (0.64, 2.29) No MMR vaccine before age 5: 3.96 (0.55, 28.39) Yes MMR vaccine before age 5: 0.98 (0.55, 1.76) Asthma (age 13): Total: 0.69 (0.43, 1.09) No MMR vaccine before age 5: 5.41 (0.62, 47.16) Yes MMR vaccine before age 5: 0.94 (0.51, 1.74)
	Confounding: Family history of eczema in children, allergic eczema, and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, daycare attendance at age 5, birth weight, and family history of chronic bronchitis/asthma						
Averina et al. (2019) Low	Norway 2010–2011	Cohort	Adolescents in their first year of high school from TFF1 and TFF2	Serum Girls: GM = 5.8 (IQR = 2.7) Boys: GM = 6.8 (IQR = 3.0)	Asthma self-reported, doctor-diagnosed	OR by quartiles of PFOS	TFF1 Q2: 1.51 (0.72, 3.18) Q3: 2.75 (1.36, 5.57); p-value = 0.005

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 675				Q4: 2.11 (1.02, 4.37); p-value = 0.044 p-value for trend = 0.02 TFF2 Q2: 2.00 (0.96, 4.15); p-value = 0.064 Q3: 2.56 (1.24, 5.30); p-value = 0.011 Q4: 1.43 (0.65, 3.12) Trend not statistically significant
	Results: Lowest quartile used as reference group. **Confounding:** Sex, age, BMI, physical activity, unemployment/disability of parents, living with adoptive parents, fish intake						
Workman et al. (2019) *Low*	Canada 2010–2012	Cohort	Mothers and their infants N = 85	Maternal plasma 2.2 (Range: 0.18–21)	Recurrent wheezing episodes	Difference in prenatal PFOS levels for wheezing vs. no wheezing (Mann-Whitney test)	No significant differences
	Confounding: None reported						

Notes: AEC = absolute eosinophil counts; BMI = body mass index; CI = confidence interval; ECP = eosinophilic cationic protein; ETS = environmental tobacco smoke; GBCA = Genetic and Biomarker study for Childhood Asthma; GM = geometric mean; IgE = immunoglobulin E; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; MMR = measles, mumps, rubella; mo = months; NY = New York; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; T2 = tertile 2; T3 = tertile 3; TFF1 = Tromsø Fit Futures; TFF2 = Tromsø Fit Futures 2; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-10. Associations Between PFOS Exposure and Allergies in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Children			
Wang et al. (2011) *Medium*	Taiwan 2004	Cohort and cross-sectional	Pregnant women and their children at age 2 N = 244 (133 boys, 111 girls)	Cord blood 5.50 (0.11–48.36)	Atopic dermatitis, IgE levels (log-KU/L)	Atopic dermatitis: OR by quartiles of PFOS IgE: Regression coefficient per ln-unit increase in PFOS	Atopic dermatitis Q2: 0.68 (0.20, 2.3) Q3: 2.34 (0.86, 6.41) Q4: 2.19 (0.78, 6.17) IgE in cord blood at birth All: 0.161 (SE = 0.147), p-value = 0.017 Boys: 0.175 (SE = 0.179), p-value = 0.053 Girls: 0.151 (SE = 0.165), p-value = 0.616 IgE in serum at age 2 All: 0.251 (SE = 0.179), p-value = 0.147 Boys: 0.359 (SE = 0.255), p-value = 0.238 Girls: 0.095 (SE = 0.325), p-value = 0.723
	Results: Lowest quartile used as reference group. **Confounding:** Gender, gestational age, maternal age. Additional confounding for atopic dermatitis: maternal history of atopy, duration of breast feeding, pre-natal ETS exposure. Additional confounding for IgE: parity.						
Okada et al. (2012) *Medium*	Japan 2002–2005	Cohort	Pregnant women and children from the Hokkaido Study on Environment and Children's Health; follow-up at 18 mo N = 343	Maternal serum 5.2 (3.4–7.2)	Food allergy, eczema, otitis media, and wheezing IgE levels (log10-IU/mL)	OR and regression coefficients per log10-unit increase in PFOS	Food allergy 3.72 (0.81, 17.10) Eczema 0.87 (0.15, 5.08) Otitis media 1.40 (0.33, 6.00) Wheezing

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							2.68 (0.39, 18.30) IgE: Linear regression −0.342 (−1.230, 0.546) Quadratic regression −0.681 (−2.50, 1.137) Cubic regression 1.464 (−5.354, 8.282) Results stratified by gender not statistically significant for boys and combined
	Confounding: maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant gender, breastfeeding period, environmental tobacco exposure, daycare attendance and blood sampling period; for IgE: maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period						
Okada et al. (2014) *Medium*	Japan 2003–2009	Cohort	Japanese women who had singleton births and their infants N = 2,062	Maternal blood 5.02 (3.71–6.83)	Total allergic diseases (eczema, wheezing, and allergic rhinoconjunctivitis symptoms)	OR by quartiles of PFOS exposure	Total allergic diseases Q2: 0.97 (0.75, 1.26) Q3: 0.80 (0.61, 1.04) Q4: 0.86 (0.66, 1.13) p-value for trend = 0.139
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, number of siblings, day care attendance, and ETS exposure in infancy at 24 months.						
Buser et al. (2016) *Medium*	United States 2005–2016	Cross-sectional	Adolescents aged 12–19 yr from NHANES N by cycle: 2005–2006: 637 2007–2010: 701	Serum 2005–2006: GM = 14.98 (10.65–22.69) 2007–2010: GM = 8.74 (5.96–13.75)	Food allergy or sensitization	OR by quartiles of PFOS	Food allergy, 2007–2010 cycle Q2: 2.22 (0.85, 5.77) Q3: 2.43 (1.05, 5.59) Q4: 2.95 (1.21, 7.24) p-value for trend = 0.27 Food sensitization, 2005–2006 cycle: No statistically significant associations or trends

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Outcome: Food sensitization defined as at least 1 food specific IgE level ≥0.35 kU/L. **Results:** Lowest quartile used as reference. **Confounding:** Age, sex, race/ethnicity, BMI, serum cotinine[c]						
Goudarzi et al. (2016a) *Medium*	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study on Environment and Children's Health N = 1,558 (765 girls, 793 boys)	Maternal blood 4.93 (3.67–6.65)	Allergic diseases, total	OR by quartiles of PFOS	Q2: 0.66 (0.48, 0.90) Q3: 0.79 (0.58, 1.07) Q4: 0.82 (0.60, 1.11) p-value for trend = 0.391 No statistically significant associations, trends, or interactions by sex
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, maternal educational level, sex, parental allergic history, number of older siblings, breast feeding, daycare attendance, environmental tobacco smoke exposure						
Stein et al. (2016b) *Medium*	United States, 1999–2000, 2003–2004, 2005–2006	Cross-sectional	Children aged 12–19 years, NHANES N = 638	Serum GM = 20.8 (95% CI: 19.1, 22.7)	Allergy and rhinitis	OR [per IQR(lnPFOS) increase (0.76 ln-ng/mL)]	Allergy 1.05 (0.80, 1.37) Rhinitis 1.16 (0.90, 1.50)
	Confounding: Age, sex, race/ethnicity, survey year; for Wheeze: age, gender, race, weight status, serum cotinine.						
Timmermann et al. (2017a) *Medium*	Faroe Islands, Recruitment: 1997–2000	Cohort	Pregnant women and infants, follow-up at ages 5, 7, and 13 yr, N = 559	Maternal serum Prenatal/At birth: 27.4 (23.3–33.3) Age 5/7: 16.8 (13.5–21.1) Age 13: 6.7 (5.2–8.5)	Allergy, allergic rhino-conjunctivitis in past 12 mo, positive skin prick test, IgE	OR per doubling of PFOS IgE: Percent change per doubling of PFOS	Allergy (age 5) OR = 0.73 (0.38,1.41) Allergic rhino-conjunctivitis in past 12 mo, age 13 1.01 (0.54, 1.89) Positive skin prick test, age 13 1.15 (0.75, 1.77) IgE, age 7: −9.38 (−37.17, 30.71)
	Confounding: Maternal parity, family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal fish intake during pregnancy, and duration of breastfeeding; for IgE: family history of eczema in						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	children, allergic eczema, and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, and daycare attendance at age 5						
Impinen et al. (2018) *Medium*	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 yr and 10 yr of age, N = 641	Cord blood 5.2 (4.0–6.6)	Rhinitis, rhino-conjunctivitis, SPT	OR per log2-unit increase in PFOS	Rhinitis, current, 10 yr 1.00 (0.72, 1.40); p-value = 0.983 Rhinitis, ever, 10 yr 1.05 (0.74, 1.48); p-value = 0.775 Rhino-conjunctivitis, ever, 10 yr 1.02 (0.72, 1.45); p-value = 0.905 Rhino-conjunctivitis, ever, spes IgE > 0.35, 10 yr 1.02 (0.71, 1.47); p-value = 0.905 SPT, any pos, 10 yr 0.87 (0.65, 1.17); p-value = 0.359 SPT + and/pr sIgE > 0.35, 10 yr 0.91 (0.69, 1.19); p-value = 0.476
	Confounding: Sex						
Impinen et al. (2019) *Medium*	Oslo, Norway, Enrollment: 1999–2008	Cohort	Pregnant women and their infants (followed to age 7), N = 921	Maternal blood 12.87 (9.92–16.63)	Allergy, food or inhaled	OR per IQR increase in PFOS	Allergy, food, current All: 1.02 (0.73, 1.41); p-value = 0.928 Boys: 1.09 (0.68, 1.74); p-value = 0.72 Girls: 0.95 (0.59,1.51); p-value = 0.815 Allergy, food, ever All: 0.99 (0.72, 1.37); p-value = 0.969 Boys: 1.11 (0.69, 1.77); p-value = 0.671 Girls: 0.91 (0.58, 1.42); p-value = 0.676

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Allergy, inhaled, current All: 1.11 (0.72, 1.69); p-value = 0.643 Boys: 0.86 (0.44, 1.71); p-value = 0.669 Girls: 1.17 (0.55, 2.48); p-value = 0.679 Allergy, inhaled, ever All: 1.27 (0.93,1.74); p-value = 0.135 Boys: 1.2 (0.79, 1.84); p-value = 0.39 Girls: 1.33 (0.84, 2.12); p-value = 0.224
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy, nursery attendance						
Ait Bamai et al. (2020) *Medium*	Hokkaido, Japan, 2003–2012	Cohort	Early pregnancy to 7 yr, N = 2,689	Maternal blood 5.12 (3.75–7.02)	Rhino-conjunctivitis	RR per ln-unit increase in PFOS, from birth to 7 yr old	0.96 (0.79, 1.15); p-value = 0.626
	Confounding: Sex, parity, maternal age at delivery, maternal smoking during pregnancy, pre-pregnancy BMI, and annual household income during pregnancy						
Kvalem et al. (2020) *Medium*	Norway, Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, age 10 yr: N = 377 Age 16 yr: N = 375	Serum All: 19.4 (IQR: 9.23) Girls: 17.52 (IQR: 8.02) Boys: 21.7 (IQR: 8.86)	Rhinitis, skin prick test (SPT)	Change in RR per IQR increase in PFOS	Rhinitis 10 yr All: 0.98 (0.74,1.30); p-value = 0.92 Boys: 0.90 (0.66, 1.23); p-value = 0.52 Girls: 0.97 (0.58, 1.62); p-value = 0.92 16 yr All: 1.03 (0.90,1.19); p-value = 0.69

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Boys: 0.92 (0.72, 1.19); p-value = 0.55 Girls: 1.15 (0.91, 1.45); p-value = 0.24 SPT 10 yr All: 1.10 (0.95, 1.26); p-value = 0.21 Boys: 0.98 (0.96, 1.01); p-value = 0.17 Girls: 0.97 (0.65, 1.44); p-value = 0.86 16 yr All: 1.09 (1.03, 1.15); p-value = 0.001 Boys: 1.07 (0.97, 1.17); p-value = 0.18 Girls: 0.99 (0.80, 1.23); p-value = 0.93
	Confounding: 10 yr: Physical activity at 10 yr, mothers' education, BMI at 10 yr; 16 yr: BMI at 16 yr, puberty status at 16 yr, mothers' education, physical activity level at 16 yr						

Notes: BMI = body mass index; CI = confidence interval; ETS = environmental tobacco smoke; GM = geometric mean; IgE = immunoglobulin E; IQR = interquartile range; MMR = measles, mumps, rubella; mo = months; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; SE = standard error; SPT = skin prick test; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-11. Associations Between PFOS Exposure and Eczema in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
General Population							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Okada et al. (2014) *Medium*	Japan 2003–2009	Cohort	Japanese women who had singleton births and their infants N = 2,062	Maternal blood 5.02 (3.71–6.83)	Eczema	OR by quartiles of PFOS exposure	Eczema Q2: 1.06 (0.77, 1.46) Q3: 0.93 (0.67, 1.29) Q4: 0.89 (0.64, 1.24) p-value for trend = 0.372
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, and ETS exposure in infancy at 24 months.						
Goudarzi et al. (2016a) *Medium*	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study on Environment and Children's Health N = 1,558 (765 girls, 793 boys)	Maternal blood 4.93 (3.67–65654)	Eczema	OR by quartiles of PFOS	Q2: 0.64 (0.44, 0.93) Q3: 0.65 (0.45, 0.95) Q4: 0.85 (0.591, 1.22) p-value for trend = 0.427 No statistically significant associations, trends, or interactions by sex
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, maternal educational level, sex, parental allergic history, number of older siblings, breast feeding, daycare attendance, environmental tobacco smoke exposure[c]						
Timmermann et al. (2017a) *Medium*	Denmark 1997–2000	Cohort	Pregnant women and infants from the CHEF study at ages 5, 7, and 13 yr N = 559	Serum Prenatal at birth: 16.8 (13.5–21.1) Age 5/7: 27.4 (23.3–33.3)	Atopic eczema at age 13	OR per doubling of PFOS at age 13	Age 5: 0.75 (0.42, 1.34) Age 13: 0.8 (0.46, 1.39) MMR vaccination before age 5 Yes: 8.94 (0.27, 299.11) No: 0.82 (0.53, 1.28)
	Confounding: Confounding: Family history of eczema in children., allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, and fish intake at age 13, birth weight, and family history of chronic bronchitis/asthma, maternal parity						
Chen et al. (2018) *Medium*	China 2012–2015	Cohort	Infants followed up at 6, 12, and 24 mo N = 687 children (328	Cord blood All: 2.48 (Range = 0.39–65.61) Female: 2.47	Atopic dermatitis	OR per log-unit increase in PFOS, or by quartiles	All: 1.23 (0.85, 1.76) Q2: 0.93 (0.56, 1.58) Q3:1 (0.59, 1.7) Q4:1.31 (0.78, 2.2) Female: 1.1 (0.64, 1.87) Q2: 0.73 (0.33, 1.61)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			female and 359 male)	(Range = 0.39–18.68) Male: 2.49 (Range = 0.62–65.61)			Q3:0.71 (0.32, 1.6) Q4: 1.08 (0.5, 2.35) Male: 1.42 (0.84, 2.42) Q2: 1.34 (0.64, 2.8) Q3: 1.3 (0.61, 2.75) Q4: 1.65 (0.79, 3.41)
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as reference group. **Confounding:** Maternal age, maternal pre-pregnancy BMI, gestational week at delivery, birth weight, maternal education, paternal education, parity, mode of delivery, family history of allergic disorders, infant sex, family income, maternal ethnicity, paternal smoking, breastfeeding						
Impinen et al. (2018) *Medium*	Norway 1992–2002	Cohort, Nested case-control	Children from the ECA study at 0, 2, and 10 yr N = 641	Cord blood 5.2 (4.0–6.6)	Atopic dermatitis diagnosed anytime between 0 and 2 yr old, or between 0 and 10 yr old	OR per log2-unit increase PFOS	Ages 0–2: 1.15 (0.88, 1.52) Ages 0–10: 0.68 (0.38, 1.2)
	Confounding: Sex						
Manzano-Salgado et al. (2019) *Medium*	Spain 2003–2015	Cohort	Pregnant women and children followed up at ages 1.5, 4, and 7 from the INMA study N = 1,188 at 1.5, N = 1,184 at 4 yr, N = 1,066 at 7 yr	Maternal plasma 6.06 (4.52–7.82)	Eczema	OR or RR per log2-unit increase in PFOS	Age 1.5: 1.02 (0.83, 1.27) Age 4: 0.8 (0.65, 0.99) Age 7: 0.82 (0.68, 0.99) Boys at ages 1.5, 4, and 7: 0.91 (0.75, 1.11) Girls at ages 1.5, 4, and 7: 0.77 (0.64, 0.94) From ages 1.5 to 7 yr: 0.86 (0.75, 0.98)
	Confounding: Age at follow-up of the child, maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth						
Wen et al. (2019a)	Taiwan 2001–2005	Cohort	Children at age 2 yr	Cord blood 3.49 (2.18–5.05)	Atopic dermatitis	OR by tertiles of PFOS	T2: 1.33 (0.57, 3.20) T3: 1.86 (0.84, 4.36)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			N = 839				
	Results: Lowest tertile used as reference. **Confounding:** Sex, family income, maternal atopy, breast feeding, and maternal age at childbirth						
Wen et al. (2019b) *Medium*	Taiwan 2001–2005	Cohort	General population, children, and adolescents < 18 yr.; Infants followed from birth up to 5 yr of age N = 863	Cord blood 3.49 (2.18–5.05)	Atopic dermatitis	Hazard ratio for PFOS ≥ 5.05 ng /mL vs. < 5.05 ng/mL	1.43 (0.82, 2.43) No statistically significant associations
	Confounding: Sex, parental education, parental atopy, breast feeding, and maternal age at childbirth						

Notes: BMI = body mass index; CD = Crohn's disease; CHEF = Children's Health and the Environment in the Faroes; CIS = clinically isolated serum syndrome; ECA = Environment and Childhood Asthma; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; MMR = measles, mumps, rubella; mo = months; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; RRMS = relapsing remitting multiple sclerosis; UC = ulcerative colitis; yr = years.

[a] Exposure levels are reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-12. Associations Between PFOS Exposure and Autoimmune Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Gaylord et al. (2020) *Medium*	United States	Case-control	Children and adolescents younger than 21 yr with (cases) and without (controls) celiac disease N = 88 (42 girls, 46 boys)	Serum Cases: 2.02 (IQR = 1.85) Controls: 1.59 (IQR = 1.64)	Celiac disease	OR per ln-unit change in PFOS	2.20 (0.78, 6.18) Girls: 12.8 (1.17, 141); p-value < 0.05 Boys: 1.02 (0.24, 4.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Genetic susceptibility score, albumin, BMI, age, race (non-Hispanic white vs. other race/ethnicity) and sex[c]						
Steenland et al. (2018) *Low*	United States 1999–2012	Case-control	Patients with UC, CD, or healthy controls N = 114 UC, 60 CD, 75 controls	Serum UC: 3.95 CD: 3.32 Neither: 4.21	UC	Change in log(PFOS) comparing cases and controls	UC vs. CD: 0.05 (0.16), p-value = 0.77 UC vs. control: −0.40 (0.21), p-value = 0.06
	Comparison: Logarithm base not specified. **Results:** Lowest quintile used as reference. **Confounding:** Age, sex, ethnic group (white or non-white), year of sample						
Sinisalu et al. (2020) *Low*	Finland 1999–2005	Cohort	Pregnant women and infants at birth and 3 mo from the Type 1 Diabetes Prediction and Prevention Study in Finland (DIPP) N = 33 (17 celiac disease, 16 controls)	Cord blood Case: 2.21 (min–max: 0.27–8.17) Control: 2.25 (min–max: 0.27–5.32) 3-mo serum Case: 2.93 (min–max: 0.27–7.66) Control: 3.40 (min–max: 0.71–6.70)	Celiac disease	Comparison of mean PFOS exposure levels	No significant differences in exposure between cases and control at birth or 3 mo
Ammitzbøll et al. (2019) *Low*	Denmark 2019	Case-control	Adults with (cases) or without (controls) RRMS or CIS N = 162 (92 women, 70 men)	Serum Cases: 7.14 (5.76–9.93) Controls: 9.41 (6.41–13.0)	Relapsing remitting multiple sclerosis (RRMS)	Percent change in PFOS comparing MS cases vs. healthy controls	−17 (−27, −6); p-value = 0.004 Females: −14 (−28, 3); p-value = 0.093 Males: −19 (−32, −3); p-value = 0.023
	Confounding: Age, sex, breastfeeding						

Notes: CD = Crohn's disease; CIS = clinically isolated serum syndrome; DIPP = Diabetes Prediction and Prevention Study in Finland; IQR = interquartile range; mo = months; OR = odds ratio; RRMS = relapsing remitting multiple sclerosis; UC = ulcerative colitis.
[a] Exposure levels are reported as median (25th–75th percentile) unless otherwise noted.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.
[c] Confounding indicates factors the models presented adjusted for.

D.5 Cardiovascular

D.5.1 Cardiovascular Endpoints

Table D-13. Associations Between PFOS Exposure and Cardiovascular Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Li et al. (2021a) *High* for gestation, birth, and childhood exposures (3-yr and 8-yr) *Medium* for exposure at 12-yr follow-up	United States 2003–2006	Cohort	Pregnant women and their children followed up at birth and ages 3, 8, and 12 from HOME study Gestation: N = 203 At birth: N = 124 Age 3: N = 137 Age 8: N = 165 Age 12: N = 190	Maternal serum Gestation: 12.9 (8.9–18.0) Cord serum At birth: 4.2 (3.0–6.5) Serum At age 3: 6.2 (4.5–9.9) At age 8: 3.6 (2.8–4.7) At age 12: 2.4 (1.8–3.2)	SBP (z-score), mean of SBP and DBP (z-score)	Regression coefficient per log2-unit IQR increase in PFOS	SBP (z-score) Gestation: 0.1 (−0.1, 0.2) At birth: 0.2 (0.0, 0.4) Age 3: 0.1 (−0.1, 0.4) Age 8: 0.1 (−0.3, 0.4) Age 12: 0.2 (−0.1, 0.5) Mean of SBP and DBP (z-score) Gestation: 0.1 (−0.1, 0.2) At birth: 0.1 (0.0, 0.3) Age 3: 0.1 (−0.1, 0.3) Age 8: 0.1 (−0.2, 0.4) Age 12: 0.2 (0.0, 0.4)
	Confounding[c]: visit, visit × PFAS, maternal age, maternal education, maternal pre-pregnancy BMI, gestational serum cotinine concentrations, and parity; and child age, sex, race, and pubertal stage. Additional confounding for analyses at age 3, age 8, and age 12: Breastfeeding duration.						
Ma et al. (2019) *Medium*	United States 2003–2012	Cross-sectional	Adolescents aged 12–20 from NHANES	Serum median = 11.1 (6.2–18.0)	DBP, SBP	Regression coefficient per log10-unit increase in PFOS	DBP Total cohort: 0.014 (−0.001, 0.030) Females: 0 (−0.02, 0.02)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 2,251 (1,048 female, 1,203 male)				Males: 0.025 (0.001, 0.049); p-value < 0.05 SBP Total cohort: 0.002 (−0.004, 0.009) Females: −0.001 (−0.009, 0.008) Males: 0.003 (−0.006, 0.012)
Warembourg et al. (2019) *Medium*	France, Spain, Lithuania, Norway, Greece, United Kingdom 1999–2015	Cohort	Pregnant women and their children at ages 6 and 11 from the HELIX Project N = 1,277 Prenatal exposure Postnatal exposure	Maternal blood: 6.4 (4.1–9.6) Plasma: 2.0 (1.3–3.2)	DBP, SBP	Regression coefficient per log2-unit IQR increase in PFOS	DBP Maternal PFOS: 0.46 (−0.34, 1.27) Childhood PFOS: 0.48 (−1.06, 0.62) SBP Maternal PFOS: −0.22 (−1.06, 0.62) Childhood PFOS: 0.23 (−0.56, 1.03)
	Confounding: Cohort of inclusion, maternal age, maternal education level, maternal pre-pregnancy BMI, parity, parental country of birth, child age, child sex, child height[c]						
Canova et al. (2021) *Medium*	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 yr and children aged 8 to 11 yr from health surveillance program in Veneto Region Adolescents: N = 6,669 Children: N = 2,693	Serum Adolescents: 3.3 (2.2–4.9) Children: 2.2 (1.6–3.0)	DBP, SBP	Regression coefficient per ln-unit increase in PFOS, or by quartiles	DBP Adolescents Per ln-unit increase: −0.44 (−0.82, 0.05) Q2: −0.54 (−1.15, 0.08) Q3: −0.66 (−1.30, −0.02) Q4: −0.78 (−1.45, −0.10) Children Per ln-unit increase: 0.03 (−0.54, 0.61) Q2: 0.67 (−0.15, 1.54) Q3: 0.91 (0.05, 1.77) Q4: −0.10 (−0.95, 0.75)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							SBP Adolescents Per ln-unit increase: −0.47 (−1.02, 0.08) Q2: −0.67 (−1.54, 0.20) Q3: −0.96 (−1.87, −0.06) Q4: −1.34 (−2.30, −0.38) Children Per ln-unit increase: −0.42 (−1.18, 0.33) Q2: −0.13 (−1.22, 0.95) Q3: 0.18 (−0.95, 1.31) Q4: −0.80 (−1.92, 0.33)
	Results: Lowest quartile used as the reference group. **Confounding:** Age, gender, country of birth, data on food consumption, degree of physical activity, salt intake, smoking status (for adolescents only), time lag between the beginning of the study and the date of enrollment.						
Papadopoulou et al. (2021) *Medium*	United Kingdom, France, Spain, Lithuania, Norway, Greece Recruitment 1999–2010, Follow-up: 2013–2015	Cohort	Mother-child pairs from the HELIX Project, children followed up around age 8 (range 6–12) N = 1,101	Maternal plasma (prenatal) 6.15 (3.99–9.16) Plasma (childhood) 1.93 (1.22–3.11)	DBP (z-score), SBP (z-score)	Regression coefficient per doubling in PFOS, or by quartiles	DBP Maternal PFOS: 0.04 (−0.06, 0.14) Q2: −0.06 (−0.23, 0.11) Q3: 0.03 (−0.16, 0.23) Q4: −0.04 (−0.29, 0.21) p-trend = 0.922 Childhood PFOS: 0.01 (−0.06, 0.08) Q2: −0.02 (−0.18, 0.13) Q3: −0.01 (−0.19, 0.17) Q4: 0.01 (−0.20, 0.23) p-trend = 0.827 SBP Maternal PFOS: 0.03 (−0.08, 0.14) Q2: −0.06 (−0.25, 0.13) Q3: 0.10 (−0.12, 0.13) Q4: −0.05 (−0.32, 0.23)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							p-trend = 0.980 Childhood PFOS: −0.01 (−0.08, 0.07) Q2: −0.04 (−0.21, 0.13) Q3: −0.03 (−0.23, 0.16) Q4: −0.03 (−0.27, 0.21) p-trend = 0.763
	Comparison: Maternal PFOS quartiles are defined as follows: Q1: 0.28–3.98; Q2: 3.99–6.15; Q3: 6.15–9.15; Q4: 9.16–47.98; childhood PFOS quartiles are defined as follows: Q1: 0.00–1.22; Q2: 1.22–1.92; Q3: 1.93–3.10; Q4: 3.11–33.83. **Results:** Lowest quartile used as the reference group. **Confounding:** Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, child gender, PFHxS, PFNA, PFOA						
Manzano-Salgado et al. (2017b) *Medium*	Spain 2003–2008	Cohort	Pregnant women and their children at ages 4 and 7 from INMA study Age 4 N = 839 (412 girls, 427 boys) Age 4 N = 386 (197 girls, 189 boys) for CMR score measurements Age 7 N = 1,086 (535 girls, 551 boys)	Maternal blood GM = 5.80 (4.52–7.84)	Blood Pressure (BP) (z-score) Cardiometabolic Risk Score (CMR)	Regression coefficient per log2-unit increase in PFOS	BP All age 4: −0.05 (−0.15, 0.06) Girls: −0.06 (−0.22, 0.09) Boys: −0.02 (−0.18, 0.14) All age 7: 0.06 (−0.04, 0.15) Girls: 0.06 (−0.09, 0.20) Boys: 0.04 (−0.08, 0.17) CMR All age 4: 0.28 (−0.33, 0.89) Girls: 0.10 (−0.73, 0.93) Boys: 0.47 (−0.44, 1.37)
	Confounding: Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child						
Lin et al. (2013) *Medium* for CIMT *Low* for Systolic BP	Taiwan 2006–2008	Cross-sectional	Adolescents and young adults ages 12–30 N = 637	Serum 8.65 (5.4–13.52)	SBP, CIMT	Mean by quartiles	SBP: No associations across quartiles; p-trend = 0.177 CIMT: Significant associations across exposure groups; p-trend < 0.002

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Females: significant associations across exposure groups; p-trend < 0.001 Males: no associations across exposure groups; p-trend = 0.401 Ages 12–19: significant associations across exposure groups; p-trend < 0.001 Ages 20–30: no associations across exposure groups; p-trend = 0.084
	Confounding: Age, gender, smoking status, alcohol drinking, BMI; for CIMT, also includes systolic blood pressure, low-density lipoprotein cholesterol, triglyceride, high sensitivity C-reactive protein, homeostasis model assessment of insulin resistance						
Geiger et al. (2014b) *Medium*	United States 1999–2000, 2003–2008	Cross-sectional	Children ages ≤ 18 yr from NHANES N = 1,655	Serum Mean (SE) = 18.4 (0.5)	Hypertension	OR per ln-unit increase in PFOS, or by quartile	Hypertension Per ln-unit increase: 0.83 (0.58, 1.19) Q2: 0.99 (0.55, 1.78) Q3: 0.73 (0.36, 1.48) Q4: 0.77 (0.37, 1.61) p-trend = 0.3625
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, race-ethnicity, BMI categories, annual household income categories, moderate activity, total cholesterol, and serum cotinine						
Averina et al. (2021) *Medium*	Norway 2010–2011	Cross-sectional	First level high school students ages 15–19 yr from TFF1 N = 940	Serum Girls: GM (IQR) = 5.71 (2.64) Boys: GM (IQR) = 6.52 (3.09)	Hypertension	OR by quartiles	Hypertension Q2: 1.40 (0.78, 2.51), p-value = 0.261 Q3: 1.01 (0.56, 1.80), p-value = 0.980 Q4: 1.86 (1.08, 3.19), p-value = 0.025
	Outcome: Hypertension defined as systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg. **Comparison:** PFOS quartiles are defined as follows: Q1: 1.28–4.86; Q2: 4.87–6.21; Q3: 6.22–7.80; Q4: 1.28–4.86. **Results:** Lowest quartile used as the reference group. **Confounding:** Sex, age, BMI and physical activity outside school						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Lin et al. (2016) *Medium*	Taiwan 1992–2000	Cross-sectional	Adolescents and young adults ages 12–30 N = 848	Serum GM = 6.44 (95% CI: 6.05–6.89)	8-OHDG (log-µg/g creatinine) CIMT CD31+ / CD42a− (log count/µL) CD31+ / CD42a+ (log count/µL) CD62E (log count/µL) CD62P (log count/µL)	Mean by quartiles	8-OHDG: No associations across exposure groups; p-trend = 0.102 CIMT Q1: 0.433 (0.423, 0.442) Q2: 0.437 (0.428, 0.446) Q3: 0.456 (0.447, 0.465) Q4: 0.453 (0.444, 0.463) p-trend <0.001 CD31+ / CD42a−: Statistically significant increase across exposure groups, 4.65–5.30 (Q3); p-trend = 0.010 CD31+ / CD42a+: Statistically significant increase across exposure groups, 8.02–8.54 (Q3); p-trend = 0.010 CD62E, CD62P: No statistically significant associations across exposure groups
	Confounding: Age, gender, smoking status, BMI, systolic blood pressure, low-density lipoprotein, triglyceride, homeostasis model assessment of insulin resistance, and high sensitivity C-reactive protein						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children ages 8–12 N = 48	Serum 2.79 (IQR = 2.10)	DBP, SBP	Regression coefficient per unit increase in PFOS	DBP: 1.17 (−0.40, 2.74) SBP: 1.53 (−0.46, 3.51)
	Confounding: Age, race, sex						
Koshy et al. (2017) *Low*	United States 2011–2012	Cross-sectional	Children and adolescents from the World Trade Center Health	Serum 3.72 (IQR = 2.82)	Augmentation Index (AI)	Regression coefficient per ln-unit increase in PFOS	AI: −0.24 (−2.02, 2.41) BAD: 0.30 (−0.01, 0.62) PWV: −0.06 (−0.23, 0.11)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Registry (WTCHR) N = 308	Comparison: 2.78 (IQR = 2.18)	Brachial Artery Distensibility (BAD) Pulse Wave Velocity (PWV)		
	Confounding: BMI category, caloric intake, cotinine concentration, physical activity, race, sex						
				Pregnant Women			
Matilla-Santander et al. (2017) *Medium*	Spain 2003–2008	Cohort	Pregnant women from INMA study N = 1,240	Plasma 6.05 (4.51–7.81)	CRP (log10 mg/dL)	Percent median change by quartiles and per log10-unit increase in PFOS	CRP −8.41 (−18.4, 3.35) By quartile: Q2: 6.18 (−11.3, 28.4) Q3: −6.76 (−22.9, 11.6) Q4: −5.82 (−22.9, 12.7)
	Results: Lowest quartile used as the reference group. **Confounding:** Sub-cohort, country of birth, pre-pregnancy BMI, previous breastfeeding, parity, gestational week at blood extraction, physical activity, relative Mediterranean Diet Score						
				General Population			
Liao et al. (2020) *High*	United States 2003–2012	Cross-sectional	Adults ages 20+ from NHANES N = 6,967 (3,439 females, 3,528 males)	Serum 12.8 (7.2–22.0)	DBP, SBP, hypertension	DBP and SBP: Regression coefficient per log10-unit increase in PFOS or around inflection point (8.20 ng/mL) Hypertension: OR by tertiles	DBP Levels ≤ 8.20 ng/mL: −2.62 (−4.73, −0.51) Levels > 8.20 ng/mL: 1.23 (−0.42, 2.88) SBP Per log10-unit change: 1.35 (0.18, 2.53) Hypertension: No statistically significant associations or trends by tertiles or age groups Males T2: 1.17 (0.93, 1.47) T3: 1.07 (0.85, 1.34) Females

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							T2: 1.08 (0.87, 1.34) T3: 1.18 (0.92, 1.51) p-value for interaction by sex = 0.016
	Outcome: Hypertension defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed antihypertensive medication. **Comparison:** Tertiles are defined as follows (in ng/mL PFOS): T1 ≤ 8.9; 8.9 < T2 ≤ 18.1; 18.1 < T3. **Results:** Lowest tertile used as the reference group. **Confounding:** Age, sex, education level, race, diabetes mellitus, consumption of at least 12 alcohol drinks/year, current smoking status, BMI, waist circumference, hemoglobin, total cholesterol, estimated glomerular filtration rate (eGFR), dietary intake of sodium, dietary intake of potassium, and dietary intake of calcium						
Mattsson et al. (2015) *High*	Sweden 1990–1991, 2002–2003	Case-control	Rural men N = 462	Serum Cases: 22.8 (IQR = 10.0) Controls: 22.0 (IQR = 10.1)	CHD	OR by quartiles	CHD Q2: 0.82 (0.46, 1.45) Q3: 1.30 (0.74, 2.26) Q4: 1.07 (0.6, 1.92)
	Results: Lowest quartile used as reference. **Confounding:** BMI, systolic blood pressure, total cholesterol, HDL, tobacco use						
Mobacke et al. (2018) *High*	Sweden Years not reported	Cross-sectional	Adults aged 70 from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study N = 801	Serum Mean (SD) = 14.9 (8.88)	Left Ventricular End-Diastolic Diameter (LVEDD) (mm) Left Ventricular Mass Index (LVMI) ($g/m^{2.7}$) Relative Wall Thickness (RWT)	Regression coefficient per ln-unit increase in PFOS	LVEDD: 0.47 (0.08, 0.87) LVMI: 0.12 (−0.73, 0.97) RWT: −0.01 (−0.01, −0.001)
	Confounding: Sex, systolic blood pressure, antihypertensive medication, HDL and LDL, cholesterol, blood glucose, waist circumference, triglycerides, BMI, education levels, exercise habits, smoking, energy, alcohol intake						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Bao et al. (2017) *Medium*	China 2015–2016	Cross-sectional	Adults aged 22–96 N = 1,612 (408 females, 1,204 males)	Serum 24.2 (14.6–37.2)	DBP, SBP, hypertension	Regression coefficient per ln-unit change in PFOS Hypertension: OR per ln-unit increase in PFOS	DBP Total: 2.70 (1.98, 3.42) Females: 2.86 (1.51, 4.20) Males: 0.45 (−0.47, 1.36) p-value for interaction by sex = 0.001 SBP Total: 4.84 (3.55, 6.12) Females: 6.65 (4.32, 8.99) Males: 1.50 (−0.17, 3.18) p-value for interaction by sex < 0.001 Hypertension Total: 1.24 (1.08, 1.44) Females: 1.63 (1.24, 2.13) Males: 1.08 (0.90, 1.29) p-value for interaction by sex = 0.016
	Outcome: Hypertension defined as mean SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, and/or use of antihypertensive medications. **Confounding:** Age, sex, BMI, education, income, exercise, smoking, drinking, family history of hypertension						
Liu et al. (2018a) *Medium*	United States 2004–2007	Controlled trial	Overweight and obese adults ages 30–70 in the POUNDS Lost study N = 621 (384 females, 237 males)	Plasma Females: 22.3 (14.3–34.9) Males: 27.2 (19.9–45.2)	DBP, SBP	Partial Spearman correlation coefficient	DBP: 0.15; p-value < 0.05 SBP: 0.07
	Confounding: Age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), dietary intervention groups						
Lin et al. (2020b) *Medium*	United States 1996–2014	Cohort	Adults from the Diabetes Prevention	Serum Baseline: 26.7 (17.4–40.3)	DBP, SBP, pulse pressure	DBP, SBP: Regression coefficient per log2-	SBP: lifestyle arm, baseline to year 2: −2.13 mmHg/year (−3.54, −0.71)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Program (DPP) and Outcomes Study (DPPOS) N = 957 at baseline, 956 at year 2, and 346 at year 14	Year 2: 27.6 (19.6–38.9) Year 14: 9.8 (5.9–14.8)	(mmHg), and hypertension	unit increase in PFOS, or by quartiles Hypertension: HR or RR per log2-unit increase in PFOS or by quartiles	DBP, pulse pressure, hypertension: No statistically significant associations by timepoint, by quartiles, or by sex
	Outcome: Hypertension defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in those without diabetes, SBP ≥ 130 mmHg, and DBP ≥ 80 mmHg in those with diabetes, self-reported hypertension diagnosis, or use of antihypertensive medication. **Confounding:** Sex, age, race/ethnicity, treatment assignment, education, income, marital status, alcohol intake, smoking, and DASH diet score						
Mitro et al. (2020) *Medium*	United States 1999–2005	Cohort	Pregnant women and their children at age 3 from Project Viva N = 761 mothers (496 ages < 35, 265 ages ≥ 35)	Plasma 24.7 (18.1–33.9)	DBP, SBP, CRP (mg/L)	Regression coefficient per log2-unit increase in PFOS Percent difference (%) per log2-unit increase PFOS	SBP: β = 1.2 (0.3, 2.2); p-value < 0.01 Ages < 35: 0.6% (−0.7, 1.8) Ages ≥ 35: 2.3% (0.9, 3.6); p-value < 0.01 DBP, CRP: No statistically significant associations
	Population: For measurements of C-reactive protein, N = 454 mothers (247 ages < 35, 207 ages ≥ 35). **Confounding:** age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity; breastfeeding in a prior pregnancy for BP measurements only						
Pitter et al. (2020) *Medium*	Italy 2017–2019	Cross-sectional	Adults aged 20–39 yr from Veneto Region with PFAS-contaminated drinking water DBP and SBP: N = 15,380 (7,428 males, 7,952 females) Hypertension risk: N = 15,786 (7,667	Serum 3.7 (2.5–5.6) Male: 4.8 (3.3–6.9) Female: 3 (2–4.4)	DBP, SBP, hypertension risk	DBP, SBP: Regression coefficient per ln-unit increase in PFOS, or by quartiles Hypertension risk: OR per ln-unit increase in PFOS, or by quartiles	DBP 0.44 (0.20, 0.68) Q2: 0.32 (−0.08, 0.72) Q3: 0.30 (−0.12, 0.71) Q4: 0.57 (0.13, 1.02) Males: 0.29 (−0.07, 0.64) Females: 0.51 (0.17, 0.84) SBP 0.57 (0.24, 0.90) Q2: −0.01 (−0.56, 0.53) Q3: 0.27 (−0.29, 0.84) Q4: 0.60 (0.00, 1.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			males, 8,119 females)				Males: 0.98 (0.47, 1.48) Females: 0.32 (−0.13, 0.77) Hypertension risk 1.12 (1.02, 1.22) Q2: 0.99 (0.85, 1.16) Q3: 1.06 (0.91, 1.24) Q4: 1.12 (0.95, 1.32) Males: 1.17 (1.05, 1.31) Females:1.06 (0.91, 1.24)
	Outcome: Hypertension defined as any self-reported diagnosis, use of antihypertensive drugs, or elevated systolic blood pressure (SBP ≥ 140 mmHg)/DBP ≥ 90 mmHg). **Results:** Lowest quartile used as the reference group. **Confounding:** Age, BMI, time lag between the enrolment and the beginning of the study, gender, physical activity, smoking habits, food consumption, salt habit, country of birth, alcohol consumption, education level and center in charge of the BP measurement						
Liu et al. (2018b) *Medium*	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1,871	Serum GM (SE) = 5.28 (1.02)	Hypertension	OR per ln-unit increase in PFOS	Hypertension: 1.08 (0.88, 1.33)
	Outcome: Hypertension defined as average SBP ≥ 130 mmHg and average DBP ≥ 85 mmHg, or self-reported use of prescribed antihypertensive medication. **Confounding:** Age, gender, ethnicity, lifestyle variables (smoking status, alcohol intake and household income), medications (antihypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents), other components of the metabolic syndrome						
Christensen et al. (2019) *Medium*	United States 2007–2014	Cross-sectional	Adults ages 20+ from NHANES N = 2,975	Serum 8.4 (4.8–14.0)	Hypertension	OR by quartiles	Hypertension No statistically significant associations
	Outcome: Hypertension defined as SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, or use of antihypertensive drug in a patient with a history of hypertension. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, alcohol intake, family income, MPAH, PFDE, PFHxS, PFOA, PFUnDA, race/ethnicity, smoking status, survey cycle						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Donat-Vargas et al. (2019b) *Medium*	Sweden 1990–2013	Cohort	Adults aged 30–60 at baseline N = 187	Plasma Baseline: 20 (15–26) Follow-up: 15 (9.7–21)	Hypertension	OR by tertiles or per SD-unit increase in PFOS	Hypertension Baseline OR per increase: 0.71 (0.56, 0.89) No other statistically significant associations Prospective: No statistically significant associations
	Outcome: Hypertension defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, self-reported diagnosis, or use of antihypertensive drugs **Results:** Lowest tertile as the reference group. **Confounding:** Gender, age, education, sample year, BMI, smoking habit, alcohol consumption, physical activity, healthy diet score						
Jeddi et al. (2021a) *Medium*	Italy 2017–2019	Cross-sectional	Residents aged 20–39 from the PFAS-contaminated Veneto region N = 15,876	Serum GM (range): 4.54 (<LOQ–142)	Elevated blood pressure	OR per ln-unit increase in PFOS	Elevated BP: 1.10 (1.03, 1.17), p-value < 0.05
	Outcome: Elevated blood pressure defined as SBP ≥ 130 mmHg or DBP ≥85 mmHg. **Confounding:** Age, gender, time lag between the beginning of the study and blood sampling center where BP has been measured, education, number of deliveries, physical activity, country of birth, diet, alcohol intake, and smoking status, and other components of metabolic syndrome						
Fry and Power (2017) *Medium*	United States 2003–2006	Cohort	Adults ages 60+ from NHANES N = 1,036	Serum 4.3 ng/g (SE = 0.2 ng/g)	Mortality by cerebrovascular or heart diseases	HR per SD-unit increase in PFOS	Mortality 0.85 (0.65, 1.12); p-value = 0.24
	Confounding: Age, education, gender, race/ethnicity, smoking status						
Lind et al. (2017b) *Medium*	Sweden 2001–2004	Cross-sectional	Adults ages 70+ in Uppsala, Sweden N = 1,016 (509 females and 507 males)	Plasma 13.23 (9.95–17.77)	CIMT, carotid artery intima-media complex grey scale median (CIM-GSM), carotid artery atherosclerotic plaque	CIMT, CIM-GSM: Regression coefficient per ln-unit increase in PFOS Plaque: OR per ln-unit increase in PFOS	CIMT, CIM-GSM, atherosclerotic plaque: no statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Sex, HDL and LDL cholesterol and serum triglycerides, BMI, BP, smoking exercise habits, energy and alcohol intake, diabetes, educational level						
Huang et al. (2018) *Medium*	United States 1999–2014	Cross-sectional	Adults from NHANES ages 18+ N = 10,859	Serum 12.40 (6.40–22.60)	CVD, angina pectoris, congestive heart disease, CHD, heart attack, stroke, CRP (mg/L)	OR by quartiles CRP: Spearman correlation coefficient	CVD Q2: 1.04 (0.78, 1.40) Q3: 1.36 (1.07, 1.74) Q4: 1.25 (0.92, 1.69) p-trend = 0.0681 Females: No statistically significant associations or trends Males Q2: 1.76 (1.11, 2.80) Q3: 2.19 (1.37, 3.51) Q4: 1.92 (1.20, 3.07) p-trend = 0.0290; p-trend for sex interaction = 0.0326 Ages < 50: No statistically significant associations or trends Ages ≥ 50 Q2: 1.01 (0.74, 1.38) Q3: 1.39 (1.08, 1.78) Q4: 1.27 (0.92, 1.75) p-trend = 0.0491; p-trend for age interaction = 0.1228 Angina pectoris: No association by quartiles, no significant trend; p-trend = 0.4211 Congestive heart disease: No association by quartiles, no significant trend; p-trend = 0.9462

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							CHD: No association by quartiles, no significant trend; p-trend = 0.0910 Heart attack Q2: 1.30 (0.90, 1.87) Q3: 1.56 (1.01, 2.43) Q4: 1.53 (0.96, 2.45) p-trend = 0.1026 Stroke: No association by quartiles, no significant trend; p-trend = 0.3084 CRP: −0.006; p-value = 0.6062
	Comparison: Age groups were defined as < 50 yr and ≥ 50 yr. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, sex, race/ethnicity, family poverty-income ratio, education levels, physical activity levels, BMI, alcohol-drinking status, smoking status, diabetes, hypertension, family history of CVD, total energy intake, log-transformed levels of serum cotinine, log-transformed levels of serum total cholesterol						
Cardenas et al. (2019) *Medium*	United States 1996–2014	Controlled trial	Prediabetic adults ages 25+ from DPP and DPPOS N = 877	Plasma GM (IQR) = 26.38 (22.8)	MVD, nephropathy, neuropathy, retinopathy	OR per log2-unit increase baseline PFOS	MVD: lifestyle arm: 1.37 (1.04, 1.84) Nephropathy, neuropathy, retinopathy: No statistically significant associations
	Confounding: Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment; baseline fasting glucose and HbA1c levels for microvascular disease only						
Hutcheson et al. (2020) *Medium*	United States 2005–2006	Cross-sectional	Adults from C8 Health Project N = 48,206	Serum With diabetes: 21.4 (13.8–31.9) Without diabetes: 20.1 (13.5–29.0)	Stroke	OR per ln-unit increase PFOS	0.90 (0.82, 0.98); p-value = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Age, BMI, C-reactive proteins, diabetes duration, eGFR, HDL, LDL, history of smoking, race, sex						
Osorio-Yanez et al. (2021) *Medium*	United States 1999	Cohort	Prediabetic adults ages 25+ enrolled in the DPP trial N = 666	Plasma 27.55 (IQR = 19.30)	CAC (Agastston score), AsAC	OR per doubling in PFOS	CAC (11–400): 1.20 (0.94, 1.53) CAC (> 400): 1.49 (1.01, 2.21), p-value < 0.05 AsAC: 1.67 (1.10, 2.54), p-value < 0.05
	Results: CAC < 11 used as reference group. **Confounding:** Sex, age, BMI, race/ethnicity, cigarette smoking, education, treatment assignment, statin use.						
He et al. (2018) *Low*	United States 2003–2012	Cross-sectional	Adults ages 20+ from NHANES N = 3,948 (females) and 3,956 (males)	Serum Female Mean (SE) = 14.51 (0.26) Male Mean (SE) = 20.80 (0.32)	DBP, SBP	Percent difference in log-transformed outcome per interquartile ratio increase PFOS by quartiles	DBP Females: Q2: −1.12 (−2.55, 0.34) Q3: 0.00 (−1.45, 1.59) Q4: 1.47 (−0.11, 3.08) p-trend = 0.022 Males: No statistically significant associations; p-trend = 0.119 SBP: Females: Q2: 0.11 (−0.90, 1.02) Q3: 0.34 (−0.56, 1.36) Q4: 1.13 (0.23, 2.16) Males: No statistically significant associations; p-trend = 0.171
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as the reference group. Interquartile ratio = 75th/25th percentiles of serum PFOS: 3.08 ng/mL. **Confounding:** None listed						
Yang et al. (2018) *Low*	China Years not reported	Cross-sectional	Adult men N = 148	Serum 3.00 (Range: 0.3–14.6)	DBP, SBP, hypertension	Regression coefficient per log-unit increase in n-PFOS	DBP, SBP, hypertension: no statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						Hypertension: OR comparing above or below median	
	Outcome: Hypertension evaluated by individual BP components **Comparison:** Logarithm base not specified. **Confounding:** Age						
Chen et al. (2019a) *Low*	Croatia 2007–2008	Cross-sectional	Adults aged 44–56 N = 122	Plasma GM = 8.91 (Range = 2.36–33.67)	DBP, SBP	Regression coefficient per ln-unit increase PFOS	DBP: 1.42 (−0.95, 3.79) SBP: 1.40 (−3.46, 6.25)
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, physical activity						
Graber et al. (2019) *Low*	United States 2016–2017	Cross-sectional	Members of community with exposed water supply (Paulsboro, NJ) ages 12+ N = 105	Serum 5.66 (3.09–9.28)	Cardiovascular conditions, self-reported	OR per unit increase in PFOS	Any condition 1.08 (0.98, 1.21)
	Confounding: Age, BMI						
			Occupational Populations				
Christensen et al. (2016a) *Low*	United States 2012–2013	Cross-sectional	Male anglers ages 50+ N = 154	Serum 19.00 (9.80–28.00)	Cardiovascular condition (any), CHD, hypertension	OR per unit increase in PFOS	Any condition: 1.00 (0.98, 1.02) CHD: 1.01 (0.98, 1.03) Hypertension: 0.99 (0.96, 1.01)
	Outcome: Hypertension was self-reported **Confounding:** Age, BMI, work status, and alcohol consumption						

Notes: 8-OHdG = 8-hydroxy-2-deoxyguanosine; AI = augmentation index; BAD = brachial artery distensibility; BMI = body mass index; BP = blood pressure; CAC = coronary artery calcium; CHD = coronary heart disease; CI = confidence interval; CIM-GSM = carotid artery intima-media complex grey scale median; CIMT = carotid artery intima-media thickness (mm); CMR = cardiometabolic risk score; CRP = C-reactive protein; CVD = cardiovascular disease; DBP = diastolic blood pressure (mmHg); DPP = Diabetes Prevention Program; DPPOS = Diabetes Prevention Program Outcomes Study; eGFR = estimated glomerular filtration rate; GM = geometric mean; HDL = high-density lipoprotein cholesterol; HELIX = Human Early-Life Exposome; IQR = Interquartile range; HOME = Health Outcomes and Measures of the Environment; HR = hazard ratio; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; LDL = low-density lipoprotein cholesterol; LVEDD = left ventricular end-diastolic diameter (mm); LVMI = left ventricular mass index (g/m2); MPAH = 2-(N-methyl-PFOSA) acetate; MVD = microvascular disease; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PFOA = perfluorooctanoic acid; PFDE = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid;

PFUnDA = perfluoroundecanoic acid; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors; POUNDS = Preventing Overweight Using Novel Dietary Strategies; PWV = pulse wave velocity; RR = risk ratio; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RWT = relative wall thickness; SBP = systolic blood pressure (mmHg); SD = standard deviation; SE = standard error; TFF1 = Tromsø Fit Futures 1; WTCHR = World Trade Center Health Registry; yr = years(s).
[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.5.2 Serum Lipids

Table D-14. Associations Between PFOS Exposure and Serum Lipid Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
				Children			
Li et al. (2021a) *High* for gestation, birth, and childhood exposures (3-yr and 8-yr) *Medium* for exposure at 12-yr follow-up	United States 2003–2006	Cohort	Pregnant women and their children followed up at birth and ages 3, 8, and 12 yr from HOME study Gestation: N = 203 At birth: N = 124 Age 3: N = 137 Age 8: N = 165 Age 12: N = 190	Maternal serum Gestation: 12.9 (8.9–18.0) Cord serum At birth: 4.2 (3.0–6.5) Serum At age 3: 6.2 (4.5–9.9) At age 8: 3.6 (2.8–4.7) At age 12: 2.4 (1.8–3.2)	Levels (mg/dL) of triglycerides and HDL; triglycerides to HDL ratio	Regression coefficient per log2-unit IQR increase in PFOS	Triglycerides Gestation: 0.0 (−0.2, 0.2) At birth: 0.1 (−0.1, 0.3) Age 3: −0.1 (−0.3, 0.1) Age 8: 0.1 (−0.1, 0.3) Age 12: 0.1 (−0.1, 0.3) HDL Gestation: 0.9 (−2.3, 4.1) At birth: 0.9 (−2.6, 4.3) Age 3: 0.4 (−3.5, 4.4) Age 8: 3.8 (−0.2, 7.7) Age 12: 6.0 (1.9, 10) Triglycerides to HDL ratio Gestation: 0.0 (−0.2, 0.2) At birth: 0.1 (−0.1, 0.3) Age 3:−0.1 (−0.3, 0.1) Age 8: 0.1 (−0.1, 0.3) Age 12: 0.1 (−0.1, 0.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	HOME = Health Outcomes and Measures of the Environment **Confounding:** visit, visit × PFAS, maternal age, maternal education, maternal pre-pregnancy BMI, gestational serum cotinine concentrations, and parity; and child age, sex, race, and pubertal stage. Additional confounding for analyses at age 3, age 8, and age 12: Breastfeeding duration.						
Lin et al. (2009) *Medium*	United States 1999–2000 and 2003–2004	Cross-sectional	Adolescents ages 12–20 yr from NHANES N = 474	Serum Mean (SEM) = 3.11 (0.05) log10-ng/mL	Metabolic syndrome HDL cholesterol and metabolic syndrome triglycerides	OR per log10-unit increase in PFOS	Metabolic syndrome HDL cholesterol Model 4: 0.89 (0.51, 1.55) Model 5: 1.38 (0.61, 3.14) Metabolic syndrome triglycerides Model 4: 0.95 (0.50, 1.80) Model 5: 0.78 (0.41, 1.49)
	Outcome: Metabolic syndrome HDL cholesterol defined as HDL ≤ 1.04 mmol/L; metabolic syndrome triglycerides defined as triglycerides ≥ 1.24 mmol/L. **Confounding:** Model 4: Age, sex, race, health behaviors (smoking status, alcohol intake, and household income), measurement data (CRP and HOMA/insulin) and medications; additional confounding for model 5: Other components of the metabolic syndrome.						
Nelson et al. (2010) *Medium*	United States 2003–2004	Cross-sectional	Adolescent girls ages 12–19 yr from NHANES N not reported	Serum Level not reported	Level (mg/dL) of HDL	Regression coefficient by quartiles	HDL Q4: 3.7 (−0.5, 7.9)
	Results: Lowest quartile used as the reference group. Quartile analyses discussed in-text only and quantitative values provided for Q4 only. **Confounding:** Not reported.						
Geiger et al. (2014a) *Medium*	United States 1999–2008	Cross-sectional	Adolescents ages 12–18 yr from NHANES N = 815	Plasma Mean (SE) = 17.7 (0.7)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides; elevated TC; elevated LDL; depressed HDL; elevated triglycerides	Lipid levels: Regression coefficient per ln-unit increase in PFOS, Mean change by tertiles	TC: 0.06 (0.02, 0.1) T2: 3.37 (−1.39, 8.13) T3: 5.85 (0.1, 11.61) p-trend = 0.051 HDL T2: 1.62 (−0.54, 3.78)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
						Elevated or depressed: OR per ln-unit increase in PFOS, or by tertiles	T3: −0.01 (−2.06, 2.04) p-trend = 0.970 LDL: 4.28 (1.6, 6.95) T2: 2.7 (−1.39, 6.78) T3: 6.99 (1.99, 11.98) p-trend = 0.0081 TG: −1.85 (−5.61, 1.91) T2: −4.79 (−11.09, 1.5) T3: −5.55 (−12.26, 1.16) p-trend = 0.110 Elevated TC: 1.35 (1.11, 1.64) T2: 1.35 (0.94, 1.95) T3: 1.53 (1.07, 2.19) p-trend = 0.018 Depressed HDL: 1.03 (0.7, 1.53) T2: 0.88 (0.52, 1.5) T3: 0.99 (0.58, 1.7) p-trend = 0.987 Elevated LDL: 1.48 (1.15, 1.9) T2: 1.43 (0.91, 2.24) T3: 1.76 (1.1, 2.82) p-trend = 0.018 Elevated TG: 0.9 (0.56, 1.43) T2: 0.82 (0.46, 1.45) T3: 0.64 (0.3, 1.37) p-trend = 0.242

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	Outcome: Elevated TC defined as TC > 170 mg/dL; elevated LDL defined as LDL > 110 mg/dL; depressed HDL defined as HDL < 40 mg/dL; elevated triglycerides defined as triglycerides > 150 mg/dL. **Results:** Lowest tertile used as the reference group. Regression coefficient for continuous analysis of HDL not reported. **Confounding:** Age, sex, race-ethnicity, BMI categories, annual household income categories, activity level, and serum cotinine						
Frisbee et al. (2010) *Medium* for TC, GDL-C, fasting TG; *low* for LDL	United States 2005–2006	Cross-sectional	Children and adolescents ages 1.0 to 17.9 yr in the C8 Health Project N = 12,470	Serum Mean (SD) = 22.7 (12.6)	Abnormal TC, abnormal HDL, abnormal LDL, and abnormal fasting triglycerides	OR by quintiles	Abnormal TC Q2: 1.3 (1.1, 1.4) Q3: 1.3 (1.2, 1.5) Q4: 1.3 (1.2, 1.6) Q5: 1.6 (1.4, 1.9) Abnormal HDL Q2: 0.9 (0.8, 1.1) Q3: 0.8 (0.7, 1.0) Q4: 0.8 (0.7, 0.9) Q5: 0.7 (0.6, 0.9) Abnormal LDL Q2: 1.2 (1.0, 1.5) Q3: 1.2 (1.0, 1.5) Q4: 1.3 (1.1, 1.6) Q5: 1.6 (1.3, 1.9) Abnormal fasting triglycerides Q2: 1.3 (0.9, 1.8) Q3: 1.0 (0.7, 1.4) Q4: 1.1 (0.7, 1.6) Q5: 1.2 (0.8, 1.5)
	Outcomes: Abnormal TC defined as TC ≥ 170 mg/dL; abnormal HDL defined as HDL < 40 mg/dL; abnormal LDL calculated for participants with a triglyceride level < 400 mg/dL regardless of fasting status and defined as LDL ≥ 110 mg/dL; fasting triglycerides defined as self-reported fasting > 6 hr before phlebotomy, and abnormal fasting triglycerides defined as fasting triglycerides ≥ 150 mg/dL. **Results:** Lowest quintile used as the reference group. **Confounding:** Age, estimated time of fasting, BMI z-score, sex, regular exercise						
Timmermann et al. (2014) *Medium*	Denmark 1997	Cross-sectional	Children ages 8–10 from Danish	Plasma	Triglycerides (mmol/L)	Percent change per 10-unit increase PFOS	Normal weight: −0.5 (−3.2, 2.4), p-value = 0.75

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
			component of EYHS N = 400 normal weight, N = 59 overweight	41.5 (Range = 6.2–132.5)			Overweight: 8.6 (1.2, 16.5), p-value = 0.02 p-value for PFOS-BMI interaction = 0.02
	Confounding: Sex, age, ethnicity, paternal income, fast-food consumption, and fitness						
Maisonet et al. (2015b) *Medium* for TC and HDL at age 7 and all lipids at age 15 *Low* for Triglycerides and LDL at age 7	United Kingdom 1991–1992	Case-control	Pregnant women and their daughters followed up at ages 7 and 15 from ALSPAC Age 7: N = 111 Age 15: N = 88	Serum 20.5 (Range = 7.6–38.2)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides (ln-mg/dL)	Regression coefficient per unit increase in PFOS in each tertile of exposure	TC Age 7 T1: 0.30 (−3.10, 3.70) T2: 2.09 (−0.64, 4.82) T3: −0.10 (−0.73, 0.54) Age 15 T1: 1.64 (−2.20, 5.48) T2: 3.41 (0.37, 6.45) T3: −0.77 (−1.40, −0.13) LDL Age 7 T1: 0.37 (−2.34, 3.08) T2: 1.02 (−1.15, 3.19) T3: 0.02 (−0.48, 0.53) Age 15 T1: 1.91 (−1.34, 5.17) T2: 2.09 (−0.50, 4.67) T3: −0.54 (−1.08, −0.003) HDL Age 7 T1: 0.76 (−0.79, 2.31) T2: 0.22 (−1.03, 1.46) T3: −0.04 (−0.33, 0.25) Age 15 T1: −0.55 (−2.34, 1.24) T2: 1.15 (−0.27, 2.57)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							T3: −0.18 (−0.47, 0.12) Triglycerides Age 7 T1: −0.031 (−0.085, 0.023) T2: 0.008 (−0.035, 0.052) T3: −0.004 (−0.015, 0.006) Age 15 T1: 0.012 (−0.032, 0.056) T2: 0.016 (−0.019, 0.051) T3: −0.004 (−0.011, 0.004)
	ALSPAC = Avon Longitudinal Study of Parents and Children **Confounding:** Previous live births, maternal education, and maternal age at delivery						
Zeng et al. (2015) *Medium*	Taiwan 2009–2010	Cross-sectional	Children ages 12–15 N = 225	Serum Median = 28.8 among males, 29.9 among females	Levels (ng/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per ln-unit increase PFOS	TC: 0.31 (0.18, 0.45) p-value < 0.001 LDL: 0.28 (0.18, 0.38) p-value < 0.001 HDL: −0.01 (−0.07, 0.05) p-value = 0.72 Triglycerides: 0.19 (0, 0.38) p-value = 0.05
	Confidence: Results for TG and LDL considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Age, gender, BMI, parental education level, exercise, environmental tobacco smoke exposure[c]						
Domazet et al. (2016) *Medium*	Denmark 1997–2009	Cohort	Members of the EYHS evaluated at ages 9 and 15 (N = 260), 9 and	Plasma Median at 9 = 44.5	Levels (mmol/L) of TG	Percent change in TG at age 15 or 21 per 10 unit	Age 9 to 15: −0.7 (−5.03, 3.77) Age 9 to 21: −1.98 (−8.17, 4.75)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
			21 (N = 175), or 15 and 21 (N = 171)	(male) or 39.9 (female) Median at 15 = 22.3 (male) or 20.8 (female) Median at 21 = 11.9 (male) or 9.1 (female)		increase in PFOS at age 9 or 15	Age 15 to 21: 0.77 (−8.28, 10.71)
	Confounding: Sex, age, and TG levels at baseline age; ethnicity, maternal parity, and maternal income in 1997 (9 yr of age). Waist circumference was adjusted for height in order to account for body size.						
Manzano-Salgado et al. (2017b) *Medium*	Spain 2003–2008	Cohort	Pregnant women and their children (age 4) from INMA study N = 627	Maternal plasma during 1st trimester GM = 5.80	Levels (z-score) of TC, LDL, HDL, and TG	Regression coefficient per log2-unit increase PFOS	TC: 0.02 (−0.10, 0.15) LDL: 0.02 (−0.10, 0.15) HDL: −0.03 (−0.14, 0.09) TG: 0.05 (−0.06, 0.17)
	Confidence: Results for TG and LDL considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child						
Jain et al. (2018) *Medium*	United States 2013–2014	Cross-sectional	Children ages 6–11 N = 458	Serum GM = 2.67 for linear PFOS, 1.35 for 1m-PFOS	Levels (log10-mg/dL) of TC, HDL, and non-HDL	Regression coefficient per log10-unit increase PFOS	Linear PFOS TC: 0.02738 p-value = 0.03 Non-HDL: −0.00357 p-value = 0.4 HDL: 0.04631 p-value = 0.1 1m-PFOS TC: 0.01241 p-value = 0.22 Non-HDL: −0.00661 p-value = 0.04 HDL: 0.04612 p-value = 0.05
	Confounding: Gender, race/ethnicity, age, poverty-income ratio, BMI percentiles, fasting time, and exposure to secondhand smoke						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Kang et al. (2018) *Medium*	Korea 2012–2014	Cross-sectional	Children aged 3–18 from Korea Environmental Health Survey in Children and Adolescents (KorEHS-C) N = 147	Serum Median = 5.68	Levels of TC (mg/dL), LDL (mg/dL), and TG (ln-mg/dL)	Regression coefficient per ln-unit increase PFOS	TC: −0.45 (−10.67, 9.77) LDL: 2.51 (−6.88, 11.89) TG: −0.020 (−0.19, 0.15) All p-value > 0.5
	Results: LDL and TG evaluated at ages 7–18 only (N = 117) Confounding: Age, sex, BMI z-score, household income, secondhand smoking						
Mora et al. (2018) *Medium*	United States 1999–2010	Cohort and cross-sectional	Pregnant women and their children from Project Viva N = 512 prenatal, 596 mid-childhood	Prenatal maternal plasma Median = 24.6 Mid-childhood plasma Median = 6.2	Levels (mg/dL) of TC, HDL, LDL, and TG	Regression coefficient per IQR increase in PFOS	Prenatal: TG: −1.4 (−4.6, 1.8) Boys:1.0 (−2.2, 4.2) Girls: −4.2 (−9.2, 0.8) p-value for interaction by sex = 0.04 Mid-childhood: TC: 1.8 (−0.2, 3.7) HDL: 1.5 (0.4, 2.5) TG: −2.5 (−4.3, −0.6) Boys: 0.5 (−1.8, 2.9) Girls: 4.0 (0.3, 7.8) No other statistically significant associations
	Confounding: maternal education, prenatal smoking, gestational age at blood draw (for prenatal data), and child's sex, race/ethnicity, and age at lipids/ALT measurements						
Jensen et al. (2020a) *Medium*	Denmark 2010–2012	Cohort	Pregnant women and their children assessed at 3 mo and 18 mo N = 260 at 3 mo, 83 at 18 mo	Maternal serum Median = 8.04	Levels (standard deviation score) of TC, LDL, HDL, and TG	Regression coefficient per unit increase in PFOS	All associations were between −0.07 and 0.05, all with p-values > 0.05
	Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI2, education, smoking, sex, and lipid outcome at 3 mo						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Spratlen et al. (2020b) *Medium*	United States 2001–2002	Cross-sectional	Pregnant women and their children from the Columbia University World Trade Center birth cohort N = 222	Cord blood Median = 6.32	Levels (mg/dL) of TC, total lipids, and TG in cord blood	Percent change per 1% increase in PFOS	TC: 0.062 (−0.004, 0.13) Total lipids: 0.067 (0.005, 0.129) p-value < 0.05 TG: 0.086 (−0.036, 0.21)
	Confounding: Maternal age, child sex, maternal education, maternal race, parity, pre-pregnancy BMI, marital status, family smoking, and gestational age						
Averina et al. (2021) *Medium*	Norway 2010–2011	Cross-sectional	First level high school students ages 15–19 yr from TFF1 N = 940	Serum Girls: GM (IQR) = 5.71 (2.64) Boys: GM (IQR) = 6.52 (3.09)	Levels (mmol/L) of TC, HDL, LDL, and TG	Regression coefficient per log10-unit increase in PFOS	TC: 0.38 (0.10, 0.66), p-value = 0.008 HDL: 0.08 (−0.03, 0.20), p-value = 0.152 LDL: 0.30 (0.05, 0.55), p-value = 0.021 TG: 0.006 (−0.18, 0.20), p-value = 0.947
	TFF1 = Tromsø Fit Futures 1 **Confounding:** Sex, age, BMI, and lifestyle and diet variables						
Blomberg et al. (2021) *Medium* for HDL and TC *Low* for LDL and TG	Faroe Islands Recruitment: 2007–2009	Cohort and cross-sectional	Children from the Faroese Birth Cohort 5 at birth, 18 mo, and 9 yr Birth: N = 459 (219 female, 240 male) 18 mo: N = 334 9 yr: N = 366	Serum Birth: 2.87 (2.13–4.04) Female: 2.82 (2.04–3.86) Male: 2.93 (2.19–4.10) 18 mo: 6.81 (4.38–9.82)	Levels (mmol/L) of TC, HDL	Regression coefficient per log2-unit increase in PFOS	TC, age 9 (PFOS age 9) 0.15 (0.025, 0.27), p-value < 0.05 Females: 0.25 (0.077, 0.43), p-value < 0.05 Males: 0.05 (−0.12, 0.22) p-value for interaction by sex = 0.104

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
				9 yr: 3.08 (2.42–4.31) Levels at 5 yr and by sex at 18 mo and 9 yr not reported			HDL, age 9 (PFOS age 9) 0.077 (0.03, 0.12), p-value < 0.05 Females: 0.07 (0.0017, 0.14), p-value < 0.05 Males: 0.083 (0.018, 0.15), p-value < 0.05 p-value for interaction by sex = 0.788
	Confounding: Child sex and maternal education; analyses except PFAS at 9 yr additionally adjusted for maternal smoking during pregnancy, maternal pre-pregnancy BMI, and parity						
Canova et al. (2021) *Medium* for TC, HDL *Low* for LDL, TG	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 yr and children aged 8 to 11 yr from health surveillance program in Veneto Region Adolescents: N = 6,669 Children: N = 2,693	Serum Adolescents: 3.3 (2.2–4.9) Children: 2.2 (1.6–3.0)	Levels (ng/mL) of TC, HDL, LDL, triglycerides	Regression coefficient per ln-unit increase in PFOS	TC Adolescents: 3.32 (2.20, 4.45) Children: 6.22 (4.32, 8.13) HDL Adolescents: 1.17 (0.71, 1.63) Children: 1.91 (1.10, 2.73) LDL Adolescents: 2.66 (1.70, 3.62) Children: 4.52 (2.80, 6.23) Triglycerides Adolescents: −0.02 (−0.04, 0.00) Children: −0.01 (−0.04, 0.02)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	Confounding: Age, gender, country of birth, data on food consumption, degree of physical activity, salt intake, smoking status (for adolescents only), time lag between the beginning of the study and the date of enrollment.						
Papadopoulou et al. (2021) *Medium*	United Kingdom, France, Spain, Lithuania, Norway, Greece Recruitment 1999–2010, Follow-up: 2013–2015	Cohort	Mother-child pairs from the HELIX Project, children followed up around age 8 (range 6–12) N = 1,101	Maternal plasma (prenatal) 6.15 (3.99–9.16) Plasma (childhood) 1.93 (1.22–3.11)	Levels (z-scores) of HDL, LDL, and triglycerides	Regression coefficient per doubling in PFOS, or by quartiles	HDL Maternal PFOS: 0.06 (−0.06, 0.18) Q2: −0.13 (−0.33, 0.07) Q3: −0.06 (−0.29, 0.17) Q4: −0.18 (−0.47, 0.12) p-trend = 0.577 Childhood PFOS: 0.00 (−0.08, 0.08) Q2: 0.23 (0.04, 0.41) Q3: 0.33 (0.11, 0.54) Q4: 0.37 (0.11, 0.63) p-trend = 0.009 LDL Maternal PFOS: −0.03 (−0.15, 0.09) Q2: −0.05 (−0.26, 0.15) Q3: −0.11 (−0.35, 0.12) Q4: 0.09 (−0.21, 0.39) p-trend = 0.990 Childhood PFOS: 0.05 (−0.03, 0.13) Q2: 0.06 (−0.13, 0.25) Q3: 0.15 (−0.06, 0.37) Q4: 0.12 (−0.14, 0.38) p-trend = 0.210 Triglycerides Maternal PFOS: −0.07 (−0.19, 0.05) Q2: −0.07 (−0.27, 0.14) Q3: −0.19 (−0.43, 0.04) Q4: −0.14 (−0.44, 0.16)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							p-trend = 0.191 Childhood PFOS: 0.04 (−0.04, 0.12) Q2: 0.02 (−0.17, 0.21) Q3: 0.03 (−0.19, 0.24) Q4: 0.13 (−0.14, 0.39) p-trend = 0.256
	Comparison: Maternal PFOS quartiles are defined as follows: Q1: 0.28–3.98; Q2: 3.99–6.15; Q3: 6.15–9.15; Q4: 9.16–47.98; childhood PFOS quartiles are defined as follows: Q1: 0.00–1.22; Q2: 1.22–1.92; Q3: 1.93–3.10; Q4: 3.11–33.83. **Results:** Lowest quartile used as the reference group. **Confounding:** Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, child gender, PFHxS, PFNA, PFOA						
Tian et al. (2020) *Medium*	China 2012	Cohort	Pregnant women and their newborn children from the S-MBCS N = 306	Maternal plasma 10.5 (7.37–16.3)	Levels (ln-mg/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per ln-unit increase in PFOS, or by tertile	TC Per ln-unit: −0.10 (−0.18, −0.02), p-value = 0.018 T2: −0.09 (−0.20, 0.03) T3: −0.15 (−0.27, −0.03), p-value < 0.05 p-trend < 0.05 LDL Per ln-unit: −0.07 (−0.18, 0.03), p-value = 0.164 T2: −0.12 (−0.27, 0.03) T3: −0.09 (−0.24, 0.06) HDL Per ln-unit: −0.11 (−0.21, −0.02), p-value = 0.021 T2: −0.11 (−0.25, 0.03) T3: −0.17 (−0.31, −0.031), p-value < 0.05 p-trend < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Triglycerides Per ln-unit: −0.05 (−0.14, 0.04), p-value = 0.287 T2: −0.08 (−0.21, 0.06) T3: −0.02 (−0.16, 0.11)
	Results: Lowest tertile used as reference group. **Confounding:** Maternal age, pre-pregnancy BMI, household income, infant sex, gestational age.						
			Pregnant Women				
Starling et al. (2014b) *Medium* for TC, HDL, and LDL *Low* for Triglycerides	Norway 2003–2004	Cross-sectional	Women in mid pregnancy (median = 18 wk of gestation) from MoBa N = 891	Plasma 13.03 (10.31–16.60)	Levels (mg/dL) of TC, HDL, LDL, and triglycerides (ln-mg/dL)	Regression coefficient per ln-unit or IQR increase in PFOS, or by quartiles	TC Per ln-unit: 8.96 (1.70, 16.22) Per IQR: 4.25 (0.81, 7.69) Q2: −3.35 (−10.34, 3.64) Q3: 3.06 (−4.93, 11.05) Q4: 7.59 (−0.42, 15.60) HDL Per ln-unit: 4.39 (2.37, 6.42) Per IQR: 2.08 (1.12, 3.04) Q2: 1.96 (−0.39, 4.31) Q3: 2.49 (0.00, 4.97) Q4: 4.45 (2.04, 6.86) LDL Per ln-unit: 6.48 (−0.07, 13.03) Per IQR: 3.07 (−0.03, 6.18) Q2: −3.23 (−9.28, 2.83) Q3: 2.60 (−4.49, 9.70)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Q4: 5.51 (−1.62, 12.64) Triglycerides Per ln-unit: −0.02 (−0.09, 0.04) Per IQR: −0.01 (−0.04, 0.02) Q2: 0.00 (−0.06, 0.07) Q3: −0.03 (−0.10, 0.05) Q4: 0.00 (−0.09, 0.04)
	Results: Lowest quartile used as reference group. **Confounding:** Age, pre-pregnant BMI, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, and oily fish consumed daily.						
Skuladottir et al. (2015) *Medium*	Denmark 1988–1989	Cross-sectional	Pregnant women N = 854	Serum Mean = 22.3	Levels (mmol/L) of TC	Regression coefficient by quintile	Q2: 0.24 (−0.04, 0.53) Q3: 0.22 (−0.07, 0.50) Q4: 0.35 (0.06, 0.64) Q5: 0.44 (0.15, 0.74) p-trend = 0.004
	Results: Lowest quintile used as reference group. **Confounding:** Age, parity, education, smoking and pre-pregnancy BMI, total caloric intake, and intake of vegetables, meat, and meat products						
Matilla-Santander et al. (2017) *Medium*	Spain 2003–2008	Cohort	Pregnant women from the Spanish INMA birth cohort N = 1240	Plasma Median = 6.05	Levels of TC (mg/dL), TG (log10-mg/dL), and C-reactive protein (log10-mg/dL)	Percent change in median lipid level per log10-unit increase in PFOS	TC: 0.88 (−0.53, 2.37) TG: −5.86 (−9.91, −1.63)
	Confidence: TG results considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Sub-cohort, country of birth, pre-pregnancy BMI, previous breastfeeding, parity, gestational week at blood extraction, physical activity, and relative Mediterranean Diet Score						
Starling et al. (2017) *Medium*	United States 2009–2014	Cohort	Pregnant women ages 16–45 from the Healthy Start study N = 598	Serum Median = 2.4	Levels of HDL (mg/dL) and TG (ln-mg-dL)	Regression coefficient per ln-unit increase PFOS	HDL: 0.79 (−0.68, 2.27) TG: 0.004 (−0.033, 0.041)
	Confounding: Maternal age, race/ethnicity, pre-pregnancy BMI, education, gravidity, smoking, and gestational age at						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	blood draw						
Yang et al. (2020b) *Medium*	China 2013–2014	Cohort	Pregnant women ages 20–40 yr in early pregnancy N = 436	Serum 6.78 (5.08–9.60)	Levels (ln-mmol/L) of TC, triglycerides, HDL, and LDL; LDL/HDL ratio	Regression coefficient per ln-unit increase in PFOS, or by quartiles	TC Per ln-unit: −0.090 (−0.274, 0.093) Q2: 0.26 (−0.33, 0.85) Q3: −0.04 (−0.44, 0.36) Q4: −0.10 (−0.52, 0.32) p-trend = 0.832 Triglycerides Per ln-unit: −0.084 (−0.307, 0.138) Q2: −0.03 (−0.48, 0.42) Q3: 0.07 (−0.38, 0.52) Q4: 0.09 (−0.35, 0.53) p-trend = 0.478 HDL Per ln-unit: 0.025 (−0.030, 0.081) Q2: 0.06 (−0.05, 0.17) Q3: 0.00 (−0.05, 0.17) Q4: 0.04 (−0.06, 0.14) p-trend = 0.600 LDL Per ln-unit: −0.116 (−0.262, 0.027) Q2: 0.02 (−0.22, 0.26) Q3: −0.05 (−0.28, 0.18) Q4: −0.11 (−0.36, 0.14) p-trend = 0.532 LDL/HDL ratio Per ln-unit: −0.039 (−0.084, 0.007)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Q2: −0.02 (−0.08, 0.04) Q3: 0.00 (−0.07, 0.07) Q4: −0.08 (−0.18, 0.02) p-trend = 0.240
	Results: Lowest quartile as reference group. **Confounding:** Age, BMI at baseline, husband smoking, GDM, parity (nulliparous, multiparous), education, career, income, energy intake and physical activity in the late term of pregnancy, gestational weeks, carbohydrate, protein, SFA, MUFA, and PUFA intake in the late term of pregnancy.						
Dalla Zuanna et al. (2021) *Medium* for TC HDL *Low* for LDL	Italy 2017–2020	Cross-sectional	Pregnant women ages 18–44 from an area exposed to PFAS through drinking water N = 319 I Trimester: N = 101 II Trimester: N = 88 III Trimester: N = 130	Serum 2.7 (1.9–3.8) I Trimester: 2.9 (2.2–3.9) II Trimester: 2.5 (1.8–3.5) III Trimester: 2.9 (1.8–4.2)	Levels (mg/dL) of TC, HDL, and LDL	Regression coefficient per ln-unit increase in PFOS, or by quartiles	TC Per ln-unit: 3.01 (−4.51, 10.53) Q2: 4.42 (−8.21, 17.05) Q3: −1.65 (−13.80, 10.50) Q4: 9.89 (−2.82, 22.59) HDL Per ln-unit: 4.84 (2.15, 7.54), p-value < 0.05 Q2: 8.60 (4.07, 13.14), p-value < 0.05 Q3: 4.81 (0.49, 9.14), p-value < 0.05 Q4: 9.20 (4.65, 13.76), p-value < 0.05 LDL Per ln-unit: −2.50 (−8.99, 3.98) Q2: −2.76 (−13.73, 8.21) Q3: −5.10 (−15.63, 5.43) Q4: 0.01 (−11.04, 11.06) First Trimester

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							TC: 15.34 (−1.08, 31.78) HDL: 8.31 (1.07, 15.55), p-value < 0.05 LDL: 6.65 (−5.90, 19.20) Second Trimester TC: −2.86 (−17.86, 12.13) HDL: 3.76 (−3.35, 10.87) LDL: −3.51 (−14.72, 7.69) Third Trimester TC: −4.51 (−18.13, 9.09) HDL: 4.25 (0.26, 8.24), p-value < 0.05 LDL: −10.05 (−22.71, 2.61)
	Results: Lowest quartile as the reference group. **Confounding:** Age, number of previous deliveries, BMI, physical activity, smoking habits, country of birth, education level, laboratory in charge of the analyses of serum lipids, gestation weeks and reported fish consumption (in tertiles)						
General Population							
Lin et al. (2009) *Medium*	United States 1999–2000 and 2003–2004	Cross-sectional	Adults ages 20+ years from NHANES N = 969	Serum Mean (SEM) = 3.19 (0.04) log10-ng/mL	Metabolic syndrome HDL cholesterol and metabolic syndrome triglycerides	OR per log10-unit increase in PFOS	Metabolic syndrome HDL cholesterol Model 4: 1.47 (1.07, 2.00), p-value < 0.05 Model 5: 1.61 (1.15, 2.26), p-value < 0.05 Metabolic syndrome triglycerides Model 4: 0.97 (0.73, 1.27)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Model 5: 0.86 (0.65, 1.16)
	Outcome: Metabolic syndrome HDL cholesterol defined as HDL < 1.03 mmol/L in men and HDL < 1.29 mmol/L in women; metabolic syndrome triglycerides defined as triglycerides ≥ 1.69 mmol/L. **Confounding:** Model 4: Age, sex, race, health behaviors (smoking status, alcohol intake, and household income), measurement data (CRP and HOMA/insulin) and medications; additional confounding for model 5: Other components of the metabolic syndrome.						
Nelson et al. (2010) *Medium*	United States 2003–2004	Cross-sectional	Adults ages 20–80 yr from NHANES N = 860	Serum 21.0 (Range = 1.4–392.0)	Levels (mg/dL) of TC, HDL, non-HDL, LDL	Regression coefficient per unit increase in PFOS, or by quartiles	TC Per unit increase: 0.27 (0.05, 0.48) Q4: 13.4 (3.8, 23.0) p-trend by quartiles = 0.01 HDL Per unit increase: 0.02 (−0.05, 0.09) Non-HDL Per unit increase: 0.25 (0.00, 0.50) LDL Per unit increase: 0.12 (−0.17, 0.41)
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, race/ethnicity, SES, saturated fat intake, exercise, time in front of a TV or computer, BMI, alcohol consumption, and smoking.						
Liu et al. (2018b) *Medium*	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1871	Serum GM = 5.28	Levels of TC (mg/dL), LDL (mg/dL), HDL (mg/dL), TG (ln-mg/dL)	Regression coefficient (SE) per ln-unit increase in PFOS	TC: 1.22 (1.91) LDL: 0.88 (1.75) HDL: 0.91 (0.70) TG: −0.08 (0.05)
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Dong et al. (2019)	United States 2003–2014	Cross-sectional	Adults age 20–80 from NHANES	Serum Mean = 15.6	Levels (mg/dL) of TC, LDL, HDL	Regression coefficient per	TC all cycles: 0.4 (0.06, 0.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Medium			N = 8,814			unit increase PFOS	p-value < 0.05 Inconsistent associations with LDL or HDL across NHANES cycles.
	Confounding: Age, gender, race, family income index, BMI, waist circumference, physical activities, diabetes status, smoking status, number of alcoholic drinks per day						
Jain et al. (2019d) *Medium*	United States 2004–2015	Cross-sectional	Members of NHANES Non-obese N = 1053 females (NF) and 1,237 males (NM) Obese N = 699 females (OF) and 640 males (OM)	Serum GMs: Female = 7.4 Male = 11.5	Levels (mg/dL) of TC, LDL, HDL, TG	Regression coefficient per log10-unit increase PFOS	TC: No clear associations LDL OF: 0.0375 (0.0024, 0.0727) p-value = 0.04 No clear associations in NF. NM, or OM HDL: No clear associations TG OF: −0.0912 (−0.153, −0.0294) p-value < 0.01 No clear associations in NF, NM, or OM
	Confounding: Race/ethnicity, smoking status, age, poverty-income ratio (PIR), fasting time, use of lipid-lowering medicine, physical exercise, survey year, daily dietary intake of total cholesterol, daily intake of total saturated fat, calories, caffeine, alcohol, protein intake						
Fan et al. (2020) *Medium*	United States 2011–2014	Cross-sectional	Adults age 20+ from NHANES N = 1,067	Serum Median = 5.14 ng/mL	Levels (mg/dL) of TC, LDL, HDL, and TG	Regression coefficient per log10-unit increase in PFOS	TC: 3.85 (1.27, 6.42) p-value = 0.003 LDL: 3.02 (0.75, 5.29) p-value = 0.009 HDL: 1.24 (0.32, 2.16) p-value = 0.009 TG: −0.01 (−0.04, 0.02) p-value = 0.505
	Confounding: Age, gender, race, education level, PIR, BMI, smoking status, alcohol use, energy intake levels, screen time						
Jain and Ducatman (2020)	United States 2007–2014	Cross-sectional	Adults age 20+ from NHANES	Serum	Apolipoprotein B (log10-mg/dL)	Regression coefficient per	Apolipoprotein B

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Medium			Non-diabetic non-LLM users: N = 2,872 Diabetic non-LLM users: N = 316 Non-diabetic LLM users: N = 519 Diabetic LLM users: N = 293	Levels not reported		log10-unit increase in PFOS	Non-diabetic non-LLM users: 0.02027, p-value = 0.02 Diabetic non-LLM users: 0.01547, p-value = 0.41 Non-diabetic LLM users: −0.01327, p-value = 0.40 Diabetic LLM users: 0.02001, p-value = 0.19
	Confounding: Gender, age, age squared, race/ethnicity, PIR, fasting time in hours, log10-transformed BMI, smoking status, survey year, daily intake of cholesterol, caffeine, alcohol, total calories, total protein, and total fat						
Steenland et al. (2009) *Medium* for TC, HDL *Low* for TG, LDL	United States 2005–2006	Cross-sectional	Adults ages 18+ from the C8 Health Project, current or former residents from areas supplied with contaminated water N = 46,494	Serum 19.6 (Range: 0.25–759.2)	Levels (ln-mg/dL) of TC, LDL, HDL, non-HDL cholesterol, and triglycerides; TC/HDL ratio; high TC	Lipid levels, ratios: Regression coefficient per ln-unit increase in PFOS High TC: OR by PFOS quartiles	TC 0.0266 (SD = 0.0014) HDL 0.00355 (SD = 0.00173) LDL 0.04172 (SD = 0.00221) Triglycerides 0.01998 (SD = 0.00402) TC/HDL ratio 0.02290 (SD = 0.00202) Non-HDL 0.03476 (SD = 0.0019) High TC Q2: 1.14 (1.05, 1.23)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Q3: 1.28 (1.19, 1.39) Q4: 1.51 (1.40, 1.64) p-trend < 0.0001
	Outcome: High TC defined as ≥ 240 mg/dL. **Results:** Lowest quartile used as the reference group; lowest decile used as the reference group. **Confounding:** Age, male gender, smoking status, education level, drinks alcohol, currently exercises, and BMI						
Château-Degat et al. (2010) *Medium*	Canada 2004	Cross-sectional	Nunavik Inuit adults Quartile analyses: N = 716 (395 women, 325 men) TC, TC/HDL ratio: N = 663 LDL: N = 651 Non-HDL: N = 670 HDL: N = 384 women, 309 men Triacylglycerols: N = 365 women, 284 men	Plasma GM (95% confidence interval): 18.6 (17.8–19.5)	Levels (mmol/L) of TC, LDL, HDL, non-HDL cholesterol, and triacylglycerols; TC/HDL ratio	Regression coefficient per unit increase in PFOS or adjusted mean by quartiles	TC 0.0009, p-value = 0.086 Q1: 4.781 (4.704, 4.864) Q2: 4.869 (4.804, 4.940) Q3: 4.969 (4.901, 5.041) Q4:5.301 (5.221, 5.381) p-trend ≤ 0.0001 LDL −0.002, p-value = 0.242 Q1: 2.750 (2.680, 2.819) Q2: 2.780 (2.730, 2.830) Q3: 2.831 (2.770, 2.891) Q4: 2.871 (2.801, 2.942) p-trend = 0.58 HDL Women: 0.0042, p-value = 0.001 Men: 0.0016, p-value < 0.001 Q1: 1.539 (1.510, 1.572) Q2: 1.619 (1.580, 1.660) Q3: 1.630 (1.580, 1.660) Q4: 1.831 (1.788, 1.868) p-trend ≤ 0.0001 Non-HDL −0.0011, p-value = 0.315

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Q1: 3.241 (3.160, 3.321) Q2: 3.241 (3.182, 3.301) Q3: 3.341 (3.271, 3.412) Q4: 3.469 (3.388, 3.549) p-trend = 0.09 Triacylglycerols Women: −0.0014, p-value = 0.04 Men: −0.0009, p-value = 0.162 Q1: 1.051 (1.009, 1.092) Q2: 1.067 (1.038, 1.096) Q3: 0.941 (0.910, 0.970) Q4: 1.000 (0.968, 1.030) p-trend = 0.42 TC/HDL ratio −0.0035, p-value < 0.001 Q1: 3.250 (3.181, 3.320) Q2: 3.210 (3.140, 3.281) Q3: 3.240 (3.170, 3.311) Q4: 3.130 (3.049, 3.211) p-trend = 0.75
	Results: Adjusted means presented with lower and upper bounds of standard error in parentheses. **Confounding:** Means adjusted for age, gender, BMI, and smoking status. All regression analyses adjusted for lipid-lowering drugs. Additional regression analyses adjustments: TC: gender, smoking status, age and n-3 PUFAs; LDL: age, BMI, smoking status, and insulinaemia; HDL: PFOS and n-3 PUFAs; non-HDL cholesterol: smoking status, age and gender; triacylglycerols: PFOS, smoking status, BMI, stratified by gender; TC/HDL ratio: smoking status and gender						
Eriksen et al. (2013) *Medium*	Denmark 1993–1997	Cross-sectional	Adults ages 50–65 from DCH N = 753	Plasma Mean = 36.1	Levels of TC (mg/dL)	Regression coefficient per IQR increase in PFOS	4.6 (0.8, 8.5) p-value = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	Confounding: Sex, education, age, BMI, smoking status, intake of alcohol, egg, and animal fat and physical activity						
Fisher et al. (2013) *Medium*	Canada 2007–2009	Cross-sectional	Adults ages 18–74 yr from CHMS, cycle 1 N = 2,700 TC, HDL, Non-HDL, TC/HDL ratio: N = 2,345 LDL, triglycerides: N = 1,168 High cholesterol: N = 1,042	Plasma GM (SD) = 8.40 (2.04)	Levels (ln-mmol/L) of TC, HDL, LDL, non-HDL, triglycerides; TC/HDL ratio (ln-transformed); high cholesterol	Lipid levels, TC/HDL ratio: Regression coefficient per ln-unit increase in PFOS High cholesterol: OR per ln-unit increase in PFOS, or by quartiles	TC 0.014 (−0.019, 0.05) HDL −0.02 (−0.07, 0.02) LDL 0.02 (−0.03, 0.08) Non-HDL 0.03 (−0.11, 0.07) Triglycerides −0.02 (−0.12, 0.07) TC/HDL ratio 0.04 (−0.008, 0.08) High cholesterol per ln-unit increase: 1.15 (0.89, 1.59) Q2: 0.97 (0.58, 1.62) Q3: 0.94 (0.58, 1.54) Q4: 1.36 (0.87, 2.12) p-trend = 0.13
	Outcome: High cholesterol defined as TC > 5.2 mmol/L. **Results:** Lowest quartile used as the reference group. **Confounding:** Lipid levels, TC/HDL ratio: Age, sex, marital status, BMI alcohol, smoking status and physical activity index; High cholesterol: Age, gender and alcohol consumption						
Fitz-Simon et al. (2013) *Medium* for TC, HDL	United States Baseline: 2005–2006; Follow-up: 2010	Cohort	Adults ages 20–60 from C8 Short-Term Follow-up Study living in West	Serum Baseline GM (SD) = 18.5 (13.5)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Percentage decrease (log10 of final and initial ratio change per	TC: 3.20 (1.63, 4.76) R^2 = 0.04 LDL: 4.99 (2.46, 7.44) R^2 = 0.07 HDL: 1.28 (−0.59, 3.12)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Low for TG, LDL			Virginia and Ohio with PFOA-contaminated drinking water N = 560 (N = 521 for LDL analysis)	Follow-up GM (SD) = 8.2 (7.1)		log10 of ratio change in PFOS)	R^2 = 0.04 Triglycerides: 2.49 (−2.88, 7.57) R^2 = 0.08
	Confounding: Age, sex, interval between measurements, and fasting status						
Donat-Vargas et al. (2019b) *Medium*	Sweden 1990–2013	Cohort	Non-diabetic adults ages 30–60 at baseline in Västerbotten Intervention Programme (VIP) N = 187	Plasma Baseline median = 20 Median at 10-yr follow-up = 15	Levels (mmol/L) of TC and TG	Regression coefficient per 1-SD change PFOS or by tertiles	Per change in PFOS TC Baseline: −0.21 (−0.39, −0.04) Follow-up: 0.01 (−0.19, 0.21) Prospective: 0.05 (−0.15, 0.21) TG Baseline: −0.05 (−0.16, 0.06) Follow-up: −0.15 (−0.28, −0.03) Prospective: −0.14 (−0.27, −0.02)
	Confounding: Gender, age, education, sample year, BMI, smoking habit, alcohol consumption, physical activity and healthy diet score						
Lin et al. (2019) *Medium*	United States 1996–2014	Cohort and cross-sectional	Prediabetic adults age 25+ from the DPP and Outcomes Study (DPPOS) N = 940 (888 not on metformin)	Plasma Median = 27.2	Levels (mg/dL) of TC, LDL, HDL, triglycerides, non-HDL, and very low-density lipids (VLDL); hypercholesterolemia, hypertriglyceridemia	Regression coefficient per doubling PFOS HR or OR for hypercholesterolemia or hypertriglyceridemia per doubling of PFOS	<u>Cross-sectional</u> TC: 2.53 (−0.10, 5.16) LDL: 1.38 (−1.02, 3.77) HDL: −0.40 (−1.19, 0.39) Triglycerides: 7.75 (0.63, 14.88) VLDL: 1.57 (0.24, 2.89) Hypercholesterolemia at baseline OR: 1.02 (0.85, 1.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Hypertriglyceridemia at baseline OR: 1.23 (1.03, 1.46) <u>Prospective</u> Hypercholesterolemia HR: 1.01 (0.91, 1.12) Hypertriglyceridemia HR: 1.09 (0.93, 1.27) Greater effect in the placebo group
	Confounding: Age, sex, race and ethnicity, marital status, educational attainment, drinking, smoking, percent of daily calorie from fat intake, daily fiber intake, physical activity level, and waist circumference at baseline						
Canova et al. (2020) *Medium*	Italy 2017–2019	Cross-sectional	Residents of PFAS "Red Area" with contaminated public water supply ages 20–39 N = 15720 (7,620 female, 8100 male)	Serum Median = 3.7 Female = 3 Male = 4.8	Levels (mg/dL) of TC, LDL, HDL, non-HDL, and triglycerides	Regression coefficient per ln-unit increase PFOS or by quartile, or by decile	TC 4.99 (4.12, 5.86) p-value for interaction by sex = 0.39 Consistently increased associations by deciles, from 4.33 to 11.77 LDL 3.97 (3.21, 4.73) Males: 5.07 (3.87, 6.27) Females: 2.43 (1.47, 3.39) p-value for interaction by sex = 0.003 Associations for deciles 2–10 consistently increase from 2.94 to 9.67 HDL 1.43 (1.1, 1.76) Males: 0.91 (0.47, 1.36)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Females: 1.95 (1.46, 2.45) p-value for associations = 0.001 Associations for deciles 2–10 moderately increase from 1.13 to 3.43 Triglycerides 0 (−0.01, 0.01) p-value for associations = 0.954 Associations for deciles 2–10 inconsistently vary from 0 to 0.02
	Results: Lowest quartile or decile used as reference group. **Confounding:** Age, BMI, time lag between enrollment and beginning of study, physical activity, smoking habits, country of birth, alcohol consumption, education level, laboratory in charge of analyses, reported food consumption						
Lin et al. (2020c) *Medium*	Taiwan 2016–2017	Cross-sectional	Adults aged 55 to 75 that resided in the study area for more than 10 yr and not taking lipid-lowering medication N = 352	Serum 16.2 (10.1–24.1)	Levels (mg/dL) of TC, HDL, LDL, and triglycerides	Regression coefficient by quartiles	TC Q2: 15.06 (4.66, 25.46), p-value < 0.05 Q3: 11.47 (1.03, 21.91), p-value < 0.05 Q4: 10.18 (−0.59, 20.94) p-trend = 0.11 HDL Q2: 3.23 (−0.79, 7.24) Q3: 1.92 (−2.11, 5.95) Q4: −2.68 (−6.84, 1.47) p-trend = 0.19 LDL Q2: 13.43 (4.05, 22.80), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							Q3: 12.32 (2.91, 21.73), p-value < 0.05 Q4: 15.29 (5.59, 24.99), p-value < 0.05 p-trend = 0.004 Triglycerides Q2: 8.93 (−9.74, 27.59) Q3: 7.58 (−11.16, 26.31) Q4: 6.76 (−12.55, 26.07) p-trend = 0.53
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, smoking status, and drinking status						
Liu et al. (2020a) *Medium*	United States 2004–2007	Randomized clinical trial	Adults from POUNDS Lost study ages 20+ N = 326	Plasma 23.5	Levels (mg/dL) of TC, triglycerides, and apolipoproteins log10-ApoB, ApoE, and ApoC-III	Least-squared means (LSM) by tertile PFOS	TC T1: 180.9 (8.0) T2: 189.3 (7.9) T3: 190.7 (7.3) p-trend = 0.21 Triglycerides T1: 126.8 (11.6) T2: 132.4 (11.4) T3: 126.1 (10.5) p-trend = 0.80
	Results: LSM are presented with standard error in parentheses. **Confounding:** Age, sex, race, educational attainment, smoking status, alcohol consumption, physical activity, BMI, regular lipid-lowering medication use, dietary intervention groups						
Han et al. (2021) *Medium*	China 2016–2017	Case-control	Adults ages 25 to 74 including type 2 diabetes cases and healthy controls N = 304	Serum Cases: 7.60 (4.47–10.55) Controls: 8.45 (5.40–11.95)	Levels (log10-mmol/L) of TC, HDL, LDL, and triglycerides	Regression coefficient per log10-unit increase in PFOS	TC: 0.06 (−0.01, 0.12) HDL −0.02 (−0.09, 0.05) LDL: 0.12 (0.03, 0.21), p-value < 0.05 Triglycerides: 0.03 (−0.13, 0.18)
	Confounding: Age, sex, BMI.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Jeddi et al. (2021a) *Medium*	Italy 2017–2019	Cross-sectional	Residents aged 20–39 from the PFAS-contaminated Veneto region N = 15,876	Serum GM (range): 4.54 (<LOQ–142)	Reduced HDL, elevated triglycerides	OR per ln-unit increase in PFOS	Reduced HDL: 0.79 (0.73, 0.86), p-value < 0.05 Elevated triglycerides: 0.97 (0.88, 1.07)
	Outcome: Reduced HDL defined as HDL< 40 mg/L for male or HDL < 50 mg/L for female; elevated triglycerides defined as triglycerides ≥ 175 mg/dL. **Confounding:** Age, gender, time lag between the beginning of the study and blood sampling center where BP has been measured, education, number of deliveries, physical activity, country of birth, diet, alcohol intake, and smoking status, and other components of metabolic syndrome						
			Occupational Populations				
Olsen et al. (2003a) *Medium*	United States, Belgium 1994–2000	Cross-sectional	Current and former workers at two fluorochemical production plants Male N = 421, Female N = 97, Regression analysis N = 174	Serum Antwerp Mean (SD) = 0.96 ppm (0.97); Decatur = 1.40 ppm (1.15)	Levels of cholesterol (ln-mg/dL), HDL (mg/dL)	Comparison of mean outcome by PFOS exposure quartile Regression coefficient per unit increase in PFOS	No significant differences between mean cholesterol or HDL by quartile among male and female employees Cholesterol 0.01 (−0.005, 0.025)
	Confounding: Age, BMI, drinks/day, cigarettes/day, location, entry period, baseline years worked						

Notes: ALSPAC = Avon Longitudinal Study of Parents and Children; ALT = alanine aminotransferase; APFO = ammonium perfluorooctanoate; ApoB = Apolipoprotein B; ApoE = Apolipoprotein E; ApoC-III = Apolipoprotein C-III; BMI = body mass index; CHMS = Canadian Health Measures Survey; DCH = Diet, Cancer and Health; DPPOS = Diabetes Prevention Program and Outcomes Study; EYHS = European Youth Study; GDM = gestational diabetes; GM = geometric mean; HDL = high-density lipids; HELIX = Human Early-Life Exposome; HOME = Health Outcomes and Measures of the Environment; hr = hours; HR = hazard ratio; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; IQR = interquartile range; KorEHS-C = Korea Environmental Health Survey in Children and Adolescents; LDL = low-density lipids; LLM = lipid lowering medication; mo = months; MoBa = Norwegian Mother and Child Cohort Study; MUFA = monounsaturated fatty acid; NF = non-obese female; NHANES = National Health and Nutrition Examination Survey; NM = non-obese male; OF = obese female; OM = obese male; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PIR = poverty income ratio; POUNDS = Preventing Overweight Using Novel Dietary Strategies; PUFA = polyunsaturated fatty acid; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; Q5 = quartile 5; S-MBCS = Shanghai-Minhang Birth Cohort Study; SD = standard deviation; SE = standard error; SEM = serum mean; SES = socioeconomic status; SFA = saturated fatty acid; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; TC = total cholesterol; TFF1 = Tromsø Fit Futures 1; TG = triglycerides; VIP = Västerbotten Intervention Programme; VLDL = very low-density lipoprotein; wk = weeks; yr = year(s).

[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.6 Endocrine

Table D-15. Associations Between PFOS Exposure and Endocrine Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
General Population							
Lebeaux et al. (2020) *High* for cord serum thyroid hormones; *Medium* for maternal thyroid hormones	United States 2003–2007	Cohort	Mother-infant pairs from Health Outcome Measures of the Environment (HOME) Study N = 256 for cord serum N = 185 for maternal serum	Cord serum 14.3 Maternal serum 5.5	Levels of TSH (µIU/L), TT4 (µg/dL), TT3 (ng/dL), FT4 (ng/dL), and FT3 (pg/mL)	Regression coefficient per log2-unit increase in PFOS	Cord serum TSH: 0.09 (−0.06, 0.25) TT4: 0.01 (−0.04, 0.07) TT3: −0.02 (−0.10, 0.06) FT4: −0.02 (−0.06, 0.02) FT3: −0.03 (−0.07, 0.02) Maternal serum TSH: 0.02 (−0.24, 0.28) TT4: 0.02 (−0.08, 0.08) TT3: −0.02 (−0.07, 0.03) FT4: 0.02 (−0.02, 0.07) FT3: −0.03 (−0.06, 0.00)
	Confounding: Individual PFAS, maternal age at delivery, race/ethnicity, marital status at baseline, maternal education level, household income, mean log10-transformed cotinine, maternal alcohol usage during pregnancy, nulliparity, maternal BMI based on pre-pregnancy weight in pounds, child's sex, gestational week at blood draw for PFAS measurement, and (for cord serum only) delivery mode						
Blake et al. (2018) *Medium*	Fernand, Ohio, USA 1991–2008	Cohort	FCC Median age 38 yr at enrollment, N = 122 for TSH measurements; 47 male and 75 female N = 144 for TT4 measurements;	Drinking water Serum 28.4	Levels of TSH (ln-µIU/mL), TT4 (ln-µg/dL)	Percent change per IQR increase in PFOS	TSH 9.75 (1.72, 18.4), p-value = 0.02 Males: 21.4 (6.55, 38.3) p-value = 0.01 Females: 5.13 (−5.29, 16.7) p-value = 0.36 TT4 −0.51 (−4, 3.1), p-value = 0.78 Males: −5.29 (−10.1, −0.26), p-value = 0.04

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			63 males and 81 females				Females: 1.69 (−3.28, 6.91), p-value = 0.52
	Confounding: Age, year of measurement, sex, education, income, marital status, BMI[c]						
Jain and Ducatman (2019b) *Medium*	United States 2007–2012	Cross-sectional	Adults from NHANES aged 20+ GF status: GF-1 = 1,653 GF-2 = 720 GF-3A = 114 GF-3B/4 = 62	Serum Levels not reported	Levels of TSH (log-µIU/mL), TGN (log-ng/mL), TT4 (log-µg/dL), FT4 (log-ng/dL), TT3 (log-ng/dL), FT3 (log-pg/mL)	Regression coefficient per log10-unit increase in PFOS	TT4 GF-1: 0.002, p-value = 0.76 GF-2: −0.008, p-value = 0.47 GF-3A: 0.058, p-value = 0.02 GF-3B/4: −0.002, p-value = 0.94
	GF Stages: GF-1: GFR ≥ 90 mL/min/1.73 m^2; GF-2: GFR between 60 and 90 mL/min/1.73 m^2; GF-3A: GFR between 45 and 60 mL/min/1.73 m^2; GF-3B/4: GFR between 15 and 45 mL/min/1.73 m^2 **Confounding:** Gender, race/ethnicity, iodine deficiency status, age, BMI, fasting time, PIR, total calories consumed during the last 24 hr, smoking status, use of drugs						
Jain (2013) *Low*	United States 2007–2008	Cohort	Adults and children from NHANES aged 12+ N = 1,540 including children	Serum Total cohort	Levels of TSH (µIU/L), FT3 (pg/L), TT3 (fg/dL), FT4 (pg/L), TT4 (pg/L), TGN	Regression coefficient per log10-unit increase in PFOS, or by tertiles	TSH, FT3, FT4, TT3, TT4, TGN: No statistically significant associations
	Results: Lowest tertile used as the reference group. **Confounding:** Gender, race, age, iodine deficiency, iodine replete						
Lewis et al. (2015) *Low*	United States 2011–2012	Cross-sectional	Men and women from NHANES ages 20–80 699 men 680 women	Serum Males 20–40: 7.75 Males 40–60: 9.28 Males 60–80: 11.1 Females 20–40: 4.20 Females 40–60: 4.93	Levels of TSH (µIU/mL), TT3 (ng/dL), FT3 (pg/mL), TT4 (µg/mL), FT4 (ng/dL)	Percent change per doubling of PFOS	TSH Males 20 to < 40: −2.9 (−8.6, 3.2) 40 to < 60: −1.3 (−8.9, 7.1) 60 to 80: −2.3 (−9.4, 5.3) Females 20 to < 40: −1.0 (−7.9, 6.4) 40 to < 60: 0.0 (−7.1, 7.7) 60 to 80: −1.5 (−9.6, 7.3) FT4

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Females 60–80: 9.50			Females 20 to < 40: 2.2 (0.5, 3.9) p-value < 0.05 40 to < 60: 1.3 (−0.5, 3.2) 60 to 80: −0.5 (−2.5, 1.5) Males: No statistically significant associations TT3, FT3, TT4: No statistically significant associations
	Confounding: Age, BMI, PIR, serum cotinine, and race/ethnicity						
Li et al. (2017) *Low*	China 2013–2014	Cross-sectional	Residents of Southern China, ages 1 mo to 90 yr, 70% with thyroid condition N = 202	Serum 1.3	Levels of TSH (µIU/mL), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per log-unit IQR increase in PFOS	TSH: 0.41 (0.05, 0.76), p-value = 0.024 FT3: −0.14 (−0.24, −0.04), p-value = 0.007 FT4: −0.13 (−0.22, −0.04), p-value = 0.004
	Comparison: Logarithm base not specified. **Confounding:** Age, sex						
Byrne et al. (2018) *Low*	St. Lawrence Island, Alaska, USA 2013–2014	Cross-sectional	Alaska Natives, aged 18–45 N = 85 38 men 47 women	Serum 4.55 Males: 6.81 Females: 3.35	Levels of TSH (ln-µIU/mL), TT3 (pg/mL), FT3 (ng/dL), TT4 (µg/dL), FT4 (ng/dL)	Regression coefficient per ln-unit increase in PFOS	TSH Males: −0.06 (−0.62, 0.51), p-value = 0.085 Females: No association TT3 Males: −10.54 (−22.28, 1.20), p-value = 0.08 Females: No association FT3 Males: −0.30 (−0.53, 0.07), p-value = 0.01 Females: 0.35 (0.05, 0.65) p-value for sex interaction = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							TT4, FT4: No statistically significant associations
	Confounding: Age, sex, smoking status						
Zhang et al. (2018b) *Low*	China 2013–2016	Cross-sectional	Women aged 20–40 yr, with (cases) or without (controls) POI N = 120	Plasma Cases: 8.18 Controls: 6.02	Levels (ng/mL) of TSH, FT3, FT4	Regression coefficient per log-unit increase in PFOS	TSH POI cases: 1.57 (0.65, 2.5) POI controls: 0.67 (0.08, 1.26) FT3 POI cases −0.88 (−1.64, −0.09) FT4 POI cases −2.99 (−4.52, −1.46) FT3 and FT4 in POI controls: No associations
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI, education, income, sleep, and parity						
			Children				
Xiao et al. (2019) *High*	Faroe Islands, Denmark 1994–1995	Cohort	Pregnant women and their infant children N = 172 and 153 for measurements in maternal and cord serum, respectively	Maternal blood Geometric mean = 20.86 μg/g	Cord serum levels of TSH (log-IU/L), T4 (log-pmol/L), FT3 (log-pmol/L), FT4, (log-pmol/L) FT3 resin uptake, FT4 index (FTI) (log-IU/L)	Regression coefficient per log2-unit increase in PFOS	TSH All children: 39.7 (7.9, 80.9) Boys: 39.5 (0.4, 94.1) Girls: 39.9 (−4.1, 104.2) FTI All children: 6.7 (−1.5, 15.6) Boys: 2.1 (−7.7, 13) Girls: 13.2 (0.9, 27.1) T4, FT3, FT4, FT3 resin uptake: No statistically significant associations
	Confounding: Child sex (in detailed results), parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Kim et al. (2020a) *High*	South Korea 2012–2017	Cohort	Children, aged 2, 4, 6 yr N = 511 for age 6 (268 boys)	Serum Age 2: 4.530 Age 4: 4.050 Age 6: 3.980	Levels of TSH (ln-µIU/mL), FT4 (ln-ng/dL), and T3 (ln-ng/dL) at age 6 Subclinical hypothyroidism	Regression coefficient per ln-unit increase in PFOS Subclinical hypothyroidism: OR per increase in PFOS	T3 at age 6 All: 0.04 (0.017), p-value < 0.05 Boys: 0.04 (0.018), p-value < 0.05 No interaction with sex Subclinical hypothyroidism at age 6 All: 0.36 (0.41, 0.96) Boys: 0.24 (0.07, 0.92) No interaction with sex TSH, FT4: No statistically significant associations between or within age groups
	Results: Comparisons for T3 are presented with standard error in parentheses. **Confounding:** Age, sex, dietary iodine intake						
Kato et al. (2016) *Medium*	Japan 2002–2005	Cross-sectional	Pregnant women and their children N = 392 Male children = 180 Female children = 212	Maternal serum Male: 5.2 Female: 5.3	Levels of TSH (log10-µU/mL), FT4 (log10-ng/mL)	Regression coefficient per log10-unit increase in PFOS LSM by quartile	TSH All infants: 0.18, p-value = 0.001 Increasing trend in LSM by quartiles p-trend = 0.024 Males: 0.21, p-value = 0.014 Females: 0.17, p-value = 0.021 FT4: No statistically significant associations
	Confounding: Maternal age at delivery, BMI, parity, educational level, thyroid antibody, intake of seaweed, blood sampling period before/after delivery for PFOS and PFOA, and gestational week at which blood sampling was obtained for TSH and FT4						
Preston et al. (2018) *Medium*	United States 1999–2002	Cohort	Pregnant women and their children N = 465 neonates (236 male, 229 female)	Maternal plasma 23.5	Levels of T4 (µg/dL)	Regression coefficient by quartiles	T4, all neonates: Q2: −0.63 (−1.64, 0.37) Q3: −0.36 (−1.36, 0.67) Q4: −1.1 (−2.13, −0.07) T4, males: Q2: −1.56 (−3.04, −0.08) Q3: −1.7 (−3.28, −0.12) Q4: −2.2 (−3.74, −0.66)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							No associations in newborn females
	Results: Lowest quartile used as the reference group. **Confounding:** Maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw						
Aimuzi et al. (2019) *Medium*	China 2012–2013	Cross-sectional	Pregnant women and their children N = 567 Male children = 305 Female children = 262	Cord blood 2.51	Levels of TSH (ln-mIU/L), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per ln-unit increase in PFOS	TSH All children: −0.05 (−0.08, −0.02) Boys: −0.047 (−0.097, 0.003) Girls: −0.048 (−0.093, −0.003)
	Confounding: Maternal age, fish intake, parity infant sex, gestational age at delivery, and maternal pre-pregnancy BMI						
Itoh et al. (2019) *Medium*	Japan 2003–2005	Cohort	Pregnant women and their children 365 male children 336 female children	Plasma 6.21	Levels of TSH (ln-μU/mL), FT3 (ln-pg/mL), FT4 (ln-pg/mL), TPOAb (ln-IU/mL), TgAb (ln-IU/mL)	Regression coefficient per ln-unit increase in PFOS	TSH All boys: 0.23 (0.07, 0.39), p-value = 0.004 Boys with TA-negative mothers: 0.39 (0.12, 0.66), p-value = 0.005 No significant association among TA-positive mother-infant pairs
	Confounding: Age at delivery, parity, educational level, alcohol consumption, smoking during pregnancy, pre-pregnancy BMI, logFT4						
Tsai (2017) *Low*	Taiwan 2004–2005	Cross-sectional	Newborns from Taiwan Birth Panel Study (TBPS) N = 118 (64 boys, 54 girls)	Cord blood Mean = 7.24	Levels of TSH (μIU/mL), T3 (ln-μg/dL), T4 (μg/dL)	Regression coefficient by quartiles or per ln-unit increase in PFOS	TSH, all newborns: Q2: 0.21 (−0.20, 0.63) Q3:0.19 (−0.22, 0.61) Q4: 0.65 (0.02, 1.28) Per increase: 0.35 (0.10, 0.59) TSH, boys: Q2: 0.63 (0.04, 1.22) Q3: 0.30 (−0.33, 0.94) Q4: 0.75 (0.13, 1.62) Per increase: 0.33 (0.01, 0.68)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							T4, all newborns: Q2: −0.50 (−1.29, 0.29) Q3: −0.28 (−1.08, 0.51) Q4: −1.03 (−2.17, −0.12) Per increase: −0.46 (−0.92, −0.001) T4, boys: Q2: −0.30 (−1.40, 0.80) Q3: 0.19 (−0.99, 1.36) Q4: −2.12 (−3.62, −0.618) Per increase: −0.67 (−1.28, −0.05)
	Results: Lowest quartile used as the reference group. **Confounding:** Maternal age at delivery, newborn sex, maternal BMI, maternal education, gestational age, and delivery type						
			Pregnant Women				
Dreyer et al. (2020) *High*	Denmark 2010–2012	Cohort	Pregnant women from Odense Child Cohort (OCC) N = 1,048	Serum 7.64	Levels of diurnal urinary (dU) cortisol (nmol/24-hr), dU-cortisone (nmol/24-hr), dU-cortisol/cortisone, serum cortisol (nmol/L)	Percent change per 2-fold increase in PFOS	dU-cortisone: −9.1 (−14.7, −3.0), p-value < 0.05 T2: −5.7 (−14.7, 4.2) T3: −16.0 (−23.9, −7.2), p-value < 0.05 p-trend < 0.01 dU-cortisol/cortisone: 9.3 (3.3, 15.6), p-value < 0.05 T2: 11.0 (1.8, 21.1), p-value < 0.05 T3: 16.6 (6.9, 27.1), p-value < 0.05 p-trend < 0.01 dU-cortisol and serum cortisol: No statistically significant associations
	Confounding: Age, parity, and offspring sex						
Xiao et al. (2019) *High*	Faroe Islands, Denmark 1994–1995	Cross-sectional	Pregnant women and their children	Maternal blood Geometric mean = 20.86 µg/g	Maternal serum levels of TSH (log-IU/L), T4 (log-pmol/L),	Regression coefficient per log2-unit	TSH in maternal serum All children: 16.4 (−7.5, 46.5) Boys: −6 (−29.6, 25.4) Girls: 54.2 (11.3, 113.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			Maternal age 28 (SD = 5.6) N = 172 and 153 for measurements in maternal and cord serum, respectively		FT3 (log-pmol/L), FT4 (log-pmol/L) FT3 resin uptake FT4 index	increase in PFOS	T4, FT3, FT4, FT3 resin uptake, FT4 index: No statistically significant associations
	Confounding: Child sex (in detailed results), parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury						
Berg (2017) *Medium*	Norway 2007–2009 or until 3 d after birth	Cohort	Pregnant women and children from the Norway Mother and Child Contaminant Cohort Study (MISA) N = 370	Serum 8.03	Levels of TSH (mIU/L), FT3 (pmol/L), T3 (nmol/L), FT4 (pmol/L), T4 (nmol/L)	Regression coefficient by quartiles	TSH Q2: 0.04 (−0.03, 0.11) Q3: 0.08 (0.01, 0.15) Q4: 0.10 (0.02, 0.17) T3, T4, FT3, or FT4: No statistically significant associations
	Results: Lowest quartile used as reference group. **Confounding:** Parity, t-uptake						
Preston et al. (2018) *Medium*	United States 1999–2002	Cross-sectional	Pregnant women and their children N = 718 women (98 TPOAb-positive and 620 TPOAb-negative)	Maternal plasma 24.0	Levels of TSH (mIU/mL), T4 (µg/dL), FT4 index	Percent difference in hormone level per IQR increase in PFOS	TSH among TPOAb-positive mothers: −16.4 (−29.8, −0.38) p-value for effect modification by TPOAb status = 0.05 FT4, TT4: No statistically significant associations
	Confounding: Maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw						
Reardon et al. (2019)	Canada 2019–2012	Cohort	Pregnant women	Maternal blood	Levels of	Regression coefficient per	TSH, linear PFOS Main effect: 0.01 (−0.03, 0.04)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Medium			recruited prior to 18 wk of gestation N = 478	Total PFOS: 4.77 Linear PFOS: 2.49 ∑Br-PFOS: 1.08	TSH (log-mIU/mL), FT3 (log-pmol/L), FT4 (log-pmol/L) by gestation status and 3 mo postpartum	unit increase in total, linear, or 1m-PFOS	3 mo postpartum: 0.06 (0.01, 0.12) TSH, ∑Br-PFOS Main effect: 0.29 (0.02, 0.56) FT3, FT4: No statistically significant associations
	Confounding: Maternal age, ethnicity, history of smoking, history of drug and alcohol use						
Kato et al. (2016) *Low*	Japan 2002–2005	Cross-sectional	Pregnant women and their children N = 392 Male children = 180 Female children = 212	Maternal serum Male: 5.2 Female: 5.3	Levels of TSH (log10-μU/mL), FT4 (log10-ng/mL)	Regression coefficient per log10-unit increase PFOS LSM by quartile	TSH All mothers: −0.21, p-value < 0.001 Decreasing trend in LSM by quartiles: p-trend < 0.001 Male: −0.25, p-value = 0.002 Female: −0.21, p-value = 0.005 FT4: No statistically significant associations
	Confounding: Maternal age at delivery, BMI, parity, educational level, thyroid antibody, intake of seaweed, blood sampling period before/after delivery for PFOS and PFOA, and gestational week at which blood sampling was obtained for TSH and FT4						

Notes: BMI = body mass index; d = day(s); FCC = Fernald Community Cohort; FTI = free thyroxine index; GF = glomerular filtration; GFR = glomerular filtration rate; HOME = Health Outcomes and Measures of the Environment; IQR = interquartile range; LSM = least square means; MISA = Norway Mother and Child Contaminant Cohort Study; NHANES = National Health and Nutrition Examination Survey; OCC = Odense Child Cohort; TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; FT3 = free triiodothyronine; FT4 = free thyroxine; hr = hour(s); mo = month(s); PCBs = polychlorinated biphenyls; PIR = poverty income ratio; POI = premature ovarian insufficiency; POUNDS = Preventing Overweight Using Novel Dietary Strategies; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; T3 = tertile 3; T4 = tertile ; TBPS = Taiwan Birth Panel Study; TgAb = thyroglobulin antibody; TPOAb = thyroid peroxidase antibody; TT3 = total triiodothyronine; TT4 = total thyroxine; TGN = thyroglobulin; USA = United States of America; wk = week(s); yr = years.

[a] Exposure levels are reported as median unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.7 Metabolic/Systemic

Table D-16. Associations Between PFOS Exposure and Metabolic Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Ashley-Martin et al. (2017) *High*	Canada, Recruitment 2008–2011	Cohort	Pregnant women and their children, from the MIREC Study N = 1,175	Maternal blood 4.6	Adiponectin, leptin	Regression coefficient per log10-unit increase in PFOS	Adiponectin, leptin: No statistically significant associations
	Confounding: Maternal age, pre-pregnancy BMI, sex, and parity[c]						
Buck et al. (2018) *High*	United States, 2003–2006	Cohort	Pregnant women and their children in the HOME study N = 230	Maternal serum 14	Adiponectin, leptin	Percent change per doubling of PFOS	Adiponectin, leptin: No statistically significant associations
	Confounding: Maternal age, race, education, income, parity, maternal BMI, serum cotinine, delivery mode, and infant sex						
Chen et al. (2019b) *High*	China, 2012–2017	Cohort	Infants followed up at age 5, N = 404	Cord blood 2.44	BMI, WC, body fat, waist-to-height ratio	Regression coefficient per ln-unit increase in PFOS, or by tertile	BMI, waist circumference, body fat, waist-to-height ratio: No statistically significant association
	Confounding: Maternal age, maternal pre-pregnancy BMI, gestational week at delivery, maternal education, paternal smoking during pregnancy, and parity						
Jensen et al. (2020a) *High*	Denmark, 2010–2012	Cohort	Pregnant women and their infants assessed at birth, 3 mo, and 18 mo, Odense Child Cohort N = 593	Maternal serum 8.04	BMI z-score, WC	Regression coefficient per unit increase in PFOS	BMI z-score, WC: No statistically significant associations
	Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI^2, education, smoking, sex, visit, adiposity marker at birth						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Minatoya et al. (2017) *High*	Japan, 2002–2005	Cohort	Pregnant women and their children N = 168	Serum 5.1	Adiponectin, leptin	Regression coefficient per log10-unit increase in maternal serum PFOS	Adiponectin: 0.12 (0.01, 0.22), p-value = 0.028 Leptin: No statistically significant association
	Confounding: Maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age, infant sex						
Alderete et al. (2019) *Medium*	United States, 2001–2012	Cohort	Obese Hispanic children (8–14 yr), SOLAR Project N = 38	Plasma 12.22	Blood glucose, insulin, 2-hr glucose (mg/dL)), 2-hr insulin, insulin resistance, insulin levels	Regression coefficient per ln-unit increase in PFOS	Glucose (2-hr) 6.2 (−2.3, 14.8) Blood glucose, insulin, 2-hr insulin, insulin resistance, insulin levels: No statistically significant associations
	Confounding: Sex, baseline social position (categorical), baseline outcome, baseline and change in age at follow-up, pubertal status (categorical), baseline and change in body fat percent at follow-up.						
Braun et al. (2016) *Medium*	United States, 2003–2006, follow-up at age 8	Cohort	Pregnant women and their children in the HOME study N = 204	Maternal serum 13	Overweight, obesity, BMI z-score, waist circumference, body fat	Percent change per doubling of PFOS	Overweight, obesity, BMI z-score, waist circumference, body fat: No statistically significant associations
	Confounding: Maternal age, race, education, income, parity, marital status, employment, depressive symptoms, BMI at 16 wk gestation, fruit/vegetable consumption, fish consumption, prenatal vitamin use, maternal serum cotinine concentrations, and child age in months						
Conway et al. (2016) *Medium*	United States, 2005–2006	Cross-Sectional	Children working or living in six PFOS-contaminated water districts, C8 Health Project N = 47	Serum Mean = 86.5	Type 1 Diabetes	OR per ln-unit increase in PFOS	Children with T1D: 0.52 (0.54, 0.87)
	Confounding: Age, sex, race, BMI, eGFR, hemoglobin, iron						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Domazet et al. (2016) *Medium*	Denmark, 1997–2009	Cohort	Children from EYHS followed through ages 9, 15, and 21, N = 176	Plasma Age 21 Males: 11.9 Females: 9.1 Age 15 Males: 22.3 Females: 20.8 Age 9 Males: 44.5 Females: 39.9	WC, HOMA-Beta, HOMA-IR, insulin, glucose, skinfold thickness, BMI	Percent change at 15 or 21 yr old per 10-unit increase in PFOS at 9 yr old	WC: Age 15 from age 9: 1.18 (0.42, 1.84) Age 21 from age 9: 1.52 (0.05, 2.91) Skinfold thickness: Age 15 from age 9: 4.03 (1.33, 6.67) Age 21 from age 9: 5.67 (0.6, 10.93) BMI: Age 15 from age 9: 1.54 (0.62, 2.4) HOMA-Beta age 21, BMI age 21, HOMA-IR, insulin, glucose: No statistically significant associations
	Confounding: Sex, age, and outcome levels at baseline (9 yr of age), and ethnicity, maternal parity, and maternal income in 1997 (9 yr of age). Waist circumference was adjusted for height in order to account for body size.						
Domazet et al. (2020) *Medium*	Denmark, 1997	Cross-sectional	Children from EYHS, 9-yr-old N = 242	Plasma Boys: 42.9 Girls: 42.0	Body fat (mm), adiponectin (ng/mL), leptin (pg/mL)	Percent change per 10% increase in PFOS	Body fat: −0.59 (−2.88, 1.24), p-value = 0.552 Adiponectin: 0.24 (−1.70, 2.21), p-value = 0.811 Leptin: −3.65 (−8.23, 1.16), p-value = 0.134
	Confounding (Adiponectin and leptin): Sex, age, parity, maternal income level **Confounding (Body fat):** Sex, age, accelerometer wear time, parity, maternal income level						
Gyllenhammar et al. (2018b) *Medium*	Sweden, 1996–2011, children followed up at age 5	Cohort	Mothers and their children from the POPUP Study N = 381	Maternal serum 13	BMI z-score	Regression coefficient per IQR increase in maternal PFOS	BMI z-score: Ages 36 Non-significant positive association (numeric results not provided) Ages 48 and 60 mo: Positive statistically significant associations.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, and total time of breastfeeding						
Hartman et al. (2017) *Medium*	United Kingdom, recruitment 1991–1992	Cohort	Pregnant women and their daughters, ALSPAC N = 319	Maternal serum 19.8	Waist circumference (WC)(cm), Trunk fat (%), BMI (kg/m^2), Total body fat (%) per high, medium, and low educational status	Regression coefficient per unit increase in PFOS	WC: −0.12 (−0.20, −0.04), p-value = 0.005 Trunk fat: −0.06 (−0.12, 0.01), p-value = 0.02 BMI: −0.04 (−0.07, 0.0), p-value = 0.03 Total body fat (%), WC, Trunk fat, and BMI for overall, low, and medium education status: No statistically significant associations
	Confounding: Sampling design, pre-pregnancy BMI (kg/m2) and maternal educational status						
Kang et al. (2018) *Medium*	Korea, 2012–2014	Cross-sectional	Children from KorEHS-C Seoul and Gyeonggi, 3–18 yr of age, N = 147	Plasma 5.68	Fasting blood glucose (mg/dL)	Regression coefficient per ln-unit increase in PFOS	Blood glucose: 0.707 (−1.921, 3.336), p-value = 0.595
	Confounding: Age, sex, BMI z-score, household income, secondhand smoking						
Karlsen et al. (2017) *Medium*	Faroe Islands, recruited 2007–2009 (at birth); follow-up at child ages 18 mo, 5 yr	Cohort	Children, 5 yr (BMI) N = 349 Children, 5 yr (overweight) N = 371 Children, 18 mo (overweight) N = 444	Serum, Maternal serum 5 yr: 4.7 18 mo: 8.25	BMI z-score, Overweight	RR (OW), or Regression coefficient per log10-unit increase in maternal PFOS, or by tertiles (BMI)	BMI z-score 18 mo: 0.2 (0.1, 0.4), p-value < 0.05 OW 18 mo:1.29 (1.01, 1.64), p-value < 0.05
	Results: Lowest tertile used as reference.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal nationality, age at delivery, pre-pregnancy BMI, smoking during pregnancy, child sex, exclusive breastfeeding duration, child's fish intake at age 5 yr						
Kobayashi et al. (2017) *Medium*	Japan, 2002–2005	Cross-sectional	Children from Hokkaido Study on Environment and Children's Health N = 176	Maternal serum 5.3	Ponderal index	Regression coefficient per ln-unit increase in PFOS	−1.07 (−1.79, −0.36), p-value = 0.004
	Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, gestational age, infant sex, and maternal blood sampling period						
Lauritzen et al. (2018) *Medium*	Norway and Sweden, Recruitment 1986–1988	Cohort	Pregnant women and their children at 5-yr follow-up N = 412	Serum Norway: 9.62 Sweden: 16.3	BMI, triceps skin fold, subscapular skinfold, overweight	Regression coefficient or OR per ln-unit increase in maternal PFOS	Regression coefficient BMI: 0.18 (0.01, 0.35) Triceps skinfold: 0.15 (0.02, 0.27) Odds ratio Overweight: 2.04 (1.11, 3.74) Subscapular skinfold: No statistically significant association
	Confounding: Age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 wk, interpregnancy interval, previous breastfeeding duration and country of residence						
Lopez-Espinosa et al. (2016) *Medium*	United States, 2005–2006	Cohort	Children, ages 6–9 yr from the C8 Health Project N = 1,123 girls and 1,169 boys	Serum Girls: 20.9 Boys: 22.4	Insulin-like growth factor 1 (IGF-1) (ln-ng/mL)	Percent difference for 75th vs. 25th percentile of ln(PFOS), or by quartiles	IGF-1 Girls: −5.6 (−8.2, −2.9) Q4: −11.4 (−16.5, −6.0) Boys: −5.9 (−8.3, −3.3) Q3: −6.3 (−11.6, −0.6) Q4: −11.5 (−16.6, −6.1) Boys Q2; Girls Q2, Q3: No statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Age and month of sampling						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Manzano-Salgado et al. (2017b) *Medium*	Spain, Recruitment 2003–2008	Cohort	Mother-child pairs, followed for 8 yr, INMA Study N = 1230	Maternal blood GM = 5.80	BMI, WC, overweight, waist-to-hip ratio	Regression coefficient per-log2-unit increase in PFOS	BMI, waist circumference, overweight, waist-to-hip ratio: No statistically significant associations
	Confounding: Maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI), age of child						
Martinsson et al. (2020) *Medium*	Sweden, 2003–2008	Case-control	Pregnant women and their children at age 4, Southern Sweden Maternity Cohort N = 1,048	Serum 16.6	Overweight	OR by quartiles	OW Q4: 1.57 (1.07, 2.3) Q2 and Q3: No statistically significant association
	Results: Lowest quartile used as reference **Confounding:** Risk strata, difference from strata-specific mean, sex						
Mora et al. (2017) *Medium*	United States, 1999–2002	Cohort	Early childhood N = 992 Mid-childhood N = 871	Maternal Plasma Early childhood: 24.8 Mid-childhood: 24.7	WC (cm), Sum of subscapular and triceps skinfold thickness (mm), BMI, waist-to-hip ratio, obesity, overweight, total fat mass index, total fat-free mass index	Regression coefficient per IQR increase in PFOS	All: Sum of subscapular and triceps skinfold thickness: −0.41 (−0.77, −0.05) Boys: Waist-to-hip ratio: −0.76 (−1.47, −0.05) Early childhood: BMI, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association Mid-childhood: Waist circumference (cm), Sum of subscapular and triceps skinfold thickness (mm), BMI, waist-to-hip

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							ratio, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association.
	Confounding: Maternal age, race/ethnicity, education, parity, pre-pregnancy BMI, timing of blood draw, household income, child sex, age at outcome assessment						
Scinicariello et al. (2020a) *Medium*	United States, 2013–2014	Cross-sectional	Children aged 3–11 yr from NHANES N = 600	Serum GM = 3.90 (SE = 0.17) Girls: GM = 3.69 (SE = 0.15) Boys: GM = 4.12 (SE = 0.27)	BMI z-score (BMIZ), height-for-age z-score (HAZ), WAZ	Regression coefficient per ln-unit increase in PFOS or by tertiles	BMIZ: −0.09 (−0.30, 0.13) T2: −0.19 (−0.41, 0.03) T3: −0.21 (−0.53, 0.11) p-value for trend = 0.17 Girls: −0.20 (−0.48, 0.07) Boys: −0.02 (−0.29, 0.24) HAZ: −0.29 (−0.49, −0.10) T2: −0.32 (−0.60, −0.04) T3: −0.39 (−0.72, −0.06) p-value for trend = 0.06 Girls: −0.34 (−0.73, 0.05) Boys: −0.22 (−0.41, −0.03) T3: −0.28 (−0.53, −0.03) WAZ: −0.25 (−0.47, −0.03) T2: −0.32 (−0.60, −0.04) T3: −0.40 (−0.76, −0.04) p-value for trend = 0.06 Girls: −0.35 (−0.72, 0.03) Boys: −0.17 (−0.37, 0.03) No other statistically significant associations or trends by quartiles stratified by sex
	NHANES = National Health and Nutrition Examination Survey **Results:** Lowest tertile used as reference **Confounding:** Age, quadratic age, race/ethnicity, PIR, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, sex						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Fleisch et al. (2017) *Medium* for metabolic function *Low* for HOMA-IR	United States, Pregnant women recruited 1999–2002, outcome assessed at mid-childhood follow-up	Cohort	Pregnant women and their children from Project Viva N = 584 Median age at follow-up = 7.7 yr	Plasma GM = 6.2	Leptin, Adiponectin, HOMA-IR	Percent change per IQR increase in PFOS, or by quartiles	HOMA-IR: Per IQR increase −10.1% (−16.4, −3.3) Q4: −24.7 (−37.8, −8.8) Females: −16.7 (−25.7, −6.7) Q4: −30.7 (−47.5, −8.4) Leptin, adiponectin: No statistically significant associations
	Results: Lowest quartile used as reference; Q4 (9.8–51.4 ng/mL), Q1 (<0.1–4.2 ng/mL) PFOS. **Confounding:** Characteristics of child (age, sex, race/ethnicity), mother (age, education), and neighborhood census tract at mid-childhood (median household income, percent below poverty)						
			Pregnant Women				
Jensen et al. (2018) *High*	Denmark, recruitment 2010–2012, outcome assessed 12–20 wk later	Cohort	Pregnant women, Odense Child Cohort N = 158	Serum 8.37	Blood glucose, insulin, c-peptide, 2-hr glucose, insulin resistance, beta-cell function, insulin sensitivity	Percent change per log2-unit increase in PFOS	Blood glucose, insulin, c-peptide, 2-hr glucose, insulin resistance, beta-cell function, insulin sensitivity: No statistically significant association
	Confounding: Age, parity, education level, pre-pregnancy BMI						
Mitro et al. (2020) *High*	United States, Recruitment 1999–2002	Cohort	Pregnant women, Project Viva N = 786	Plasma 24.8	WC (cm), BMI (kg/m^2), Adiponectin (µg/mL), Skinfold thickness, Arm circumference, HbA1c, Leptin	Percent difference per log2-unit increase in PFOS	Skinfold thickness All: 1.2 (0.1, 2.2), p-value < 0.05 Women < 35 at pregnancy: 1.5 (0.1, 3), p-value < 0.05 WC, BMI, Adiponectin, arm circumference, HbA1c, leptin: No statistically significant associations
	Confounding: Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, breastfeeding in a prior pregnancy						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Preston et al. (2020) *High*	United States, 1999–2002	Cohort	Pregnant women from Project Viva N = 1,533	Serum 25.7	Gestational diabetes, glucose tolerance, hyperglycemia, glucose blood level	Regression coefficient by quartiles	Glucose blood level, All Q4: 4.3 (0.5, 8.0) < 35 yr Q4: 6.5 (2.1, 10.9) Q3: 5.2 (0.8, 9.7) Q2: 5.2 (0.8, 9.6) Gestational diabetes, glucose tolerance, hyperglycemia: No statistically significant association
	Results: Lowest quartile used as reference; Q1 (0.1–18.8 ng/mL), Q2 (18.9–25.7 ng/mL), Q3 (25.8–34.9 ng/mL), Q4 (35.0–185.0 ng/mL). **Confounding:** Pre-pregnancy BMI, prior history of gestational diabetes/parity, race/ethnicity, smoking, and education, maternal age (Full group only)						
Starling et al. (2017) *High*	United States, 2009–2014	Cohort	Pregnant women and their children in the Healthy Start study N = 628	Maternal serum 2.4	Maternal glucose	Regression coefficient per unit increase in PFOS and by tertile	Maternal glucose: No statistically significant associations
	Confounding: Maternal age, pre-pregnancy BMI, race/ethnicity, education, smoking during pregnancy, gravidity, and gestational age at blood draw						
Ashley-Martin et al. (2016) *Medium*	Canada, Pregnant women recruited 2008–2011, outcome assessed at birth	Cohort	Pregnant women from MIREC N = 1,609	Serum 0.15	GWG (kg)	Regression coefficient per log2-unit increase in PFOS	Underweight/normal BMI: 0.39 (0.02, 0.75) Overweight and obese BMI: No statistically significant association
	Confounding: Age, income, parity						
Jaacks et al. (2016) *Medium*	United States, 2005–2007	Cohort	Pregnant women N = 218	Serum Mean = 14.81	GWG (kg)	Regression coefficient and OR per SD-unit increase in PFOS	GWG 0.26 (−0.66, 1.18) OR for excessive GWG: 1.01 (0.72, 1.4)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Pre-pregnancy non-fasting serum lipids, BMI						
Liu et al. (2019) *Medium*	China, 2013–2015	Case-control	Pregnant women without history or family history of diabetes N = 189	Serum 3.13	Gestational diabetes (GDM), glucose homeostasis	Regression coefficient per ln-unit increase or by tertiles sum m-PFOS or L-PFOS	GDM: m-PFOS Per ln-unit increase: 1.36 (0.88, 2.11) T2: 1.53 (0.7, 3.34) T3: 1.23 (0.56, 2.72) L-PFOS Per ln-unit increase: 1.58 (0.89, 2.79) T2: 1.34 (0.62, 2.93) T3: 1.37 (0.62, 3.02) Glucose homeostasis: No statistically significant association
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, BMI in early pregnancy, fetal sex, serum triglyceride, total cholesterol						
Marks et al. (2019) *Medium*	United Kingdom 1991–1992	Cohort	Mothers from ALSPAC N = 905	Serum Mothers of sons: 13.8 Mothers of daughters: 19.8	GWG (absolute)	Regression coefficient per 10% increase in log-unit PFOS	GWG: No statistically significant associations
	Comparison: Logarithm base not specified. **Confounding:** Maternal education, prenatal smoking, maternal age at delivery, parity, pre-pregnancy BMI, gestational age at delivery, gestational age at sample						
Rahman et al. (2019) *Medium*	United States, 2009–2013	Cohort	Pregnant women with singleton pregnancies N = 2,292	Plasma GM = 5.21	GDM	RR per SD-unit increase in PFOS	GDM: No statistically significant associations
	Confounding: Maternal age, enrollment BMI, education, parity, race/ethnicity, serum cotinine						
Ren et al. (2020)	China, 2012	Cross-sectional	Pregnant women,	Plasma 10.7	Glucose (1 hr, fasting)	Regression coefficient per	Glucose (1 hr tolerance test): 0.31 (0.11, 0.50), p-value = 0.003

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			Shanghai-Minhang Birth Cohort Study N = 705			ln-unit increase in PFOS	Glucose after fasting, glucose after 1 hr tolerance test by gestational weeks: No statistically significant association
	Confounding: Maternal age at enrollment, pre-pregnancy BMI, per capita household income, education level, passive smoking, pregnancy complication, history of abortion and stillbirth, parity						
Shapiro et al. (2016) *Medium*	Canada, 2008–2011	Cohort	Pregnant women N = 1,195	Urine Normal glucose GM = 4.58 Gestational impaired glucose tolerance GM = 4.29 Women with GDM GM = 4.74	GDM, gestational impaired glucose tolerance	OR per quartile PFOS	Gestational diabetes, gestational impaired glucose tolerance: No statistically significant association
	Confounding: Maternal age, race, pre-pregnancy BMI, and education						
Valvi et al. (2017) *Medium*	Faroe Islands, 1997–2000	Cohort	Pregnant women and their children N = 604	Maternal serum 27.2	Gestational diabetes	OR per doubling of PFOS, or by tertiles	Gestational diabetes: Per doubling: 0.86 (0.43, 1.7) T2: 0.85 (0.43, 1.7) T3: 0.56 (0.26, 1.19)
	Results: Lowest tertile used as the reference group **Confounding:** Maternal age at delivery, education, parity, pre-pregnancy BMI, smoking during pregnancy						
Wang et al. (2018c) *Medium*	China 2013	Case-control	Pregnant women with (cases) and without (controls) GDM N = 242	Serum n-PFOS Cases: 2.70 Controls: 2.81 1m-PFOS Cases: 0.14 Controls: 0.14 3m+4m-PFOS Cases: 0.44 Controls: 0.42	Fasting blood glucose, GDM	Fasting blood glucose: OR by tertiles of PFOS isomer GDM: OR per unit increase in PFOS isomer	Fasting blood glucose n-PFOS T2: 1.94 (1.05, 3.58), p-value < 0.05 T3: 1.59 (0.85, 2.96) 1m-PFOS T2: 1.86 (1.00, 3.48), p-value < 0.05 T3: 2.07 (1.09, 3.93), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				5m-PFOS Cases: 0.36 Controls: 0.36 6m-PFOS Cases: 0.29 Controls: 0.31			3m + 4m-PFOS T2: 1.81 (0.98, 3.33) T3: 1.88 (1.00, 3.52), p-value < 0.05 5m-PFOS T2: 1.94 (1.05, 3.80), p-value < 0.05 T3: 2.45 (1.24, 4.64), p-value < 0.05 6m-PFOS T2: 1.24 (0.67, 2.28) T3: 1.42 (0.83, 2.77) GDM: No statistically significant associations
	Results: Lowest tertile used as reference. **Confounding:** Fasting blood glucose: BMI, age, GDM status; GDM: BMI, GWG, ethnic groups, maternal education, parity, maternal drinking during pregnancy, household income						
Wang et al. (2018a) *Medium*	China, 2013–2014	Cohort	Pregnant women aged 20–40 N = 385	Serum 5.4	Fasting blood glucose, fasting insulin, HOMA-IR, gestational diabetes, oral glucose tolerance	LSM by tertiles	Fasting blood glucose: T2: 1.47 (1.45, 1.48), p-value < 0.05 T3: 1.47 (1.45, 1.48), p-value < 0.05 Oral glucose tolerance: 1.88 (1.84, 1.91), p-value < 0.05 Fasting insulin, HOMA-IR, gestational diabetes: No statistically significant association
	Results: Lowest tertile used as reference. **Confounding:** Pregnant age, diabetes mellitus history of relatives, husband smoking status, family per capita income, baby sex, averaged intake of meat, vegetable, and aquatic products, averaged physical activity, and averaged energy intake, pre-pregnant maternal BMI						
Xu et al.(2020b) *Medium*	China, 2017–2019	Nested case-control	Pregnant women	Serum Cases: 6.69 Controls: 6.45	GDM	OR per unit increase in PFOS; OR per	GDM Q2: 0.69 (0.34, 2.07) Q3: 0.72 (0.48, 1.90)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 165 cases, 330 controls			log10-unit increase in PFOS	Q4: 1.07 (0.51, 1.32) p-trend = 0.27 logPFOS: 0.61 (0.42, 1.65), p-value = 0.21
	Confounding: Maternal age, sampling time, parity, BMI, educational level, and serum lipids						
			General Population				
Cardenas et al. (2017) *High*	United States, Recruitment July 1996–May 1999, outcome assessed annually until May 2001	Cohort	Adults at high risk of Type 2 diabetes N = 956	Plasma GM = 26.38	Adiponectin (µg/mL), HbA1c (%), Insulin (fasting) (µU/mL), Glucose (fasting) (µU/mL), HOMA-IR, Insulin (30 min, µU/mL), Proinsulin (fasting, pM), HOMA-B, Insulin (corrected response), Insulinogenic index, Diabetes, HOMA-IR, glucose (30 min), glucose (2 hr), BMI	Regression coefficient per doubling of PFOS	HbA1c: 0.03 (0.002, 0.07), p-value = 0.04 Insulin (fasting): 1.37 (0.41, 2.34), p-value = 0.005 Glucose (fasting): 0.55 (0.03, 1.06), p-value = 0.04 HOMA-IR: 0.39 (0.13, 0.66), p-value = 0.004 Insulin (30 min): 4.63 (0.89, 8.36), p-value = 0.02 Proinsulin (fasting): 1.37 (0.5, 2.25), p-value = 0.002 HOMA-B: 9.62 (1.55, 17.7), p-value = 0.02 Diabetes, glucose (30 min), glucose (2 hr), BMI, adiponectin, insulin (corrected), insulinogenic index: No statistically significant association
	Confounding: Sex, race/ethnicity, BMI, age, marital status, education, smoking history.						
Blake et al. (2018)	United States, 1991–2008	Cohort	Adults living in a community	Serum 28.4	BMI	Percent change per IQR	BMI: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			with water supply from a PFAS-contaminated aquifer N = 192			increase in PFOS	
	Confounding: Age, year of measurement, sex, education, income, marital status, and BMI						
Cardenas et al. (2019) *Medium*	United States, 1996–2014	Controlled trial	Adults older than 25 without diabetes and with elevated fasting and postload glucose, DPP N = 956	Plasma GM = 26.38	T2D	Hazard ratio per log2-unit increase in baseline PFOS and by PFOS tertiles	T2D: HR: 1.05 (0.94, 1.18) T2: 0.94 (0.75, 1.17) T3: 0.94 (0.75, 1.18)
	Confounding: Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment						
Christensen et al. (2016b) *Medium*	United States, 2011–2013	Cross-sectional	Male anglers N = 154	Serum 19.0	Diabetes, pre-diabetes	OR per unit in PFOS	Diabetes, pre-diabetes: No statistically significant associations.
	Confounding: Age, BMI, employment status, number of alcoholic drinks consumed per month						
Conway et al. (2016) *Medium*	United States, 2005–2006	Cross-sectional	All individuals working or living in six PFOS-contaminated water districts with diabetes N = 6,460	Serum All participants mean = 86.5	T1D, T2D, Uncategorized Diabetes	OR per ln-unit increase in PFOS	T1D: 0.73 (0.67, 0.79) T2D: 0.92 (0.88, 0.96) Children with T1D: 0.52 (0.54, 0.87) Adults with T1D: 0.77 (0.71, 0.84) Uncategorized diabetes: No statistically significant association
	Confounding: Age, sex, race, BMI, eGFR, hemoglobin, iron						
Donat-Vargas et al. (2019a) *Medium*	Sweden, 1990–2003, 2001–2012	Case-control	Adults with (cases) and without (controls) type 2 diabetes living in Sweden	Plasma Cases: 19.0 Controls: 20.0	T2D	OR per SD log10-unit increase in baseline PFOS, or by tertiles	T2D OR: 0.7 (0.47, 1.03) T2: OR: 0.79 (0.34, 1.87) HOMA-B and HOMA-IR: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 248				
	Results: Lowest tertile used as reference; T1 (13, 11–16 ng/mL), T2 (21, 19–23 ng/mL). **Confounding:** Gender, age, sample year, red and processed meat intake, fish intake, BMI						
Duan et al. (2020) *Medium*	China, 2017	Cross-sectional	Adults, 19 to 87 yr old N = 252	Serum 14.24	Fasting glucose (nmol/L), HbA1c	Regression coefficient per 1% increase in serum PFOS	HbA1c 55+: 0.02819 (0.00557, 0.04965) HbA1c < 55, fasting glucose: No statistically significant association
	Confounding: Sex, age, BMI, smoking and alcohol-drinking status, exercising status, education level, and family history of diabetes						
Jain et al. (2019e) *Medium*	United States, 2011–2014	Cohort	Adults from NHANES, 20 and older N = 2,883	Serum Non-obese GM = 2.2 Obese GM = 2.0	Obesity	Comparison of GM of PFOS levels for non-obese vs. obese	Obesity: p-value = 0.01
	Confounding: Not reported						
Jeddy et al. (2018) *Medium*	England, mothers recruited 1991–2002, outcome assessed at age 17	Nested case-control studies	Pregnant mothers and their 17-yr old daughters, ALSPAC N = 221	Maternal serum 20.2	Fat mass	Regression coefficient per unit increase in PFOS	Fat mass: No statistically significant association
	Confounding: Maternal pre-pregnancy BMI, maternal education, maternal age at delivery, gestational age at sample collection, and ever breastfed status at 15 mo						
Liu et al. (2018a) *Medium* for adiposity/weight change *Uninformative* for insulin resistance	Boston, Massachusetts and Baton Rouge, Louisiana, 2004–2007	Controlled Trial	Overweight and obese patients from the POUNDS Lost Trial, Ages 30–70, N = 621	Plasma, glucose Males: 27.2 Females: 22.3	Body weight (kg), Resting metabolic rate (RMR) (kcal/24 hr), HbA1c, insulin, glucose, fat mass, WC, leptin, HOMA-IR	Partial Spearman correlation with baseline PFOS (insulin, leptin) Regression coefficient per log10-unit increase in PFOS, or by tertile	Spearman correlations Body weight: 0.8, p-value < 0.05 Body weight, months 6–24 All: T1: 1.5, p-trend = 0.007 T2: 3.5, p-trend = 0.007 T3: 3.2, p-trend = 0.007 Women: T1: 2.1, p-trend = 0.01 T2: 4.1, p-trend = 0.01 T3: 4.0, p-trend = 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Per log10-umit increase in PFOS 0.8, p-value < 0.05 RMR First 6 mo, all T1: −5.0, p-trend = 0.005 T2: −24.7, p-trend = 0.005 T3: −45.4, p-trend = 0.005 Months 6–24, all T1: 94.6, p-trend < 0.001 T2: 67.3, p-trend < 0.001 T3: 0.9, p-trend < 0.001 First 6 mo, women T1: −19.2, p-trend = 0.01 T2: −29.7, p-trend = 0.01 T3: −60.4, p-trend = 0.01 Months 6–24, men T1: 46.8, p-trend = 0.05 T2: 60.8, p-trend = 0.05 T3: −40.2, p-trend = 0.05 Months 6–24, women T1: 141.6, p-trend = 0.001 T2: 90.1, p-trend = 0.001 T3: 47.7, p-trend = 0.001 HbA1c, glucose, fat mass, WC, leptin: No statistically significant association
	Results: Lowest tertile used as reference; Tertile 1 (<19.2 ng/mL), tertile 2 (19.2–32.1 ng/mL), tertile 3 (> 32.1 ng/mL) PFOS. **Confounding:** Age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups.						
Liu et al. (2018b) *Medium*	United States, 2013–2014	Cross-sectional	Adults from NHANES N = 1,871	Serum GM = 5.28	Fasting blood glucose, 2-hr glucose, HbA1c, insulin levels, HOMA-IR,	Regression coefficient per ln-unit increase in PFOS	Fasting blood glucose: 1.96 (SE = 0.79) 2-hr glucose, HbA1c, insulin levels, HOMA-IR, beta-cell function,

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					beta-cell function, metabolic syndrome, WC		metabolic syndrome, WC: No statistically significant associations
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, WC, and medications (antihypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Mancini et al. (2018) *Medium*	France, 1990–2012	Cohort	Women aged 40–60, E3N Cohort N = 71,294	Food Mean = 0.49 ng/kg body weight/day	T2D	Hazard ratio per decile PFOS	T2D: No statistically significant association
	Confounding: Smoking status, physical activity, education level, hypertension, hypercholesterolemia, family history of diabetes, energy intake, alcohol intake, adherence to the Western diet and adherence to the Mediterranean diet, water consumption, dairy product consumption						
Su et al. (2016) *Medium*	Taiwan, 2009–2011	Cross-Sectional	Adults aged 20–60 living in Taiwan N = 571	Plasma 8.0	Diabetes, Fasting blood glucose (ng/mL), blood glucose (120 min) (ln) (ng/mL), glucose AUC (ng/mL), HbA1c (ln) (%)	OR and GM ratio (GMR) per doubling of PFOS, or by quartiles	Diabetes: OR: 2.39 (1.52, 3.76) OR Q4: 3.37 (1.18, 9.56) Glucose (Fasting): GMR: 1.03 (1.01, 1.04) GMR Q4: 1.05 (1.02, 1.09) Glucose (120 min) GMR: 1.08 (1.05, 1.12) GMR Q4: 1.17 (1.08, 1.25) Glucose AUC: GMR: 1.06 (1.04, 1.09) GMR Q4: 1.12 (1.06, 1.19)
	Results: Lowest quartile used as reference; Q1 (<2.4 ng/mL); Q4 (> 4.8 ng/mL). **Confounding (Diabetes):** Age, sex, education, smoking (ever vs. never), alcohol (ever vs. never), BMI, hypertension, total cholesterol, regular exercise **Confounding (Other):** Age, sex, education, smoking, alcohol, BMI, hypertension, total cholesterol, regular exercise						
Sun et al. (2018) *Medium*	United States, recruitment 1989, blood sample	Case-control	Female nurses drawn from the Nurses' Health	Plasma Cases: 35.7 Controls:	T2D hemoglobin, insulin, adiponectin	Regression coefficient SD log10-unit	T2D Per SD increase: 1.15 (0.98, 1.35), p-value = 0.008 OR for T2: 1.63 (1.25, 2.12)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	collection 1995–2000, outcome assessed during biennial follow-up through June 2011		Study II cohort study, N = 1,586	33.1		increase in PFOS OR by tertiles	OR for T3: 1.62 (1.09, 2.41) Partial Spearman correlation coefficient for hemoglobin, insulin, and adiponectin: No statistically significant association
	Results: Lowest tertile used as reference. **Confounding:** Age, month of sample collection, fasting status, menopausal status, postmenopausal hormone use, family history of diabetes, oral contraceptive use, breastfeeding duration at blood draw, number of children delivered after 1993, states of residence, smoking status, alcohol intake, physical activity, baseline BMI, and Alternative Healthy Eating Index (AHEI) score						
Chen et al. (2019a) *Medium* for metabolic syndrome *Low* for all other outcomes	Croatia 2007–2008	Cross-sectional	Residents of Hvar ages 44–56 yr N = 122	Plasma GM = 8.91 (Range: 2.36–33.67)	BMI, fasting insulin (µIU/mL), fasting plasma glucose (mmol/L), glycated HbA1c (%), hip circumference (cm), homeostatic model assessment of beta-cell function (HOMA-β), homeostatic model assessment of insulin resistance (HOMA-IR), metabolic syndrome defined by the ATP III criteria,	Metabolic syndrome: OR per ln-unit increase in PFOS All other outcomes: regression coefficient per ln-unit increase in PFOS	Metabolic syndrome: 1.89 (0.93, 3.86); p-value = 0.08 All other outcomes: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					waist circumference (cm)		
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity						

Notes: AHEI = Alternative Healthy Eating Index; ALSPAC = Avon Longitudinal Study of Parents and Children; AUC = area under the curve; BMI = body mass index; BMIZ = BMI z-score; DM = diabetes mellitus; DPP = Diabetes Prevention Program; EYHS = European Youth Heart Study; GDM = gestational diabetes mellitus; GM = geometric mean; GWG = gestational weight gain; HAZ = height-for-age z-score; HbA1c = Hemoglobin A1c; HOMA-Beta = homeostatic model assessment of β-cell function; HOMA-IR = homeostatic model assessment for insulin resistance; HOME = Health Outcomes and Measures of the Environment; hr = hour; IGF = insulin-like growth factor; INMA = INfancia y Medio Ambiente (Environment and Childhood) Project; IQR = interquartile range; IR = insulin resistance; KorEHS-C: Korea Environmental Health Survey in Children and Adolescents; LSM = least square mean; min = minutes; MIREC = Maternal-Infant Research on Environmental Chemicals; mo = months; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; OW = overweight; PIR = poverty income ratio; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; POUNDS = Preventing Overweight Using Novel Dietary Strategies; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RMR = resting metabolic rate; RR = risk ratio; SD = standard deviation; SE = standard error; SOLAR = Study of Latino Adolescents at Risk of Type 2 Diabetes; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; T1D = type 1 diabetes; T2D = type 2 diabetes; vs. = versus; WC = waist circumference; wk = weeks; yr = years.

[a] Exposure levels are reported as median in ng/mL unless otherwise noted.

[b] Results are reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.8 Nervous

Table D-17. Associations Between PFOS Exposure and Neurological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Harris et al. (2018) *High*	United States, Recruitment: 1999–2002; Follow-up at early- and mid-childhood	Cohort	Pregnant women and their children from Project Viva N = 853	Plasma Maternal: 24.9 (18.4–34.4) Child: 6.2 (4.2–9.7)	Both age groups: Wide Range Assessment of Visual Motor Abilities (WRAVMA) score	Mean difference by quartiles of PFOS exposure	Visual-Motor Mid-childhood (maternal plasma) Q2: −1.6 (−4.7, 1.6) Q3: −1.4 (−4.7, 1.8) Q4: −3.2 (−6.6, 0.2) Mid-childhood (child plasma) Q2: −1.6 (−5.5, 2.2) Q3: −4.6 (−8.7, −0.5) Q4: −2.0 (−6.3, 2.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Early childhood only: Peabody Picture Vocabulary Test (PPVT-III) score Mid-childhood only: Kaufman Brief Intelligence Test Second Edition (KBIT-2) nonverbal and verbal IQ, (WRAML2) design memory and picture memory		Nonverbal IQ Mid-childhood (maternal plasma) Q2: −0.7 (−3.8, 2.3) Q3: −1.8 (−5.0, 1.4) Q4: 1.6 (−1.8, 4.9) Mid-childhood (child plasma) Q2: −0.4 (−4.0, 3.2) Q3: 1.6 (−2.3, 5.4) Q4: −0.1 (−4.1, 3.8) Verbal IQ Mid-childhood (maternal plasma) Q2: −2.1 (−4.5, 0.2) Q3: −1.7 (−4.2, 0.7) Q4: 0.8 (−1.8, 3.4) Mid-childhood (child plasma) Q2: 0.9 (−2, 3.8) Q3: −0.4 (−3.4, 2.7) Q4: −0.2 (−3.4, 3.0) Design memory Mid-childhood (maternal plasma) Q2: −0.1 (−0.7, 0.4) Q3: 0.3 (−0.3, 0.8) Q4: 0.6 (0, 1.2) Mid-childhood (child plasma) Q2: 0.1 (−0.5, 0.7) Q3: 0.1 (−0.6, 0.7) Q4: −0.2 (−0.9, 0.5) Picture memory Mid-childhood (maternal plasma) Q2: −0.3 (−0.9, 0.2) Q3: −0.1 (−0.7, 0.5) Q4: 0.4 (−0.2, 1.0) Mid-childhood (child plasma)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q2: −0.1 (−0.8, 0.5) Q3: 0.1 (−0.6, 0.9) Q4: 0 (−0.7, 0.8) Early childhood: No statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Year of pregnancy blood collection gestational age at time of pregnancy blood collection, estimated glomerular filtration rate at blood draw, maternal race/ethnicity, age, education, KBIT-2 score, pre-pregnancy BMI, smoking status, paternal education, annual household income in mid-childhood, HOME-SF score, child's sex and age at mid-childhood cognitive testing, proxy for breastfeeding of a prior child[c]						
Niu et al. (2019) *High*	China, Recruitment: 2012; Follow-up at age 4 yr	Cohort	Pregnant women and their children from the Shanghai-Minhang Birth Cohort N = 533 (236 Females; 297 Males)	Maternal serum 10.8 (7.6–15.8)	ASQ-3 skill scales: communication, gross motor, fine motor, problem solving, personal-social	RR per ln-unit increase in PFOS and by tertiles	Communication Overall: 1.01 (0.77, 1.34) Females: 1.04 (0.65, 1.68) T2: 0.52 (0.26, 1.04); p-value <0.10 T3: 1.10 (0.63, 1.92) Males: 1.00 (0.70, 1.44) T2: 1.16 (0.76, 1.77) T3: 0.89 (0.53, 1.51) p-value for interaction by sex = 0.350 Gross Motor 1.22 (0.79, 1.89) No statistically significant associations, trends, or interactions by sex Fine Motor Overall: 1.25 (0.79, 1.96) No statistically significant associations, trends, or interactions by sex Problem Solving Overall: 1.02 (0.71, 1.47) Females: 1.16 (0.63, 2.15)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							T2: 0.55 (0.15, 2.07) T3: 2.00 (0.77, 5.17) Males: 0.93 (0.59, 1.47) T2: 1.21 (0.65, 2.28) T3: 0.66 (0.29, 1.48) p-value for interaction by sex = 0.010 Personal-Social Skills Overall: 1.34 (0.91, 1.96) Females: 2.56 (1.2, 5.45) T2: 0.32 (0.04, 2.77) T3: 2.97 (0.90, 9.84); p-value < 0.10 p-trend < 0.10 Males: 1.05 (0.67, 1.64) T2: 1.47 (0.76, 2.84) T3: 1.18 (0.57, 2.44) p-value for interaction by sex = 0.039
	Outcome: Neuropsychological problems defined as scores ≤ 10th percentile. **Results:** Lowest tertile used as reference **Confounding:** Maternal age at enrollment, pre-pregnancy BMI, maternal education, paternal education, parity, per capita household income, maternal passive smoking, maternal prenatal depressive symptoms, gestational age, child's sex						
Oulhote et al. (2016) *High*	Faroe Islands, Recruitment: 1997–2000, Follow-up at ages 5 and 7	Cohort	Children at 5 yr (N = 508) and 7 yr (N = 491)	Serum Maternal: 27.35 (23.19–33.13) 5 yr: 16.78 (13.52–21.05) 7 yr: 15.26 (12.38–18.99)	Strengths and Difficulties Questionnaire (SDQ) scores: Total score (hyperactivity/inattention, conduct problems, peer relationship problems, emotional	Mean difference (autism, internalizing, externalizing, total) or mean ratio (hyperactivity/inattention, conduct, peer relationship, emotional, prosocial) per	SDQ total score Prenatal: 0.46 (−0.78, 1.7), p-value = 0.47 5-yr serum: 0.51 (−0.5, 1.52), p-value = 0.32 7-yr serum: 0.18 (−0.95, 1.31), p-value = 0.76 Hyperactivity/Inattention Prenatal: 1.03 (0.80, 1.31), p-value = 0.84

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					symptoms), prosocial behavior, internalizing problem, externalizing problems, autism screening (peer problems minus prosocial)	doubling of PFOS	5-yr serum: 1.05 (0.86, 1.29), p-value = 0.64 7-yr serum: 0.88 (0.70, 1.11), p-value = 0.27 Conduct Prenatal: 1.03 (0.81, 1.32), p-value = 0.80 5-yr serum: 1.00 (0.81, 1.23), p-value = 0.98 7-yr serum: 1.01 (0.80, 1.26), p-value = 0.95 Peer Relationship Prenatal: 1.31 (0.87, 1.96), p-value = 0.19 5-yr serum: 1.28 (0.91, 1.80), p-value = 0.15 7-yr serum: 1.17 (0.82, 1.69), p-value = 0.39 Emotional Prenatal: 1.10 (0.84, 1.44), p-value = 0.49 5-yr serum: 1.14 (0.90, 1.45), p-value = 0.26 7-yr serum: 1.22 (0.94, 1.58), p-value = 0.13 Prosocial Prenatal: 1.00 (0.91, 1.09), p-value = 0.96 5-yr serum: 0.98 (0.91, 1.06), p-value = 0.70 7-yr serum: 1.01 (0.92, 1.10), p-value = 0.88

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Internalizing Prenatal: 0.35 (−0.35, 1.05), p-value = 0.32 5-yr serum: 0.44 (−0.15, 1.02), p-value = 0.15 7-yr serum: 0.48 (−0.16, 1.13), p-value = 0.14 Externalizing Prenatal: 0.11 (−0.68, 0.89), p-value = 0.79 5-yr serum: 0.08 (−0.58, 0.73), p-value = 0.82 7-yr serum: −0.31 (−1.03, 0.42), p-value = 0.41 Autism screening Prenatal: 0.2 (−0.37, 0.77), p-value = 0.49 5-yr serum: 0.33 (−0.14, 0.8), p-value = 0.17 7-yr serum: 0.06 (−0.46, 0.58), p-value = 0.82
	Confounding: Age, sex, maternal age, pre-pregnancy BMI, parity, socioeconomic status, alcohol, and smoking during pregnancy						
Braun et al. (2014) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 4–5 yr	Cohort	Pregnant women and their children from the HOME study N = 175 (80 Females; 95 Males)	Maternal Serum 13 (9.3–18)	Social Responsiveness Scale (SRS) total score	Regression coefficient per log10-unit/2SD increase in PFOS	SRS 2.1 (0.2, 3.9) Females: 0.9 (−1.5, 3.3) Males: 3.8 (1.3, 6.3) p-value for interaction by sex = 0.08
	Confounding: Maternal race, maternal age, maternal education, marital status, annual household income, maternal depressive symptoms, maternal IQ, child sex, caregiving environment score, maternal serum						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Chen et al. (2013) *Medium*	Taiwan, Recruitment: 2004–2005; Follow-up at age 2 yr	Cohort	Pregnant women and their children from the Taiwan Birth Panel Study N = 239	Cord blood Mean = 7.0 (SD = 5.8)	CDI skill quotients: cognitive, fine motor, gross motor, language, self-help, social, whole test	Regression coefficient per IQR increase in ln-transformed PFOS	Cognitive: −0.8 (−2.8, 1.1) Fine Motor: −1.8 (−3.8, 0.1) Gross Motor: −3.7 (−6.0, −1.5) Language: −0.9 (−2.9, 1.2) Self Help: −2.2 (−4.8, 0.3) Social: −1.0 (−3.7, 1.6) Whole Test: −2.1 (−4.1, −0.2)
	Confounding: Maternal education, family income, infant sex and gestational age, breastfeeding, HOME score at 24 mo of age, cord blood cotinine levels, postnatal environmental tobacco smoke exposure						
Ghassabian et al. (2018) *Medium*	United States, 2008–2010	Cohort	Children aged 7 yr from Upstate KIDS Study N = 788	Blood 1.74 (IQR = 1.33)	SDQ scores: total behavioral difficulties–total score, borderline problems; hyperactivity, conduct, peer, or emotional problems; difficulties in prosocial behavior	Regression coefficient (total behavioral difficulties, problem scores) and OR (borderline behavioral difficulties, problem scores, difficulties in prosocial behavior) per log-SD increase in PFOS and by quartiles	Total Behavioral Difficulties (β) 0.04 (−0.02, 0.10) Q2: 0.14 (−0.01, 0.28) Q3: 0.04 (−0.11, 0.19) Q4: 0.17 (0.01, 0.32) Conduct problems (OR) 1.22 (0.97, 1.52) Q2: 1.78 (0.97, 3.27) Q3: 0.86 (0.43, 1.74) Q4: 2.22 (1.18, 4.15) Conduct problems (β) 0.02 (−0.08, 0.13) Q2: 0.14 (−0.10, 0.39) Q3: −0.07 (−0.33, 0.19) Q4: 0.19 (−0.07, 0.46) Emotional problems (OR) 1.31 (1.04, 1.63) Q2: 2.08 (1.13, 3.80) Q3: 0.89 (0.47, 1.68) Q4: 2.28 (1.24, 4.18) Emotional problems (β) 0.09 (0, 0.18) Q2: 0.24 (0.03, 0.45) Q3: 0.01 (−0.20, 0.22)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: 0.27 (0.05, 0.49) Borderline Behavioral Difficulties (OR) 1.30 (1.03, 1.65) Q2: 1.67 (0.84, 3.34) Q3: 1.73 (0.87, 3.43) Q4: 2.47 (1.29, 4.72) Difficulties in Prosocial Behavior (OR) 1.26 (0.92, 1.72) Q2: 0.86 (0.35, 2.15) Q3: 1.72 (0.65, 4.52) Q4: 1.87 (0.70, 4.98) Hyperactivity problems, peer problems: No statistically significant associations
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as reference. **Confounding:** Child's age and sex, maternal age, pre-pregnancy BMI, race/ethnicity, education, marital status, history of smoking in pregnancy, having private insurance, parity, and infertility treatment						
Goudarzi et al. (2016b) *Medium*	Japan, 2002–2005	Cohort	Pregnant women and their infants at 6 and 18 mo from the Hokkaido Study on Environment and Children's Health N = 173 (90 Females; 83 Males)	Maternal serum 5.7 (4.4–7.4)	Bayley Scales of Infant Development, Second Edition (BSID-II) Mental Development Index (MDI), Psychomotor Development Index (PDI)	Regression coefficient log10-unit increase in PFOS	MDI 6 Months: 0.018 (−4.52, 5.59) Females: 0.072 (−5.19, 9.38) Males: −0.141 (−11.26, 3.45) 18 Months: 0.052 (−9.91, 16.66) PDI 6 Months: 0.039 (−6.38, 10.37) Females: 0.031 (−11.66, 15.09) Males: 0.120 (−5.24, 15.60) 18 Months: −0.023 (−13.45, 10.72)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Gestational age, parity, maternal age, smoking during pregnancy, alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education level, blood sampling period, breast feeding, total dioxin levels						
Jeddy et al. (2017) *Medium*	Great Britain. Recruitment: 1991–1992; Follow-up at ages 15 and 18 mo	Cohort	Mothers and daughters aged 15 and 38 mo from ALSPAC N = 353	Maternal serum 19.8 (15.0–24.95)	MacArthur Communicative Development Inventories (MCDI): communicative, intelligibility, language, nonverbal communication, social development, verbal comprehension, and vocabulary comprehension scores	Regression coefficient per unit increase in PFOS	Nonverbal, 15 mo.: 0.02 (−0.01, 0.05) Social, 15 mo.: 0.02 (−0.03, 0.08) Verbal, 15 mo.: 0.03 (0.01, 0.05) Maternal age ≤ 30: No statistically significant associations Maternal age > 30: 0.04 (0.01, 0.08) Vocabulary, 15 mo.: 0.02 (−0.39, 0.44) Communicative, 38 mo.: 0 (−0.01, 0.01) Intelligibility, 38 mo.: −0.01 (−0.01, 0) Maternal age < 25: 0.02 (0.01, 0.03) Maternal age ≥ 25: No statistically significant associations Language, 38 mo.: −0.29 (−0.54, −0.05) Nonverbal, social, vocabulary, communicative, language: No statistically significant associations stratified by maternal age at delivery
	Confounding: Parity, maternal age, maternal education, maternal smoking status, gestational age at sample collection, total maternal Crown-Crisp Experiential Index						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Liew et al. (2015) *Medium*	Denmark, Recruitment: 1996–2002; Follow-up at average age 10.7 yr	Case-control	Mother-child pairs from Danish National Birth Cohort 215 Cases (39 Females; 176 Males) 545 Controls (33 Females; 180 Males)	Maternal plasma Cases: 25.40 (18.73–32.40) Controls: 27.40 (20.40–35.60)	ADHD, ASD	RR and OR (stratified by quartile or by sex) per ln-unit increase in PFOS or by quartiles	ADHD: 0.87 (0.74, 1.02) Q4: 0.79 (0.64, 0.98) ASD: 0.92 (0.69, 1.22) No other statistically significant associations by quartiles or by sex
	Results: Lowest quartile used as reference **Confounding:** Maternal age at delivery, SES, parity, smoking and drinking during pregnancy, psychiatric illnesses, gestational week of blood drawn, child's sex, birth year						
Liew et al. (2018) *Medium*	Denmark, Recruitment: 1996–2002; Follow-up at age 5 yr	Cohort	Pregnant women and their children from the Danish National Birth Cohort N = 1,592	Maternal plasma 28.10 (21.60–35.80)	Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) full-scale IQ, performance IQ, verbal IQ	Regression coefficient for mean difference per ln-unit increase in PFOS and by quartiles	Full-Scale IQ Q2: −0.4 (−3.2, 2.5) Q3: 1.1 (−1.8, 4.0) Q4: −0.5 (−3.5, 2.6), p-trend = 0.87 Performance IQ Q2: 0.6 (−2.3, 3.5) Q3: 1.6 (−1.2, 4.5) Q4: −0.1 (−3.1, 2.8), p-trend = 0.93 Verbal IQ Q2: −1.0 (−3.9, 1.9) Q3: −0.2 (−3.3, 2.9) Q4: −0.7 (−3.9, 2.4), p-trend = 0.76
	Results: Lowest quartile used as reference. **Confounding:** Maternal age at childbirth, parity, maternal socioeconomic status, maternal IQ, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal pre-pregnancy BMI, gestational week of blood draw						
Long et al. (2019) *Medium*	Denmark, Recruitment: 1982–1999; Follow-Up: 1993–2009	Case-control	Pregnant women and their children from the Historic Birth Cohort at	Amniotic fluid Cases: 0.61 (Range: 0.61–2.98)	ASD	OR per unit increase in PFOS	0.410 (0.174, 0.967), p-value = 0.042 Females: 0.027 (0, 4.755), p-value = 0.171 Males: 0.586 (0.192, 1.782), p-value = 0.346

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Statens Serum Institute 37 Cases (7 Females; 29 Males) 50 Controls (15 Females; 35 Males)	Controls: 1.44 (Range: 0.61–4.22)			
	Confounding: Child's birth year, child sex, mother's age at delivery, father age at childbirth, birth weight, gestational week at sampling, gestational age at birth, Apgar score, parity, congenital malformation						
Lyall et al. (2018) *Medium*	United States, 2007–2009	Case-control	Children and adolescents aged 4.5–9 yr from EMA study 985 (553 Cases; 432 Controls)	Maternal serum Cases: GM = 17.5 (95% CI = 16.8–18.3) Controls: GM = 17.9 (95% CI = 17.0–18.7)	ASD measured by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR), intellectual disability	OR per ln-unit increase in PFOS and by quartiles	ASD: 0.77 (0.58, 1.01) Q2: 0.85 (0.58, 1.23) Q3: 0.74 (0.50, 1.09) Q4: 0.64 (0.43, 0.97), p-trend = 0.03 Intellectual Disability: 0.67 (0.45, 0.98) Q2: 0.61 (0.36, 1.05) Q3: 0.80 (0.46, 1.38) Q4: 0.59 (0.32, 1.09), p-trend = 0.17
	Results: Lowest quartile used as reference. **Confounding:** Matching factors, parity, maternal age, race/ethnicity, weight at sample collection, and maternal birthplace						
Oulhote et al. (2019) *Medium*	Faroe Islands, Recruitment: 1997–2000; Follow-up at age 7 yr	Cohort	Children N = 419	Blood Maternal: 27.69 (23.22–33.35) 5 Years: 16.8 (13.5–21.13)	Boston Naming Test with and without cues, SDQ total score	Regression coefficient per IQR increase in PFOS	Boston Naming Test With Cues Prenatal: −0.11 (−0.27, 0.01) 5-yr serum: 0.00 (−0.08, 0.07) Without Cues Prenatal: −0.04 (−0.19, 0.06) 5-yr serum: 0.00 (−0.06, 0.06) SDQ Prenatal: 0.15 (0.08, 0.23) 5-yr serum: 0.02 (−0.03, 0.08)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: None reported						
Quaak et al. (2016) *Medium*	Netherlands, Recruitment: 2011–2013; Follow-up through age 18 mo	Cohort	Pregnant women and their children from the LINC cohort 54 (20 Females; 34 Males)	Cord blood 1,600.0 ng/L (Range: 570–3,200 ng/L)	Child Behavior Checklist 1.5–5 (CBCL 1.5–5) measures of ADHD, externalizing behavior	Regression coefficient by tertiles	ADHD T2: −0.33 (−1.75, 1.17), p-value = 0.66 T3: −0.87 (−2.06, 0.42), p-value = 0.19 Females T2: 0.17 (−1.50, 1.67), p-value = 0.85 T3: −0.73 (−2.36, 0.90), p-value = 0.43 Males T2: −0.55 (−2.84, 1.57), p-value = 0.64 T3: −0.99 (−3.03, 0.92), p-value = 0.35 Externalizing Behavior T2: −1.23 (−5.68, 3.85), p-value = 0.62 T3: −2.43 (−6.55, 1.93), p-value = 0.31 Females T2: −2.63 (−8.21, 4.33), p-value = 0.44 T3: −2.98 (−8.08, 2.23), p-value = 0.31 Males T2: 0.72 (−5.77, 6.59), p-value = 0.81 T3: −0.94 (−6.72, 5.12), p-value = 0.74
	Results: Lowest tertile used as reference. **Confounding:** Alcohol use, smoking, family history of ADHD, education						
Shin et al. (2020)	United States,	Case-Control	Mother-child pairs from	Maternal serum 5.81 (3.86–9.11)	ASD measured by Autism	OR per increase (ln-transformed	By modeled prenatal exposure ln-transformed: 1.18 (0.77, 1.80)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium	Recruitment: 2002–2009; Follow-up: 2009–2017		CHARGE with children aged 2–5 yr N = 453 (239 Cases; 214 Controls; 88 Females; 365 Males)		Diagnostic Interview-Revised (ADI-R)	or linear scale) in modeled, maternal, prenatal PFOS or measured, maternal, postnatal PFOS and by quartiles	No statistically significant associations or interactions by sex Linear: 1.03 (0.99, 1.08); p-value < 0.10 Females: 0.96 (0.85, 1.08) Males: 1.05 (1.00, 1.10), p-value < 0.05 Interaction p-value = 0.38 By measured postnatal levels ln-transformed: 1.21 (0.80, 1.83) Linear: 1.05 (0.97, 1.13); p-value < 0.10 No statistically significant associations or trends by quartiles
	Confounding: Child's age, child's sex, regional center, child's birth year, parity, gestational age at delivery, maternal race/ethnicity, maternal birthplace, mother's age at delivery, maternal pre-pregnancy BMI, periconceptional maternal vitamin intake, homeownership, breastfeeding duration						
Skogheim et al. (2019) *Medium*	Norway, Recruitment: 1999–2008; Follow-up: 2007–2011	Cohort	Mother-child pairs from MoBa N = 943	Maternal plasma 11.51 (8.77–14.84)	Nonverbal and Verbal Working Memory measured by Stanford Binet Intelligence Scales	Regression coefficient per unit increase in PFOS and by quintiles	Nonverbal Working Memory Q2: 0.06 (−0.14, 0.26) Q3: −0.10 (−0.30, 0.10) Q4: −0.02 (−0.22, 0.18) Q5: −0.26 (−0.48, −0.06) Verbal Working Memory Q2: −0.05 (−0.27, 0.17) Q3: 0.09 (−0.14, 0.31) Q4: 0.10 (−0.12, 0.33) Q5: −0.01 (−0.24, 0.22)
	Results: Lowest quintile used as reference. **Confounding:** Maternal education, age, parity, fish intake, child sex, child age at testing, maternal ADHD symptoms						
Spratlen et al. (2020a) *Medium*	United States, Recruitment: 2001–2001;	Cohort	Pregnant women and their children	Cord blood GM = (Range:)	BSID-II scores: MDI and PDI), Full IQ,	Regression coefficient of mean difference	MDI Year 1: −0.61 (−3.17, 1.95) Year 2: 2.36 (−1.23, 5.94)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up at age 1, 2, and 3 yr		from the Columbia University Birth Cohort N = 302 (150 Females; 152 Males)		Performance IQ, Verbal IQ	per log-unit increase in maternal PFOS	Females: 5.52 (0.64, 10.4) Males: −1.35 (−7.09, 4.39) Interaction p-value = 0.04 Year 3: 1.96 (−1.24, 5.16) PDI Year 1: −0.07 (−4.56, 4.43) Year 2: −1.34 (−4.26, 1.57) Year 3: −0.55 (−5.34, 4.23) Full IQ Year 4: −0.41 (−4.25, 3.43) Year 6: 2.81 (−1.84, 7.46) Performance IQ Year 4: −0.05 (−4.56, 4.46) Year 6: 2.81 (−2.29, 7.91) Verbal IQ Year 4: −0.19 (−4.50, 4.12) Year 6: 2.67 (−2.56, 7.90) No other statistically significant associations or interactions by sex
	Comparison: Logarithm base not specified. **Confounding:** Maternal age, material hardship, parity, pre-pregnancy BMI, maternal IQ, maternal race, maternal education, family smoking status, child age at testing, child's gestational age at birth, maternal demoralization, trimester on 9/11, child's sex, child's breastfeeding history						
Strøm et al. (2014) *Medium*	Denmark Recruitment: 1988–1999 Follow-up: 2010	Cohort	Pregnant women and their children, from the DaFO88 cohort N = 876	Maternal serum Median = 21.4 (IQR = 9.0)	Depression, ADHD, scholastic achievement	Depression, ADHD: Hazard ratio (depression and ADHD) by tertile Scholastic achievement: Regression	Depression T2: 1.61 (0.99, 2.61) T3: 1.16 (0.69, 1.95) p-value = 0.14 ADHD T2: 1.05 (0.43, 2.53) T3: 0.54 (0.19, 1.53) p-value = 0.38

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						coefficient per unit increase in PFOS and by tertiles	Scholastic Achievement: −0.01 (−0.03, 0.01), p-value = 0.57 T3: −0.11 (−0.50, 0.28), p-trend = 0.59
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, parity, maternal smoking during pregnancy, maternal education, maternal cholesterol, maternal triglycerides, offspring sex						
Vuong et al. (2016) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 5 and 8 yr	Cohort	Children ages 5 and 8 yr from the HOME study N = 218	Serum 13.2 (8.8–17.8)	BRIEF measures of behavioral regulation, metacognition, global executive composite indices, inhibit, shift, emotional control, working memory, plan/organize, initiate, organization of materials, monitor	All outcomes: OR for score ≥ 60 per unit increase in PFOS Index and compositive scores only: Regression coefficient per ln-unit increase in PFOS and by quartiles	Behavioral Regulation: 3.14 (0.68, 5.61) Metacognition: 3.10 (0.62, 5.58) Global Executive Function: 3.38 (0.86, 5.90) No statistically significant interactions by age; no statistically significant trends by quartiles Inhibit: 2.59 (1.23, 5.41) Shift: 1.50 (0.72, 3.11) Emotional control: 1.97 (0.84, 4.64) Working memory: 1.87 (1.01, 3.48) Plan/organize: 3.54 (1.65, 7.60) Initiate: 1.89 (0.80, 4.45) Organization: 1.84 (0.82, 4.13) Monitor: 3.39 (1.42, 8.08)
	Confounding: Maternal age, race, education, income, maternal serum cotinine, maternal depression, HOME score, maternal IQ, marital status, child sex						
Vuong et al. (2018b) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 3 and 8 yr	Cohort	Children from the HOME study N = 204	Serum 3 yr: 6.2 (4.5–10.0) 8 yr: 3.6 (2.7–4.9)	BRIEF measures of behavioral regulation, metacognition, global executive	OR per ln-unit increase in PFOS	Behavioral Regulation 3 yr: 0.66 (0.29, 1.51) 8 yr: 0.40 (0.14, 1.14) Metacognition 3 yr: 0.83 (0.42, 1.63)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					composite indices		8 yr: 1.53 (0.67, 3.52) Global Executive Function 3 yr: 0.95 (0.45, 2.01) 8 yr: 1.04 (0.41, 2.68)
	Confounding: Maternal age, race/ethnicity, household income, maternal smoking status, maternal alcohol consumption, maternal depression, HOME Score, marital status, maternal marijuana use, maternal IQ, maternal serum PCBs, maternal blood lead levels, child sex						
Vuong et al. (2018a) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 3 and 8 yr	Cohort	Mother-child dyads from the HOME study 204	Serum Prenatal: 12.9 (8.8–17.6) 3 yr: 6.2 (4.5–9.9) 8 yr: 3.6 (2.7–4.8)	Conners' Continuous Performance Test-II commissions t-score, omissions t-score, hit reaction time, tau (ms) Virtual Morris Water Maze (VMWM) scores for visual-spatial learning distance (pool units), learning time (s), memory retention distance (%), and memory retention time (s)	Regression coefficient per ln-unit increase in PFOS	Conners' Commissions Prenatal: −0.1 (−2.0, 1.8) 3 Years: 1.0 (−1.5, 3.5) 8 Years: 1.3 (−1.0, 3.6) Omissions Prenatal: −0.8 (−5.2, 3.5) 3 Years: −0.1 (−4.4, 4.2) 8 Years: −0.8 (−5.3, 3.8) Females: 4.3 (−1.2, 9.9) Males: −7.3 (−13.0, −1.7) Hit reaction time Prenatal: −1.5 (−4.2, 1.2) 3 yr: −0.4 (−3.2, 2.5) 8 yr: −2.5 (−6.0, 1.1) Tau Prenatal: 6.0 (−23.2, 35.2) 3 yr: 13.4 (−9.8, 36.5) 8 yr: 5.8 (−22.1, 33.7) Visual-spatial scores (VMWM) Learning distance Prenatal: 0.2 (−1.6, 1.7) 3 yr: −0.7 (−2.2, 0.7) 8 yr: −0.2 (−1.7, 1.3) Learning time Prenatal: −0.1 (−2.8, 2.6) 3 yr: −1.1 (−3.5, 1.2) 8 yr: −2.1 (−4.9,0.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Memory retention distance Prenatal: 2.8 (−1.3, 6.8) 3 yr: 0.3 (−4.7, 5.4) 8 yr: 2.1 (−2.9, 7.0) Memory retention time Prenatal: 0.4 (−1.1, 1.9) 3 yr: −0.4 (−2.1, 1.3) 8 yr: 0.5 (−1.3, 2.3)
	Confounding: Maternal age, race/ethnicity, household income, maternal smoking status, maternal alcohol consumption, maternal depression, HOME Score, marital status, maternal marijuana use, maternal IQ, maternal serum ΣPCBs, maternal blood lead levels, child sex						
Vuong et al. (2019) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 3 and 8 yr	Cohort	Pregnant women and their children from the HOME study N = 221	Serum Maternal: GM = 12.4 8 Years: GM = 3.9	Wechsler Intelligence Scale for Children–Fourth Edition (WISC-IV): full-scale IQ, perceptual reasoning, processing speed, verbal comprehension, working memory	Regression coefficient per ln-unit increase in PFOS	Full-Scale IQ Prenatal: 2.2 (−0.9, 5.2) 3 Years: 0.8 (−2.4, 4.0) 8 Years: 1.6 (−2.7, 5.8) Perceptual Reasoning Prenatal: 1.4 (−1.8, 4.7) 3 Years: 1.0 (−2.6, 4.5) 8 Years: 2.8 (−2.1, 7.7) Processing Speed Prenatal: 1.3 (−2.0, 4.7) 3 Years: 1.6 (−1.9, 5.1) 8 Years: 3.7 (−1.2, 8.5) Verbal Comprehension Prenatal: 1.4 (−1.7, 4.5) 3 Years: 0.1 (−3.3, 3.5) 8 Years: −1.7 (−5.2, 1.8) Working Memory Prenatal: 2.6 (−0.8, 5.9) 3 Years: −0.1 (−3.4, 3.2) 8 Years: 2.9 (−0.8, 6.5)
	Confounding: Maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum ΣPCBs and cotinine, maternal depression, vitamin use, maternal IQ, marital status, HOME score, child sex, breastfed						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Vuong et al. (2020a) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 8 yr	Cohort	Mother-child pairs with children aged 8 yr from the HOME study N = 161	Maternal serum Mean = 13.9 (SD = 7.9)	Wide Range Achievement Test 4 (WRAT-4) reading composite score	Regression coefficient per log10-unit increase in PFOS	7.0 (−2.9, 16.9)
	Confounding: Maternal age, race/ethnicity, education, household income, marital status, maternal depression, maternal serum cotinine, maternal blood lead levels, maternal fish consumption, maternal IQ, child sex, HOME score						
Wang et al. (2015b) *Medium*	Taiwan, Recruitment: 2000–2001; Follow-up at ages 5 yr	Cohort	Pregnant women and their children aged 5 and 8 yr from TMICS N = 120	Serum 5 Years: 13.25 (9.75–17.50) 8 Years: 12.28 (9.50–16.30)	Full-Scale IQ, Performance IQ, Verbal IQ	Regression coefficient per log2-unit increase in PFOS	Full-Scale IQ 5 Years: −1.9 (−4.3, 0.5) 8 Years: −1.9 (−4.3, 0.4) Performance IQ 5 Years: −2.2 (−4.7, 0.3) 8 Years: −1.6 (−4, 0.7) Verbal IQ 5 Years: −1.7 (−4, 0.7) 8 Years: −1.3 (−3.6, 1.1)
	Confounding: Maternal education, family annual income, children's age, sex, HOME score at IQ assessment						
Zhang et al. (2018a) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 3, 5, and 7 yr	Cohort	Pregnant women and their children aged 3, 5, and 7 yr from the HOME study N = 167	Serum Maternal: 13.0 (9.1–17.8) 3 yr: 6.6 (4.6–10.2) 8 yr: 3.6 (2.7–4.9)	Basic reading, brief reading, letter word identification, passage comprehension measured by Woodcock Johnson Test of Achievement-III (WJ-III) Reading composite, word reading,	Regression coefficient per ln-unit increase PFOS	Basic Reading Maternal Serum: 3.2 (−2.0, 8.3) Year 3 Serum: 1.1 (−4.8, 7.0) Brief Reading Maternal Serum: 2.9 (−2.2, 8.1) Year 3 Serum: 3.2 (−2.6, 9.1) Letter Word Identification Maternal Serum: 2.0 (−2.7, 6.8) Year 3 Serum: 2.1 (−3.4, 7.5) Passage Comprehension Maternal Serum: 1.7 (−1.9, 5.3) Year 3 Serum: 3.5 (−0.5, 7.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					sentence Comprehension measured by Wide Range Achievement Test 4 (WRAT-4)		Word Attack Maternal Serum: 4.1 (−1.2, 9.5) Year 3 Serum: 2.8 (−2.8, 8.4) Reading Composite Maternal Serum: 3.1 (−1.3, 7.5) Year 3 Serum: 1.6 (−3.1, 6.4) Year 8 Serum: 2.6 (−1.7, 6.9) Word Reading Maternal Serum: 3.1 (−1.0, 7.3) Year 3 Serum: −0.3 (−4.8, 4.3) Year 8 Serum: 4.4 (0.3, 8.4) Sentence Comprehension Maternal Serum: 3.2 (−1.8, 8.2) Year 3 Serum: 2.5 (−3.1, 8.1) Year 8 Serum: 1.6 (−3.3, 6.5)
	Confounding: Maternal age, race, education, household income, parity, smoking (serum cotinine concentration), maternal IQ, breastfeeding duration, HOME score						
			General Population				
Ding and Park (2020) *Medium*	United States, 2003–2016	Cross-sectional	Adults aged 20–69 yr from NHANES N = 2,731	Serum 6.2 (3.5–10.5)	High and low frequency hearing impairment (HFHI and LFHI)	OR per log2-unit increase in PFOS and for ≥ 90th percentile vs. < 90th percentile	HFHI OR (per doubling): 0.96 (0.85, 1.10) OR (90th percentiles): 1.31 (0.75, 2.27) LFHI OR (per doubling): 0.87 (0.73, 1.03) OR (90th percentiles): 0.72 (0.29, 1.75)
	Confounding: Age, age square, sex, race/ethnicity, education level, PIR, smoking status, BMI, noise exposures (occupational, recreational, firearm noise), NHANES cycles						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Gallo et al. (2013) *Medium*	United States, 2005–2006	Cross-sectional	Adults aged 50+ years from the C8 Health Project N = 21,024	Serum Range = 0.25–759.2	Memory impairment (self-reported)	OR per doubling of PFOS and by quintiles	0.93 (0.90, 0.96) Q2: 0.96 (0.87, 1.07) Q3: 0.86 (0.78, 0.96) Q4: 0.87 (0.78, 0.96) Q5: 0.85 (0.76, 0.94) p-trend < 0.001
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as reference. **Confounding:** Age, ethnicity, gender and school level, household income, physical activity, alcohol consumption, cigarette smoking						
Lenters et al. (2019) *Medium*	Norway, Recruitment: 2003–2009; Follow-up: 2008–2016	Cohort	Children and adults from HUMIS N = 1,199	Breast milk 117.732 ng/L (80.000–160.000 ng/L)	ADHD	OR per IQR increase in ln-unit PFOS	1.75 (1.11, 2.76), p-value = 0.017
	Confounding: Maternal age, childbirth year, maternal education, parity, smoking during pregnancy, small-for-gestational age, preterm birth, maternal pre-pregnancy BMI, single mother around perinatal period, maternal fish intake						
Li (2020) *Medium*	United States, 1999–2016	Cross-sectional	Adults aged 20+ years from NHANES N = 2,525	Serum 8.00 (Range: 0.14–392)	Hearing threshold > 25 d B by frequency	OR by quartiles	2,000 Hz Q2: 0.70 (0.46, 1.06) Q3: 1.12 (0.76, 1.65) Q4: 1.60 (1.09, 2.37), p-trend < 0.0001 3,000 Hz Q2: 0.76 (0.53, 1.08) Q3: 1.00 (0.71, 1.41) Q4: 1.20 (0.85, 1.71), p-trend = 0.02 4,000 Hz Q2: 0.69 (0.50, 0.97) Q3: 0.89 (0.65, 1.24) Q4: 1.02 (0.73, 1.44), p-trend = 0.14
	Results: Lowest quartile used as reference. **Confounding:** Age, sex, BMI, education, ethnicity group, family income, sample weights						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Shrestha et al. (2017) *Medium*	United States, 2000–2002	Cross-sectional	Residents aged 55–74 yr who lived adjacent to Hudson River N = 126	Serum 33.7 (23.3–50.8)	Affective state: Beck Depression Inventory (BDI) total score, State-Trait Anxiety Inventory state and trait t-scores Attention: Trail making test Part A (ln-transformed time to complete) Executive function: Stroop color word test t-score, Trail making test part B (ln-transformed time to complete), Wisconsin Card Sorting Test preservative ln-transformed error and response Memory and learning: California	Regression coefficient per IQR increase in ln-unit PFOS	Depression: 0.25 (−0.77, 1.26), p-value = 0.63 CVLT-Total score: −0.14 (−0.59, 0.31) Wisconsin Card Sorting Test Perseverative Error: −0.14 (−0.30, 0.02), p-value = 0.09 Perseverative Response: −0.16 (−0.34, 0.01), p-value = 0.07 Wechsler Memory Scale Logical Memory Immediate Recall: −0.7 (−1.92, 0.52), p-value = 0.26 Delayed Recall: −0.14 (−1.29, 1.01), p-value = 0.81 Visual Reproduction Immediate Recall: 0.56 (−0.16, 1.29), p-value = 0.13 Delayed Recall: 0.79 (0.03, 1.55), p-value = 0.04 No statistically significant associations: State-Trait Anxiety Inventory, Stroop color word test, trail-making tests, motor function outcomes, visuospatial outcomes

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Verbal Learning Test total and subscores, Wechsler Memory Scale logical memory and visual reproduction immediate and delayed recall scores Motor function (dominant and non-dominant hands): finger tapping test average scores, grooved pegboard test ln-transformed times to completion, static motor steadiness test ln-transformed total numbers of contacts and times touching Dominant hand reaction time Visuospatial function: Wechsler Adult		

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Intelligence Scale-Revised total scores for block design and digit symbol coding		
	Confounding: Age, sex, education, serum total PCB						
			Pregnant Women				
Vuong et al. (2020b) *Medium*	United States Recruitment: 2003–2006 Follow-up: ~20 wk gestation and postpartum (4 wk, 1, 2, 3, 4, 5, and 8 yr)	Cohort	Pregnant women from the HOME study N = 355	Maternal serum 13.3 (9.0–17.9)	Beck Depression Inventory-II (BDI-II)	Relative risk and OR per ln-unit increase in PFOS	Medium Score Trajectory: 0.9 (0.6, 1.5) High Score Trajectory: 0.6 (0.3, 1.2) OR for score > 13 from pregnancy to 8 yr postpartum: 1.0 (0.7, 1.5)
	Confounding: Age, race/ethnicity, household income, maternal marijuana use, serum cotinine and PCBs, IQ, marital status, parity						

Notes: ADHD = attention deficit hyperactivity disorder; ADI-R = Autism Diagnostic Interview-Revised; ALSPAC = Avon Longitudinal Study of Parents and Children; ASD = autism spectrum disorder; ASQ-3 = Ages and Stages Questionnaire-3; BDI = Beck Depression Inventory; BDI-II = Beck Depression Inventory II; BMI = body mass index; BRIEF = Behavior Rating Inventory of Executive Function; BSID-II = Bayley Scales of Infant Development, Second Edition; CDI = Comprehensive Developmental Inventory; CHARGE = Childhood Autism Risk from Genetics and Environment; CI = confidence interval; CVLT P = California Verbal Learning Test; DaFO88 = Danish Fetal Origins 1988; CRP = C-reactive protein; DSM-IV-TR = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EMA = Early Markers for Autism; GM = geometric mean; HFHI = high frequency hearing impairment; HOME = Health Outcomes and Measures of the Environment; HUMIS = Human Milk Study; ID = intellectual disability; IQ = intelligence quotient; IQR = interquartile range; KBIT-2 = Kaufman Brief Intelligence Test Second Edition; LINC = Linking Maternal Nutrition to Child Health; LFHI = low frequency hearing impairment; MCDI = MacArthur Communicative Development Inventories; MDI = Mental Development Index; mo = months; MoBa = Mother, Father, and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PCBs = polychlorinated biphenyls; PDI = Psychomotor Development Index; PFOS = perfluorooctane sulfonic acid; PPVT-III = Peabody Picture Vocabulary Test; RR = risk ratio; SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire; SES = socioeconomic status; TMICS = Taiwan Maternal and Infant Cohort Study; VMWM = Virtual Morris Water Maze; WISC-IV = Wechsler Intelligence Scale for Children–Fourth Edition; SRS = Social Responsiveness Scale; T2 = tertile 2; T3 = tertile 3; WJ-III = Woodcock Johnson Test of Achievement-III; WPPSI-R = Wechsler Primary and Preschool Scales of Intelligence-Revised; WRAML2 = Wide Range Assessment of Memory and Learning Second Edition; WRAT-4 = Wide Range Achievement Test 4; WRAVMA = Wide Range Assessment of Visual Motor Abilities; yr = year(s).

[a] Exposure levels are reported as median unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.9 Renal

Table D-18. Associations Between PFOS Exposure and Renal Effects in the General Population

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			General Population				
Blake et al. (2018) *Low*	United States, 1991–2008	Cohort	Adults and children from FCC N = 192 (115 females, 77 males)	Serum 28.4 (21.6–35.7)	eGFR	Percent change per IQR increase in PFOS	All: Repeated measures model: −0.68 (−1.9, 0.54); p-value = 0.27 Latent model: −1.72 (−3.29, −0.15); p-value = 0.03 Females: −1.32 (−3.37, 0.73), p-value = 0.64 Males: 0.71 (−2.75, 4.16), p-value = 0.69 p-value for interaction by sex = 0.46
	Confounding: Age, year of measurement, sex, education, income, marital status, and BMI[c]						
Lin et al. (2013) *Low*	Taiwan, 2006–2008	Cross-sectional	Adolescents and young adults from YOTA study, 12–30 yr, N = 644	Serum 8.65 (5.41–13.52)	Uric acid (mg/dL)	Mean concentration by PFOS percentiles	≤ 25th percentile: 6.09 (0.13) 25th–50th: 6.13 (0.13) 50th–75th: 6.04 (0.13) > 75th: 6.12 (0.13) p-value for trend = 0.891
	Results: Effect estimates are provided with standard error in parentheses. **Confounding:** Age, gender, smoking status, alcohol drinking, BMI						
Conway et al. (2018) *Low*	United States, 2005–2006	Cohort	Adults, C8 Health Project, Diabetic = 5,210, non-diabetic = 48,440	Serum Diabetic: 21.2 (13.7–31.4) Non-diabetic: 20.2 (13.6–29.1)	CKD (eGFR of <60 mL/min/1.73 m^2)	OR per ln-unit increase in PFOS	Diabetics: 0.81 (0.73, 0.9) Non-diabetic: 1.09 (1.03, 1.16)
	Confounding: Age, sex, BMI, HDLc, LDLc, white blood cell count, CRP, hemoglobin, and iron						
Liu et al. (2018b) *Low*	United States, 2013–2014	Cross-sectional	Adults from NHANES, 18+ years,	Serum GM = 5.28 (SE = 1.02)	Total protein (g/dL)	Regression coefficient per	0.05 (SE = 0.02); p-value < 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 1871			ln-unit increase in PFOS	
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Arrebola et al. (2019) *Low*	Spain, 2009–2010	Cross-sectional	Adults, BIOAMBIENT.ES study N = 342	Serum 7.23 (5.14–10.11)	Uric acid (mg/dL), hyperuricemia	OR (hyperuricemia), or regression coefficient per log-unit increase in PFOS	Uric acid Wet-basis and lipid-basis models: 0.06 (−0.03, 0.16); p-value = 0.192 Wet-basis model with adjustment for serum lipids: 0.06 (−0.03, 0.157); p-value = 0.207 Hyperuricemia Wet-basis and lipid-basis models: 1.70 (0.86, 3.49); p-value = 0.138 Wet-basis model with adjustment for serum lipids: 1.67 (0.84, 3.41); p-value = 0.151
	Outcome: Hyperuricemia defined as at least one of a) serum uric acid levels ≥ 7.0 mg/dL in males or ≥ 6.0 mg/dL in females, at recruitment or in previous screenings, b) had been prescribed any pharmacological treatment for lowering uric acid levels, and/or c) had been diagnosed with gout by a clinician. **Comparison:** Logarithm base not specified. **Confounding:** Sex, age, BMI, weight loss during the last 6 mo, region of recruitment, smoking habit, alcohol consumption, education, place of residence						
Chen et al. (2019a) *Low*	Croatia, 2007–2008	Cross-sectional	Adults, 44–56 yr N = 122	Plasma GM = 8.91 (range = 2.36–33.67)	Uric acid (µmol/L), creatinine (µmol/L)	Regression coefficient per ln-unit increase in PFOS	Uric acid: −4.87 (−25.63, 15.89) Creatinine: −3.36 (−7.96, 1.24)
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity						
Jain and Ducatman (2019c) *Low*	United States, 2005–2014	Cross-sectional	Adults from NHANES, ≥ 20 yr N = 8,220	Serum Levels not reported	Levels of albumin in urine (log10-µg/mL), creatinine in urine (log10-mg/dL),	Regression coefficient per log10-unit increase in PFOS, or percent change	Albumin in urine Per log10-unit increase: −0.08 p-value < 0.01 Negative associations across eGFR stages

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
					albumin-to-creatinine ratio in urine (log10-mg/g), albumin in serum (log10-mg/dL), creatinine in serum (log10-mg/dL)	per 10% increase in PFOS	Percent change per 10% increase: −0.75, p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.10 Creatinine in urine Per log10-unit increase: 0.04 p-value = 0.01 Positive associations across eGFR stages Percent change per 10% increase: 0.38 p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.02 Albumin-to-creatinine ratio in urine Per log10-unit increase: −0.12 p-value < 0.01 Negative associations across eGFR stages Percent change per 10% increase: −1.13 p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.73 Albumin in serum Per log10-unit increase: 0.01 p-value < 0.01 Positive associations across eGFR stages Percent change per 10% increase: 0.11 p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							p-value for gender and race/ethnicity interaction = 0.68 Creatinine in serum Per log10-unit increase: 0.01 p-value = 0.01 Positive associations in GF-1, GF-2, GF-3A Negative association in GF-3B/4 Percent change per 10% increase: 0.11 p-value < 0.05 p-value for gender and race/ethnicity interaction < 0.01
	GF Stages: GF-1: GFR ≥ 90 mL/min/1.73 m^2; GF-2: GFR between 60 and 90 mL/min/1.73 m^2; GF-3A: GFR between 45 and 60 mL/min/1.73 m^2; GF-3B/4: GFR between 15 and 45 mL/min/1.73 m^2. **Confounding:** Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), PIR, NHANES survey period						
Jain and Ducatman (2019a) *Low*	United States, 2007–2014	Cross-sectional	Adults from NHANES, ≥ 20 yr, Males = 3,330, females = 3,506	Serum Males: GM = 10.51 (9.88–11.18) Females: GM = 6.58 (6.22–6.96)	Uric acid (mg/dL) by glomerular filtration (GF) stage	Regression coefficient per log10-unit increase in PFOS	Males GF-1: 0.01, p-value = 0.01 GF-2: 0.02, p-value = 0.05 GF-3A: −0.01, p-value = 0.66 GF-3B: −0.04, p-value < 0.01 Females GF-1: 0.02, p-value = 0.04 GF-2: 0.01, p-value = 0.52 GF-3A: 0.04, p-value < 0.01 GF-3B: 0.01, p-value = 0.64
	GF Stages: GF-1: eGFR > 90 mL/min per 1.73 m^2; GF-2: 60 < eGFR ≤ 90 mL/min per 1.73 m^2; GF-3A: 45 < eGFR ≤ 60 mL/min per 1.73 m^2; GF-3B/4: 15 < eGFR ≤ 45 mL/min per 1.73 m^2. **Confounding:** Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), PIR, NHANES survey period						
Wang et al. (2019b) *Low*	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project	Serum 24.22 (14.62–37.19)	CKD, eGFR	OR (CKD) or regression coefficient per ln-unit increase	CKD (OR) Per ln-unit increase: 1.71 (0.92, 1.49), p-value = 0.205 Q2: 1.19 (0.67, 2.09)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 1612 (males = 1204, females = 408)			in PFOS, or by quartiles	Q3: 1.42 (0.82, 2.47) Q4: 1.34 (0.77, 2.33) p-value for trend = 0.617 eGFR Per ln-unit increase: All: −0.91 (−1.83, 0), p-value = 0.05 Males: −0.73 (−1.82, 0.37) p-value = 0.193 Females: −0.62 (−0.24, 1.15) p-value = 0.491 p-value for interaction by sex = 0.419 Q2: −1.25 (−3.14, 0.63) Q3: −1.59 (−3.53, 0.35) Q4: −1.77 (−3.74, 0.19) p-value for trend = 0.086
	Outcome: CKD defined as eGFR < 60 mL/min per 1.73 m^2. **Results:** Lowest quartile used as reference group. **Confounding:** Age, sex, BMI, education, annual income, regular exercise, cigarette smoking, drinking alcohol, family history of CKD, total cholesterol						
Zeng et al. (2019c) *Low*	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project N = 1612 (males = 1204, females = 408)	Serum 24.22 (14.62–37.19)	Hyperuricemia, uric acid (mg/dL)	OR (hyperuricemia) or regression coefficient (uric acid) per log10-unit increase in PFOS	Hyperuricemia All: 1.17 (0.99, 1.39) Males: 1.11 (0.92, 1.34) Females: 1.27 (0.8, 2) p-value for interaction by sex = 0.118 Uric acid All: 0.1 (0.02, 0.18), p-value = 0.017 Males: 0.07 (−0.03, 0.18) Females: 0.11 (−0.01, 0.18) p-value for interaction by sex = 0.209

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Outcome: Hyperuricemia defined as serum uric acid levels > 7.0 mg/dL in males or > 6.0 mg/dL in females. **Confounding:** Age, sex, BMI, income, drinking, smoking, career, exercise, offal consumption, fish and seafood consumption, serum creatinine						
Scinicariello et al. (2020b) *Low*	United States, 2009–2014	Cross-sectional	Adults from NHANES N = 4915 (no CKD = 4,103; CKD = 874)	Serum GM = 6.98 (SE = 0.23)	Uric acid (mg/dL), hyperuricemia, gout	OR (hyperuricemia, gout), or regression coefficient (uric acid) by quartiles	Uric acid Overall population Q2: 0.13 (0.01, 0.24) Q3: 0.21 (0.05, 0.37) Q4: 0.29 (0.14, 0.44) p-value for trend = 0.003 Participants with CKD Q2: 0.6 (0.15, 1.05) Q3: 0.31 (−0.02, 0.7) Q4: 0.38 (0.06, 0.83) p-value for trend = 0.08 Participants without CKD Q2: 0.03 (−0.1, 0.15) Q3: 0.13 (−0.02, 0.28) Q4: 0.2 (0.06, 0.34) p-value for trend = 0.02 Hyperuricemia Overall population Q2: 1.1 (0.84, 1.45) Q3: 1.27 (0.92, 1.76) Q4: 1.45 (1.03, 2.03) p-value for trend = 0.15 Participants with CKD Q2: 1.93 (0.91, 4.06) Q3: 0.85 (0.4, 1.77) Q4: 1.15 (0.53, 2.5) p-value for trend = 0.12 Participants without CKD Q2: 0.94 (0.68, 1.3) Q3: 1.26 (0.89, 1.79) Q4: 1.35 (0.92, 1.99) p-value for trend = 0.19

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Gout Overall population Q2: 1.17 (0.54, 2.53) Q3: 1.23 (0.54, 2.53) Q4: 1.46 (0.67, 3.16) p-value for trend = 0.79 Participants with CKD Q2: 0.88 (0.26, 2.92) Q3: 1.08 (0.38, 3.07) Q4: 1.08 (0.39, 2.94) p-value for trend = 0.97 Participants without CKD Q2: 1.73 (0.6, 4.94) Q3: 1.56 (0.51, 4.78) Q4: 1.93 (0.71, 5.22) p-value for trend = 0.58
	Outcomes: CKD defined as eGFR < 60 mL/min per 1.73 m^2 and/or albuminuria. Hyperuricemia defined as serum uric acid levels ≥ 7.0 mg/dL in males or ≥ 6.0 mg/dL in females. Gout was self-reported diagnosis from a health professional. **Results:** Lowest quartile used as reference group. **Confounding:** Race/ethnicity, age, sex, education, alcohol, smoking, serum cotinine, BMI, diabetes, hypertension, CKD						
Children and Adolescents							
Geiger et al. (2013) *Low*	United States, 1999–2000; 2003–2008	Cross-sectional	Children and adolescents from NHANES, 12–18 yr, N = 1,772	Serum Mean = 18.4 (SE = 0.5)	Hyperuricemia, uric acid (mg/dL)	OR (hyperuricemia) or regression coefficient (uric acid) per ln-unit increase in PFOS or by quartiles	Hyperuricemia Per ln increase: 1.37 (1.06, 1.76) Q2: 1.17 (0.8, 1.72) Q3: 1.18 (0.74, 1.87) Q4: 1.65 (1.1, 2.49) p-value for trend = 0.022 Uric acid Per 1-ln increase: 0.09 (0.02, 0.17) Q2: 0.03 (−0.1, 0.16) Q3: 0.09 (−0.04, 0.21) Q4: 0.12 (−0.01, 0.26) p-value for trend = 0.058

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Outcome: Hyperuricemia defined as serum uric acid levels ≥ 6 mg/dL. **Results:** Lowest quartile as reference group. **Confounding:** Age, sex, race/ethnicity, BMI, annual household income, moderate activity, total cholesterol, serum cotinine						
Kataria et al. (2015) *Low*	United States, 2003–2010	Cross-sectional	Children and adolescents from NHANES, 12–19 yr, NHANES N = 1,962	Serum 3.5 (2.5–4.7)	eGFR (min/mL/1.73 m^2), uric acid (mg/dL), creatinine (mg/dL)	Regression coefficient by quartiles	eGFR Q2: −5.24 (−9.75, −0.73), p-value < 0.05 Q3: −7.21 (−12.21, −2.21), p-value < 0.01 Q4: −9.47 (−14.68, −4.25), p-value < 0.001 Uric acid Q2: 0.095 (−0.081, 0.27) Q3: 0.046 (−0.1, 0.19) Q4: 0.19 (0.032, 0.34), p-value < 0.05 Creatinine Q2: 0.021 (−0.007, 0.049) Q3: 0.038 (0.008, 0.068), p-value < 0.05 Q4: 0.04 (0.01, 0.071), p-value < 0.01
	Results: Lowest quartile as reference group. **Confounding:** Sex, PIR, caregiver education, serum cotinine, prehypertension, insulin resistance, BMI, hypercholesterolemia, race/ethnicity categories						
Qin et al. (2016) *Low*	Taiwan, 2009–2010	Cross-sectional	Children from GBCA Study, 12–15 yr, N = 225 (123 girls, 102 boys)	Serum All: 28.9 (14.1–43.0) Boys: 29.9 (13.0–43.8) Girls: 28.8 (14.8–42.6)	Uric acid (mg/dL), hyperuricemia	Regression coefficient per ln-unit increase in PFOS (uric acid); OR scaled with increasing quartiles (hyperuricemia)	Uric acid All: 0.05 (−0.03, 0.13) Boys: 0.05 (−0.04, 0.15) Girls: 0.01 (−0.14, 0.16) Hyperuricemia (OR) All: 1.35 (0.95, 1.93) Boys: 1.4 (0.88, 2.21) Girls: 1.51 (0.79, 2.89)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Outcome: Hyperuricemia defined as uric acid level ≥ 6 mg/dL. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, gender, BMI, parental education level, exercise, environmental tobacco smoke exposure, and serum creatinine						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children, 8–12 yr N = 40	Serum 2.79 (IQR = 2.10)	Creatinine (mg/dL)	Regression coefficient per unit increase in PFOS	0 (−0.02, 0.03)
	Confounding: Age, sex, race						
			Pregnant Women				
Nielsen et al. (2020) *Low*	Sweden, 2009–2014	Cohort	Pregnant women, PONCH study N = 73	Serum Early pregnancy: 5.6 (5th–95th percentile = 2.6 –11.5) Late pregnancy: 4.8 (5th–95th percentile = 1.9 –8.4)	eGFR: LMrev, CKD-EPI$_{creatinine}$, CAPA, CKD-EPI$_{cystatin\ C}$, mean of LMrev and CAPA, mean of CKD-EPI$_{creatinine}$ and CKD-EPI$_{cystatin\ C}$ Glomerular pore size	Spearman's correlation coefficient	Cross-sectional correlations consistently weak and non-significant Early to late pregnancy changes: No significant associations eGFR: LMrev: 0.02, p-value = 0.85 CKD-EPI$_{creatinine}$: 0.02, p-value = 0.87 CAPA: −0.04, p-value = 0.73 CKD-EPI$_{cystatin\ C}$: −0.05, p-value = 0.66 mean of LMrev and CAPA: −0.04, p-value = 0.76 mean of CKD-EPI$_{creatinine}$ and CKD-EPI$_{cystatin\ C}$: −0.06, p-value = 0.63 Glomerular pore size: CAPA/LMrev: −0.05, p-value = 0.68 CKD-EPI$_{cystatin\ C}$/CKD-EPI$_{creatinine}$: −0.06, p-value = 0.63
	Outcome: Glomerular pore size is estimated as the ratio between eGFR$_{cystatin\ C}$ and eGFR$_{creatinine}$ and was calculated by the two ratios provided. **Confounding:** Number of days between sampling, pregnancy-induced change in BMI						
			Occupational Populations				

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Rotander et al. (2015) *Low*	Australia, 2013	Cross-sectional	Firefighters with past exposure to AFFF, 17–66 yr old N = 137 (97% male)	Serum 66 (range = 3.1–391)	Uric acid (µmol/L)	Regression coefficient per log10-unit increase in PFOS	0.045 (SE = 0.047), p-value = 0.342
	Confounding: Age, sex, BMI, smoking status, total serum protein, PFOA, PFHxS						

Notes: AFFF = aqueous film-forming foam; BMI = body mass index; CAPA = Caucasian Asian Pediatric Adult; CKD = chronic kidney disease; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration study; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate (mL/min per 1.73 m2); FCC = Fernald Community Cohort; GBCA = Genetic Biomarkers Study for Childhood Asthma; GF = glomerular filtration; GM = geometric mean; HDLc = high-density lipoprotein cholesterol; IQR = interquartile range; LDLc = low-density lipoprotein cholesterol; LMrev = Lund Malmö Revised; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PIR = poverty income ratio; PONCH = Pregnancy Obesity Nutrition and Child Health study; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SD = standard deviation; SE = standard error; YOTA = Young Taiwanese Cohort Study; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.10 Hematological

Table D-19. Associations Between PFOS Exposure and Hematological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)	Outcome	Comparison	Select Results[a]
General Population							
Etzel et al. (2019) *Medium*	United States, 2003–2010	Cross-sectional	Children and adults from NHANES, ≥ 12 yr of age, N = 7,040	Serum, Median = 15.1 (9.1–23.8)	Vitamin D deficiency (<50 ng/mL), 25-hydroxy Vitamin D (25(OH)D, nmol/L)	Regression coefficient or prevalence OR (POR) per doubling of PFOS, or by quintiles	Per doubling of PFOS: Vitamin D deficiency POR: 1.05 (0.97, 1.13) 25-hydroxy Vitamin D −0.9 (−1.5, −0.2) Q5: −2.8 (−4.7, −0.8) 60+ years: −1.7 (−2.9, −0.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)	Outcome	Comparison	Select Results[a]
							No other statistically significant associations or trends
	Results: Lowest quintile used as reference group. **Confounding:** Gender, race/ethnicity, age, BMI category, vitamin D supplement use, poverty to income ratio, smoking status, 6-mo examination time period[c]						
Chen et al. (2019a) *Medium*	Croatia 2007–2008	Cross-sectional	Adults, 44–56 yr of age, N = 122	Plasma, GM = 8.91(min = 2.36, max = 33.67)	Calcium in serum (mmol/L)	Regression coefficient per ln-unit increase in PFOS	–0.05 (−0.09, −0.01), p-value < 0.05
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity						
Jain (2020a) *Medium*	United States 2003–2016	Cross-sectional	Adults from NHANES, ≥ 20 yr of age, N = 11,251	Serum, Non-anemic males: GM = 12.0 (95% CI: 11.5, 12.7) Non-anemic females: GM = 8.1 (95% CI: 7.7, 8.5) anemic males: GM = 10.7 (95% CI: 9.2, 12.5) anemic females: GM = 5.0 (95% CI: 4.4, 5.8)	Whole blood hemoglobin (WBHGB) (log10-g/dL)	Regression coefficient per log10-unit increase in PFOS	Non-anemic males: 0.009, p-value < 0.01 Non-anemic females:0.006, p-value < 0.01 Anemic males: 0.023, p-value < 0.01 Anemic females: 0.024, p-value < 0.01
	Confounding: Age, BMI, PIR, serum cotinine, survey year, daily alcohol intake						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)	Outcome	Comparison	Select Results[a]
Khalil et al. (2018) *Low*	United States, 2016	Cross-sectional	Children with obesity, 8–12 yr of age, N = 47	Serum, Median = 2.79 (IQR = 2.10)	25-hydroxy vitamin D (ng/mL)	Regression coefficient per unit increase in PFOS	–0.10 (−1.54, 1.33)
	Confounding: Age, sex, race						
van den Dungen et al. (2017) *Low*	The Netherlands, 2015	Cross-sectional	Dutch men, 40–70 yr of age, with habitual eel consumption of at least one portion a month, N = 37	Serum, Median = 40 ng/g wet weight (15–93)	Hemoglobin (Hb), Hematocrit (Ht), Retinol (units not provided)	Regression coefficient	Hb: −0.112 (−0.477, 0.250) Ht: −0.095 (−0.455, 0.263) Retinol: 0.205 (−0.146, 0.561)
	Confounding: Age, waist-to-hip ratio						

Notes: aPTT = activated partial thromboplastin time; BMI = body mass index; CI = confidence interval; GM = geometric mean; HIV = human immunodeficiency virus; Hb = hemoglobin; Ht = hematocrit; IQR = interquartile range; mo = month; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POR = prevalence odds ratio; PPT = prothrombin time; WBHGB = whole blood hemoglobin; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.11 Respiratory

Table D-20. Associations Between PFOS Exposure and Respiratory Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Agier et al. (2019) *Medium*	France, Greece, Lithuania, Norway, Spain, United Kingdom 2003–2009	Cohort	Pregnant women and their children, ages 6–12 yr, N = 1,033	Maternal and child's serum, plasma, or whole blood Prenatal (maternal) Median = 6.6 (IQR = 5.8)	FEV1	Regression coefficient per log2-unit increase in PFOS	Prenatal: 0.1 (−1.1, 1.3), p-value = 0.89 Postnatal: 0.5 (−0.6, 1.6), p-value = 0.38

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Postnatal (child) Median = 2.1 (IQR = 1.9)			
	Confounding: Center of recruitment, child's sex, child's age, child's height, parental country of birth, breastfeeding duration, season of conception, presence of older siblings, parental education level, maternal age, maternal pre-pregnancy BMI, postnatal passive smoking status, prenatal maternal active, passive smoking status[c]						
Gaylord et al. (2019) *Medium*	New York, U.S. 2014–2016	Cross-sectional	Adolescents and young adults, ages 13–22 yr, N = 287	Serum, Comparison group: median = 2.75 (range : 0.60, 27.80) WTCHR group: median = 3.72 (range: 1.01, 14.20)	FEV1 FVC FEV1/FVC TLC RV FRC Resistance at an oscillation frequency of 5 Hz, 5–20 Hz, 20 Hz	Regression coefficient per log-unit increase in PFOS	No statistically significant differences observed between groups for the measured outcomes, p-values > 0.05
	Comparison: Logarithm base not specified. **Confounding**: Sex, race/ethnicity, age, BMI, tobacco smoke exposure						
Impinen et al. (2018) *Medium*	Norway 1992–2002	Cohort	Infants followed up at 2 yr and 10, N = 641	Cord blood, Median = 5.2 (4.0, 6.6)	Oslo Severity Score (1–5 vs. 0) Oslo Severity Score (6–12 vs. 0) Reduced lung function at birth	OR per log2-unit increase in PFOS	1.71 (1.16, 2.53), p-value = 0.007 1.15 (0.71, 1.84), p-value = 0.576 0.86 (0.43, 1.72), p-value = 0.680
	Outcome: Reduced lung function at birth: Lung function (tPTEF/tE) with standardized z-score, and binary variable of decreased lung function (cutoff < 0.20). **Confounding**: Sex						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Manzano-Salgado et al. (2019) *Medium*	Spain 2003–2015	Cohort	Pregnant women and their children, followed up at ages 1.5, 4, and 7 yr, N = 503 (4 yr) N = 992 (7 yr)	Maternal blood, Median = 6.06 (4.52, 7.82)	FEV1, FVC FEV1/FVC, FEF25%–75%	Regression coefficient per log2-unit increase in PFOS	No statistically significant associations for the measured outcomes
	Confounding: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth						
Qin et al. (2017) *Medium*	Taiwan, 2009–2010	Case-control	Children with asthma and without asthma, ages 10–15, N = 132 (with asthma) N = 168 (without asthma)	Serum, Children with asthma: Median = 31.51 (19.60, 91.69) Children without asthma: Median = 28.83 (12.39, 42.02)	FEV1 FVC FEF25%–75% PEF	Regression coefficient per ln-unit increase in PFOS	Statistically significant associations in children with asthma: FEV1: −0.06 (−0.10, −0.02), p-value < 0.05 FVC: −0.06 (−0.10, −0.01), p-value < 0.05
	Confounding: Age, sex, BMI, parental education level, exercise, environmental tobacco smoke exposure, and month of survey						

Notes: BMI = body mass index; IQR = Interquartile range; FEF25%–75% = Forced Expiratory Flow at 25%–75%; FEV1 = Forced Expiratory Volume in 1 s; FRC = Functional Residual Capacity; FVC = Forced Vital Capacity; IQR = interquartile range; OR = odds ratio; PEF = Peak Expiratory Flow rate; RV = residual volume; TLC = total lung capacity; U.S. = United States; WTCHR = World Trade Center Health Registry; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.12 Musculoskeletal

Table D-21. Associations Between PFOS Exposure and Musculoskeletal Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Children and Adolescents							

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Jeddy et al. (2018) *Medium*	England, 1991–2009	Cohort	Females from the ALSPAC Study, Age 17, N = 221	Maternal serum 20.2 (15.6–25.5)	Area adjusted BMC (g), bone area (cm^2), BMC (g), BMD, cortical bone area (cm^2), cortical BMC (mg), cortical BMD (mg/cm^2), cortical thickness (mm), endosteal circumference (mm), height (cm), periosteal circumference (mm), total femoral neck BMD (g/cm^2), total hip BMD (g/cm^2), total lean mass (g)	Regression coefficient per unit increase in PFOS	Height: −0.11 (−0.19, −0.02) Total lean mass: −75.61 (−131.12, −20.1) Bone area: −4.07 (−7.38, −0.76) BMC: −5.94 (−10.96, −0.92) No other statistically significant associations
	Confounding: Maternal pre-pregnancy BMI, maternal education, maternal age at delivery, gestational age at sample collection, ever breastfed status at 15 mo[c]						
Cluett et al. (2019) *Medium*	United States, 1999–2010	Cross-sectional	Children from Project Viva, Ages 6–10, N = 531	Plasma 6.4 (IQR = 5.6)	Areal bone mineral density (aBMD) z-score, BMC z-score	Regression coefficient per log2-unit increase in PFOS	aBMD z-score −0.08 (−0.16, −0.01) No statistically significant associations or interactions by sex BMC z-score: No statistically significant associations
	Confounding: Maternal age, education, census tract median household income, individual household income, and child age, sex, race/ethnicity, year of blood draw, dairy intake, physical activity						
Khalil et al. (2018)	United States 2016	Cross-sectional	Obese children, ages 8–12	Serum	BMD measured as broadband	Regression coefficient per	BMD (broadband ultrasound attenuation)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Low			N = 23	2.79 (IQR = 2.10)	ultrasound attenuation (dB/MHz) and speed of sound (m/s), stiffness index (%)	unit increase in PFOS	−1.03 (−5.35, 3.29) BMD (speed of sound) −5.22 (−11.2, 0.79) Stiffness index −2.15 (−5.56, 1.26)
	Confounding: Age, sex, race						
Di Nisio et al. (2019) *Low*	Italy 2017–2018	Cross-sectional	Male high school students N = 100 (50 controls, 50 exposed)	Serum Controls: 0.82 (0.4–1.3) Exposed: 1.11 (0.8–1.3) Semen Controls: 0.11 (0.08–0.13) Exposed: 0.11 (0.01–0.14)	Arm span (cm)	Mann-Whitney test (Exposed vs. Controls)	Arm span Controls: 182.75 (178.0, 185.8) Exposed: 179.00 (174.2, 187.0) Adjusted p-value for comparison of medians = 0.738
	Results: Values for each outcome are reported as median (25th, 75th percentile). **Confounding:** None reported						
			General Population				
Uhl et al. (2013) *Medium*	United States, 2003–2008	Cross-sectional	Adults from NHANES, Ages 20–84, N = 3,809, Females N = 1,921	Serum Adults: Weighted mean = 21.23 Females: Weighted mean = 18.17	Osteoarthritis	OR per ln-unit increase in PFOS or by quartiles	Adults 20–84 1.15 (0.94, 1.40) Q2: 1.04 (0.58, 1.85) Q3: 1.99 (1.14, 3.49), p-value < 0.05 Q4: 1.77 (1.05, 2.96), p-value < 0.05 Females 20–49 2.37 (1.35, 4.16), p-value < 0.01 Q2: 0.65 (0.19, 2.20) Q3: 1.11 (0.29, 4.30)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Q4: 4.99 (1.61, 15.4), p-value < 0.01 No other statistically significant associations
	Results: Lowest quartile used as the reference group. **Confounding:** Age, race/ethnicity, SES, smoking, BMI, vigorous reactional activity, prior wrist, hip, or spine fracture						
Lin et al. (2014) *Medium*	United States, 2005–2006, 2007–2008	Cross-sectional	Adults from NHANES Ages ≥ 20, Males N = 1,192, Females N = 842, Females in menopause N = 305	Serum GM = 15.32 (SD = 17.58)	Total BMD (g/cm^2) in hip or lumbar spine; fractures in hip, wrist, spine, or all types	Regression coefficient per ln-unit increase in PFOS	Total BMD in lumbar spine Women not in menopause: −0.022 (−0.038, −0.007), p-value = 0.006 Other outcomes: No statistically significant associations
	Confounding: Age, race/ethnicity, BMI, smoking, drinking, treatment for osteoporosis, use of prednisone or cortisol daily						
Khalil et al. (2016) *Medium*	United States, 2009–2010	Cross-sectional	Adolescents and adults from NHANES, Ages 12–80, Males N = 956, Females N = 958	Serum Mean = 12.7 (SE = 1.20)	BMD (g/cm^2) of total femur, femoral neck, lumbar spine; Osteoporosis among females	BMD: Regression coefficient per ln-unit increase in PFOS and by quartiles Osteoporosis: OR per ln-unit increase in PFOS and by quartiles	Total femur Females: −0.018 (−0.034, −0.002), p-value < 0.05 Q2: −0.007 (−0.038, 0.023) Q3: −0.009 (−0.037, 0.019) Q4: −0.044 (−0.074, −0.014), p-value < 0.05 Males: Not statistically significant Femoral neck Females: −0.016 (−0.029, −0.002), p-value < 0.05 Q2: 0.001 (−0.019, 0.019) Q3: −0.001 (−0.025, 0.025) Q4: −0.034 (−0.059, −0.009), p-value < 0.05

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Males: −0.013 (−0.024, −0.002), p-value < 0.05 Q2: −0.036 (−0.077, 0.006) Q3: −0.027 (−0.063, 0.009) Q4: −0.046 (−0.078, −0.015), p-value < 0.05 Lumbar spine, osteoporosis: No statistically significant associations
	Results: Lowest quartile used as the reference group. **Confounding**: Age, ethnicity, BMI, serum cotinine, physical activity, milk consumption, blood lead concentration						
Hu et al. (2019) *Medium*	United States, 2004–2007	Cohort and cross-sectional	Adults from the POUNDS Lost study, Ages 30–70, N = 294	Plasma Mean = 32.2 (16.8–43.1)	BMD and 2-yr ΔBMD (g/cm^2) of spine, total hip, femoral neck, hip trochanter, hip intertrochanteric area, and Ward's triangle area	Regression coefficient per SD increase in PFOS	Spine BMD analyses Cross-sectional: −0.02 (−0.037, −0.003) Total hip BMD analyses 2-yr ΔBMD: −0.005 (−0.009, −0.001), p-value < 0.05 Hip intertrochanteric area BMD analyses 2-yr ΔBMD: −0.008 (−0.013, −0.003), p-value < 0.05 Femoral neck, hip trochanter, Ward's triangle area: no statistically significant associations No statistically significant associations or interactions by sex
	Confounding: For cross-sectional, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group; For cohort, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group, baseline BMD, 2-yr weight change						

Notes: aBMD = areal bone mineral density; ALSPAC = Avon Longitudinal Study of Parents and Children; BMC = bone mineral content; BMD = bone mineral density; BMI = body mass index; GM = geometric mean; IQR = interquartile range; mo = months; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POUNDS Lost = Prevention of Obesity Using Novel Dietary Strategies Lost clinical trial; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SD = standard deviation; SE = standard error; SES = socioeconomic status; vs. = versus; yr = year.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.13 Gastrointestinal

Table D-22. Associations Between PFOS Exposure and Gastrointestinal Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Timmerman et al. (2020) *Medium*	Guinea-Bissau 2012–2015	Cohort	Children aged <2 yr previously enrolled in a RCT for measles vaccination N = 236 (113 girls, 123 boys)	Serum 0.77 (0.53–1.02)	Diarrhea	OR per doubling of PFOS at inclusion or 9-mo visit	At inclusion: 1.14 (0.66, 1.96) At 9 mo: 1.2 (0.62, 2.31) No statistically significant associations or interactions by sex
	Confounding: Weight and age at inclusion, sex, maternal education, breastfeeding without solids[c]						
Dalsager et al. (2016) *Low*	Denmark 2010–2015	Cohort	Pregnant women and their children from the Odense Child Cohort, Ages 1–4 yr N = 346	Serum 8.07 (Range: 2.36–25.10)	Diarrhea, vomiting (number of days with symptom or proportion of days under/above median)	Incidence rate ratio (number of days) or OR (proportion of days) by tertiles of PFOS exposure	Diarrhea Number of days with symptom T2: 1.41 (0.79, 2.51) T3: 1.19 (0.67, 2.12) Proportion of days under/above median T2: 0.89 (0.51, 1.56) T3: 1.04 (0.59, 1.82) Vomiting Number of days with symptom T2: 1.18 (0.8, 1.74) T3: 0.87 (0.58, 1.31) Proportion of days under/above median T2: 1.47 (0.86, 2.54) T3: 0.78 (0.45, 1.35)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, maternal educational level, parity, and child age						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Hammer et al. (2019) *Low*	Faroe Islands Enrollment: 1986–2009; follow-up until 2017	Cohort	Children and adults from CHEF N = 2,843	Blood Low exposure: GM = 2.33 (1.93–2.90) High exposure: GM = 26.88 (21.90–32.24)	Inflammatory bowel disease	Incidence rate ratio for highest vs. lowest tertile of PFOS exposure	0.30 (0.08, 1.07)
	Confounding: Age, calendar period						
Xu et al. (2020d) *Low*	Sweden 2014–2016	Cohort	Residents of Ronneby municipality Ronneby panel study: N = 57 Ronneby resampling: N = 113 Karlshamn: N = 19	Serum Ronneby panel study: 216 (118–300) Ronneby resampling: 271 (147–449) Karlshamn: 5 (4–7)	Inflammatory bowel disease (ln-ng/mL levels of calprotectin or zonulin)	Regression coefficient per unit increase in PFOS	Calprotectin Panel study: −0.0008 (−0.0033, 0.0018) Resampling: −0.0006 (−0.0016, 0.0005) Karlshamn: −0.045 (−0.14, 0.05) Zonulin Panel study: 0.0007 (−0.0012, 0.0025) Resampling: −0.0001 (−0.0008, 0.0005) Karlshamn: −0.019 (−0.1, 0.063)
	Confounding: Age, BMI, gender						

Notes: BMI = body mass index; CHEF = Children's Health and the Environment in the Faroes; GM = geometric mean; mo = month(s); OR = odds ratio; PFOS = perfluorooctane sulfonate; RR = risk ratio; RCT = randomized controlled trial; T2 = tertile 2; T3 = tertile 3; vs. = versus; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

D.14 Dental

Table D-23. Associations Between PFOS Exposure and Dental Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Puttige Ramesh et al. (2019) *Medium*	United States 1999–2002	Cross-sectional	Adolescents from NHANES aged 12–19 yr N = 2,869	Serum Median = 13 (7.2–22)	Dental caries	OR per log2-unit increase in PFOS and by quartiles	0.99 (0.92, 1.07) Q2: 0.91 (0.72, 1.16) Q3: 1.02 (0.81, 1.31) Q4: 0.92 (0.72, 1.17)
	Results: Lowest quartile used as reference **Confounding:** Gender, race, education level of parent/guardian, family-poverty-to-income ratio, blood lead level, serum cotinine level[c]						
Wiener and Waters (2019) *Medium*	United States 2013–2014	Cross-sectional	Children from NHANES aged 3–11 yr N = 629	Serum GM = 3.88 (95% CI: 3.53, 4.27)	Dental caries experience	OR per IQR increase in PFOS	1.41 (0.97, 2.05); p-value = 0.069
	Confounding: Age, sex, race/ethnicity, ratio of family-income-to-poverty guidelines, tooth brushing frequency, dental visit, percentages of sugar in the diet, fluoride in the water						

Notes: CI = confidence interval; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; yr = years.
[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results are reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.15 Ocular

Table D-24. Associations Between PFOS Exposure and Ocular Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Zeeshan et al. (2020) *Medium*	China, 2016	Cross-sectional	Adults from the Isomers of C8 Health Project, ages 22–96 yr, N = 1,202	Serum Median = 24.07 (14.13–36.41)	Visual impairment, synechia, macula disorder, corneal pannus, shallow anterior chamber, vitreous disorder, retinal disorder,	OR per ln-unit increase in PFOS	Visual impairment 3.11 (2.3, 4.2); p-value < 0.05 Eye disease, combined ≤ 65 yr: 1.52 (1.21, 1.91); p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					lens opacity, conjunctival disorder, combined eye disease		> 65 yr: 0.91 (0.55, 1.51) All other outcomes: No statistically significant associations
	Confounding: Age, sex, BMI, education, income, career, exercise time, drinking, smoking[c]						

Notes: BMI = body mass index; OR = odds ratio; yr = years.
[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results are reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.16 Dermal

Table D-25. Associations Between PFOS Exposure and Dermal Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Ernst et al. (2019) *Medium*	Denmark 1999–2017	Cohort	Pregnant women and their children from the Puberty Cohort within the DNBC N = 555 girls, 565 boys	Maternal blood (1st trimester) Girls Sample 1: 32.3 (19.3–50.8) Girls Sample 2: 27.9 (16.5–42.2) Boys Sample 1: 31.9 (19.2–51.2) Boys Sample 2: 27.2 (16.7–45.2)	Acne, age at occurrence (months)	Regression coefficient per log2-unit increase in PFOS, and by tertiles	Girls: −1.73 (−5.24, 1.77) T2: 0.09 (−4.69, 4.87) T3: −1.96 (−6.89, 2.97) Boys:−1.52 (−4.52, 1.48) T2:−1.33 (−5.02, 2.36) T3:−0.7 (−4.75, 3.35)
	Results: Lowest tertile used as a reference group. **Confounding:** Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy BMI, daily number of cigarettes smoked in first trimester[c]						

Notes: DNBC = Danish National Birth Cohort; T2 = tertile 2; T3 = tertile 3.
[a] Exposure levels reported as median (10th–90th percentile).
[b] Results reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.17 Cancer

Table D-26. Associations Between PFOS Exposure and Cancer in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Grice et al. (2007) *Medium*	United States 1997–1998	Cohort	Employees of a PFOS-based chemical and film manufacturing plant, 2,083	Modeled Non-exposed: GM range = 0.11–0.29 ppm; Low-exposed: GM range = 0.39–0.89 ppm; High-exposed: GM range = 1.30–1.97 ppm	Cancers: colon, melanoma, and prostate	OR by PFOS exposure category	Colon cancer: Ever exposed: 1.21 (0.51, 2.87) Low or high-exposed: 1.37 (0.57, 3.30) High-exposed: 1.69 (0.68, 4.17) Melanoma: Ever exposed: 1.08 (0.31, 3.72) Low or high-exposed: 0.90 (0.24, 3.43) High-exposed: 1.01 (0.25, 4.11) Prostate cancer: Ever exposed: 1.34 (0.62, 2.91) Low or high-exposed: 1.36 (0.61, 3.02) High-exposed: 1.08 (0.44, 2.69)
	Results: Non-exposed used as the reference group **Confounding:** Age and gender						
Eriksen et al. (2009) *Medium*	Denmark 1993–2006	Cohort	Adults with no previous cancer diagnosis, Ages 50–65 at enrollment, Prostate cancer, 1,393; Bladder cancer, 1,104; Pancreatic cancer, 900; Liver cancer, 839	Serum Mean (5th–95th percentile): Cases, men: 35.1 (17.4–60.9); Controls, men: 35.0 (16.8–62.4); Cases, women: 32.1 (14.0–58.1);	Cancers: prostate, bladder, pancreatic, liver	IRR per unit increase in PFOS, or by quartiles	Prostate cancer: Q2: 1.35 (0.97, 1.87) Q3: 1.31 (0.94, 1.82) Q4: 1.38 (0.99, 1.93) Per unit increase: 1.05 (0.97, 1.14) Bladder cancer: Q2: 0.76 (0.50, 1.16) Q3: 0.93 (0.61, 1.41) Q4: 0.70 (0.46, 1.07) Per unit increase: 0.93 (0.83, 1.03)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Controls, women: 29.3 (14.2–55.6)			Pancreatic cancer: Q2: 1.02 (0.57, 1.84) Q3: 1.24 (0.67, 2.31) Q4: 0.91 (0.51, 1.65) Per unit increase: 0.99 (0.86, 1.14) Liver cancer: Q2: 0.62 (0.29, 1.33) Q3: 0.72 (0.33, 1.56) Q4: 0.59 (0.27, 1.27) Per unit increase: 0.97 (0.79, 1.19)
	Results: Lowest quartile used as the reference group **Confounding:** Prostate cancer: years of school attendance, BMI, dietary fat intake, and vegetable intake; Bladder cancer: smoking status, smoking intensity, smoking duration, years of school attendance, occupation associated with risk for bladder cancer; Pancreatic cancer: smoking status, smoking intensity, smoking duration, dietary fat intake, and fruit and vegetable intake; Liver cancer: smoking status, years of school attendance, alcohol intake, and occupation associated with risk for liver cancer						
Bonefeld-Jorgensen et al. (2011) *Medium*	Greenland 2000–2003	Case-control	Greenlandic Inuit women with and without breast cancer, 76	Plasma Cases: 45.6 (Range = 11.6–124) Controls: 21.9 (Range = 1.5–172)	Breast cancer	OR per ln-unit increase in PFOS	1.030 (1.001, 1.070), p-value = 0.05
	Confounding: Age, BMI, pregnancy, cotinine, breastfeeding, and menopausal status						
Ghisari et al. (2014) *Medium*	Greenland 2000–2003	Case-control	Women of Greenland Inuit descent aged 18–80 years. Cases were diagnosed with breast cancer, 100	Serum	Breast cancer	OR (for high serum PFOS vs low)	CYP1A1; Ile/Val + Val/Val: 12.1 (1.29, 115); p = 0.029 CYP1B1; Leu/Leu: 11.2 (1.8, 71.1); p = 0.011 COMT; Val/Met + Met/Met 16.8 (1.68, 167); p = 0.016

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							CYP17; A1/A2 + A2/A2: 18.2 (1.67, 198.8); p = 0.017 CYP19_CT; CC: 9.6 (1.48, 62.4); p = 0.018 CYP19_TTTA; (TTTA)8–10: 29.3 (2.89, 298); p = 0.004
	Confounding: Age and cotinine.						
Ducatman et al. (2015) *Medium*	United States 2005–2006	Cross-sectional	Men from C8 Health Study, Ages 20–49, 9,169; Ages 50–69, 3,819	Serum Mean (SD): 22.18 (1.97)	Prostate-specific antigen (PSA) level	Regression coefficient (β) per ln-unit increase in PFOS GM ratio (GMR) (PSA < 4.0 ng/mL vs. PSA ≥ 4.0 ng/mL)	Age 20–49 β = 1, p-value = 0.71; GMR = 0.95 (0.71, 1.28) Age 50–69 β = 1, p-value = 0.99; GMR = 1.1 (0.98, 1.23)
	Confounding: Age, smoking status, average alcohol intake, and BMI[c]						
Ghisari et al. (2017) *Medium*	Denmark 1996–2002	Nested case-control	Adult women, 283	Serum Cases: 27.80 Controls: 28.77	Breast cancer	Relative risk ratio (RR) per ln-unit increase in PFOS, compared across genotypes: CYP1A1 (Ile462Val), CYP1B1 (Leu432Val), COMT (Val158Met), CYP17 (−34T > C), CYP19 (C > T)	Cohort: 1.15 (0.64, 2.08) CYP19 CC: 6.42 (1.08, 38.3), p-value < 0.05 No significant associations observed for remaining genotypes
	Results: Lowest tertile used as the reference group **Confounding:** Age at blood draw, BMI before pregnancy, total number of gravidities, oral contraceptives use, age of menarche, smoking status and alcohol intake during pregnancy, physical activity, maternal education.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Hurley et al. (2018) *Medium*	California, U.S. 2011–2015	Nested case-control	Adult women, 1,760	Serum Median (min–max): Cases: 6.695 (0.046–39.400) Controls: 6.950 (0.046–99.800)	Breast cancer (invasive)	OR per log10-unit increase in PFOS, or by tertiles	T3: 0.898 (0.695, 1.161) T2: 0.883 (0.691, 1.129) Per unit increase: 0.934 (0.683, 1.277), p-value = 0.67
	Confounding: Age at baseline enrollment, race/ethnicity, region of residence, date of blood draw, season of blood draw, total smoking pack-years, BMI, family history of breast cancer, age at first full-term pregnancy, menopausal status at blood draw, and pork consumption						
Cohn et al. (2020) *Medium*	United States 1959–2013	Nested case-control	Adult daughters of women in CHDS cohort, 310 controls, 102 cases	Perinatal serum Cases: 30.5 (14.1–55.8) Controls: 32.1 (14.9–58.2)	Breast cancer	OR per log2-unit increase in PFOS	0.3 (0.1, 0.9), p-value = 0.02
	Confounding: Maternal: cholesterol, age at pregnancy, history of breast cancer, primiparity, overweight at first prenatal visit, serum levels of DDTs and metabolite DDE, African American status, whether daughter was breastfed						
Mancini et al. (2020) *Medium*	France 1990–2013	Nested case-control	Postmenopausal women, Ages 40–65 in 1990, 194 cases, 194 controls	Serum 17.51 (5.83–85.26)	Breast cancer	ORs by quartiles, and by estrogen (ER) or progesterone receptor (PR) status	Overall: Q2: 1.94 (1, 3.78) Q3: 2.03 (1.02, 4.04) Q4: 1.72 (0.88, 3.36) p-trend = 0.25 ER positive: ORs of 1.8–2.4 p-trend = 0.04 ER negative: ORs of 4.7–15 p-trend = 0.72 PR positive: ORs of 1.8–2.7 p-trend = 0.02 PR negative: ORs of 1.7–3.5 p-trend = 0.93
	Results: Lowest quartile used as the reference group						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Total serum lipids, BMI, smoking status, physical activity, education level, personal history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of menopausal hormone therapy						
Shearer et al. (2021) *Medium*	United States 1993–2002	Nested case-control	Adults, 55–74, 648 Ages 55–59, 190 Ages 60–65, 224 Ages 65+, 234 Males 432 Females 216	Serum 38.4 (26.3–49.9)	Renal cell carcinoma	ORs per log2-unit increase in PFOS or by quartiles (total cohort only)	Q2: 1.67 (0.84, 3.3) Q3: 0.92 (0.45, 1.88) Q4: 2.51 (1.28, 4.92) p-trend = 0.009 Per doubling increase: 1.39 (1.04, 1.86)
	Results: Lowest quartile used as the reference group **Confounding:** BMI, smoking, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, calendar year of blood draw; sex, race and ethnicity, study year of blood draw, study center						
Fry and Power (2017) *Medium*	U.S. NHANES 2003–2006	Cohort	Adults, Ages 60+, 1,036	Serum Median (SE): 4.3 (0.2) ng/g lipid	Cancer mortality	Hazard ratio per SD-unit increase in PFOS	1.01 (0.86, 1.19), p-value = 0.88
	Confounding: Age, gender, race/ethnicity, and smoking status						
Goodrich et al. (2022) *Medium*	United States MEC Study Recruitment: 1993–1996	Nested case-control	Adults, 100 (50 cases, 50 controls)	Plasma GM (GSD): Cases: 29.2 (2.37) Controls: 29.2 (1.95)	Hepatocellular carcinoma	OR for >54.9 µg/L vs. ≤ 54.9 µg/L-PFOS, or per SD increase in PFOS	4.50 (1.20, 16.00), p-value = 0.02 Per SD increase: 1.20 (0.91, 1.60), p-value = 0.18
	Results: PFOS cutoff of 54.9 µg/L is the 85th percentile of PFOS in the study, and corresponds to the 90th percentile of PFOS exposures in the 1999–2000 NHANES **Confounding:** Age, sex, race, and study site						
Christensen et al. (2016a) *Low*	Wisconsin, U.S., 2012–2013	Cross-sectional	Male anglers, Ages 50+, 154	Serum 19.00 (9.80–28.00)	Cancer (any)	OR per unit increase in PFOS	0.99 (0.96, 1.01)
	Confounding: Age, BMI, work status, alcohol consumption						
Lin et al. (2020a) *Low*	China 2014–2017	Case-control	Children, <16, 84	Serum	Germ cell tumors	OR per unit increase in PFOS	1.08 (0.96, 1.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				4.47 (2.48–8.26)			
	Confounding: Infectious disease, cosmetics usage, barbecued food consumption, filtered water use, indoor decorating, living near farmland (maternal behaviors/factors during pregnancy)						
Tsai et al. (2020) *Low*	Taiwan 2014–2016	Case-control	Adult women, 239 Age 50 or younger, 120 Age over 50, 119	Plasma Mean (GM): 5.64 (4.77)	Breast cancer	OR per ln-unit increase in PFOS	Total cohort: 1.07 (0.64, 1.79) Age 50 or younger: 2.34 (1.02, 5.38), p-value < 0.05 ER+: 3.25 (1.29, 8.23) Age over 50: 0.62 (0.29, 1.29), p-value > 0.05
	Confounding: Pregnancy history, oral contraception use, abortion, BMI, menopause, and education level						
Itoh et al. (2021) *Low*	Japan 2001–2005	Case-control	Adult women, Ages 20–74, 802 (401 breast cancer cases, 401 controls)	Serum 14.27 (10.24–19.24)	Breast cancer	OR by quartiles	Q2: 0.38 (0.18, 0.82), p-value < 0.05 Q3: 0.31 (0.14, 0.69), p-value < 0.05 Q4: 0.15 (0.06, 0.39), p-value < 0.05 p-trend = 0.0001
	Results: Lowest quartile used as the reference group **Confounding:** Age, residential area, BMI, height, menopausal status, age at menopause, age at first childbirth, family history of breast cancer, smoking status, strenuous physical activity in the past five years, moderate physical activity in the past five years, age at menarche, number of births, breastfeeding duration, alcohol intake, isoflavone intake, education level, serum total concentrations of PCBs, fish and shellfish intake, vegetable intake, and calendar year of blood sampling						
Liu et al. (2021) *Low*	China 2016–2017	Case-control	Adult men, 96 Adult women, 223	Serum Case: 5.5 (3.6–8.8); Control: 7.5 (4.7–10.8)	Thyroid cancer	OR by quartiles	Total Q2: 0.81 (0.42, 1.53) Q3: 0.26 (0.12, 0.57) Q4: 0.28 (0.12, 0.66) p-trend = 0.001 Male: Q2: 1.13 (0.30, 4.23) Q3: 0.15 (0.02, 1.04)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Q4: 0.62 (0.15, 2.65) p-trend = 0.284 Female: Q2: 1.10 (0.52, 2.34) Q3: 0.33 (0.13, 0.80) Q4: 0.24 (0.09, 0.64) p-trend = 0.001
	Results: Lowest quartile used as the reference group **Confounding:** Age, sex, and diabetes status						
Omoike et al. (2021) *Low*	United States 2005–2012	Cross-sectional	Adults from NHANES, Ages ≥ 20 yr, 6,652	Serum 11.40 (6.45–19.68)	Cancers: ovarian, breast, uterine, and prostate	OR per unit increase in PFOS, or by quartiles	Ovarian cancer: Q2: 0.08 (0.08, 0.084) Q3: 1.64 (1.62, 1.66) Q4: 2.25 (2.22, 2.28) p-trend < 0.001 Per unit increase: 1.012 (1.012, 1.013) Breast cancer: Q2: 0.87 (0.86, 0.89) Q3: 1.06 (1.05, 1.06) Q4: 1.47 (1.46, 1.48) p-trend < 0.001 Per unit increase: 1.011 (1.011, 1.011) Uterine cancer: Per unit increase: 0.945 (0.944, 0.945) Prostate cancer: Per unit increase: 0.994 (0.994, 0.994)
	Results: Lowest quartile used as the reference group **Confounding:** Age, sex, education, race/ethnicity, PIR, BMI, and serum cotinine						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Cao et al. (2022) *Low*	China 2019–2021	Case-control	Adults and children, Ages 12–84 yr, 406 (203 cases, 203 controls)	Serum Cases: 7.2 (3.8–15) Controls: 5.5 (3.0–11)	Liver cancer	OR per log-ng/mL increase in PFOS	2.609 (1.179, 4.029) p-trend = 0.001
	Results: Logarithm base not specified **Confounding:** Age, education level, BMI, annual household income, sex, smoking habit, and medical history						

Notes: BMI = body mass index; CHDS = The Child Health and Development Studies; DDE = dichlorodiphenyldichloroethylene; DDT = dichloro-diphenyl-trichloroethane; ER = progesterone receptor; GM = geometric mean; GMR = geometric mean ratio; GSD = geometric standard deviation; IRR = incidence rate ratio; MEC = Multiethnic Cohort study; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PR = progesterone receptor; PSA = Prostate-specific antigen; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; T2 = tertile 2; T3 = tertile 3; U.S. = United States; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) in ng/mL unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval).

[c] Confounding indicates factors the models presented adjusted for.

Appendix E. Benchmark Dose Modeling

E.1 Epidemiology Studies

For the epidemiological studies considered for dose-response assessment, the U.S. Environmental Protection Agency (EPA) used multiple modeling approaches to determine points of departure (PODs), depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies, EPA used a hybrid modeling approach that involves estimating the prevalence of the outcome above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control (U.S. EPA, 2012) because EPA was able to define a level considered clinically adverse for these outcomes. Details are provided in the following sections. In addition, EPA re-expressed the reported regression (β) coefficients when modeling results for decreased birthweight when regression coefficients were reported per log-transformed units of exposure (see details in Section E.1.2). Sensitivity analyses to evaluate the potential impact of re-expression in a hybrid approach when modeling hepatic and serum lipid studies for perfluorooctane sulfonic acid (PFOS) showed little impact on benchmark doses (lower confidence limit) (BMDLs) (see Sections E.1.3 and E.1.4).

EPA also performed benchmark dose (BMD) modeling and provided study lowest-observed-adverse-effect levels/no-observed-adverse-effect levels (LOAELs/NOAELs) for the hepatic and serum lipid dose-response studies as sensitivity analyses of the hybrid approach. For the immune studies, where a clinically defined adverse level is not well defined, EPA used the results from the multivariate models provided in the studies and determined a benchmark response (BMR) according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012) (see Section E.1.1). For specific approaches used to determine PODs please see Table E-1.

Table E-1. Summary of Modeling Approaches for POD Derivation from Epidemiological Studies

Endpoint	Studies[a]	Reported Result or Beta (Units)	LogP FOS	Re-Expression (Yes/No)	Approach	BMR (SD or Cutoff)
Anti-tetanus and anti-diphtheria antibody response	**Budtz-Jørgensen and Grandjean (2018a)**	BMD = $\log_2(1-BMR)/\beta$ $\log_2$(tetanus or diphtheria) per ng/mL PFOS	Yes	No	BMD modeling BMD = $\log_2(1-BMR)/\beta$	0.5 SD and 1 SD
Anti-tetanus and anti-diphtheria antibody response	Timmerman et al. (2021)	Percent difference =$(10^{\beta}-1)*100$ $\log_{10}$(tetanus or diphtheria) per ng/mL PFOS	No	No	BMD modeling BMD = $\log_{10}(1-BMR)/\beta$	0.5 SD and 1 SD
Anti-rubella antibody response	Granum et al. (2013)	Rubella (IU/mL) per ng/mL PFOS	No	No	BMD modeling	0.5 SD and 1SD
Anti-rubella antibody response	Zhang et al. (2023c)	Percent difference = $(2.71^{\beta}-1)*100$ ln(rubella) per ln(ng/mL) PFOS	Yes	No; sensitivity analysis with re-expressed values	BMD modeling	0.5 SD and 1 SD

Endpoint	Studies[a]	Reported Result or Beta (Units)	LogP FOS	Re-Expression (Yes/No)	Approach	BMR (SD or Cutoff)
Decreased birth weight	**Wikström et al. (2020)** Chu et al. (2020) Sagiv et al. (2018) Starling et al. (2017) Darrow et al. (2013) Yao et al. (2021)	BW per ln(ng/mL) PFOS or per IQR PFOS	Yes	Yes	Hybrid	5% and 10%
Elevated ALT	**Nian et al. (2019)**	Percent difference = $(e^{\beta}-1)*100$ ln(ALT) per ln(ng/mL) PFOS	Yes	No; sensitivity analysis with re-expressed values	Hybrid	5% and 10%
Elevated ALT	Gallo et al. (2012)	ln(ALT) per ln(ng/mL) PFOS	Yes	No	Hybrid	5% and 10%
Increased total cholesterol	**Dong et al. (2019)**	TC per ng/mL PFOS	No	No	Hybrid	5% and 10%
Increased total cholesterol	Steenland et al. (2009)	ln(TC) per ln(ng/mL) PFOS	Yes	No; sensitivity analysis with re-expressed values	Hybrid Sensitivity analyses: LOAEL, BMDS	5% and 10%
Increased total cholesterol	Lin et al. (2019)	mean difference in TC (mg/dL) per quartile of PFOS (ng/mL)	No	No	BMDS	0.5 SD and 1 SD

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDS = Benchmark Dose Software; BMR = benchmark response; BW = birth weight; IQR = interquartile range; IU = international units; LOAEL = lowest-observed-adverse-effect levels; POD = point of departure; SD = standard deviation; TC = total cholesterol.

[a] Bolded study name identifies study result that advanced as the POD or selected approach.

E.1.1 Modeling Results for Immunotoxicity

E.1.1.1 Modeling Results for Decreased Tetanus Antibody Concentrations

E.1.1.1.1 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Tetanus Antibody Concentrations at 7 Years of Age and PFOS Exposure Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOS 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 perfluorooctanoic acid (PFOA) (as $\log_2$(PFOA)) (also called multi-PFAS models), and without PFOA (also called single-PFAS models). 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 PFOS concentration, 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 PFOS exposure without adjustment for PFOA using a likelihood ratio test ($p = 0.60$; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOA ($\log_2$(PFOA)) ($p = 0.71$).

Table E-2 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for PFOS at age 5 years and tetanus antibodies at age 7 years. These regression coefficients (β) and their standard errors (SE) were calculated by EPA from the authors Budtz-Jørgensen and Grandjean (2022, 2018a). As Budtz-Jørgensen and Grandjean (2018a) $\log_2$-transformed the outcome variable, the BMR measured in unit of $\log_2$(tetanus antibody concentration) was $\log_2(1-0.05) = 0.074$ $\log_2$(IU/mL)).

Table E-2. Results Specific to the Slope from the Linear Analyses of PFOS Measured at Age 5 Years and $\log_2$(Tetanus Antibody Concentrations) Measured at Age 7 Years from Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOA Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope (β_{LB}) ng/mL
PFOS at Age 5	Linear	No[a]	−0.0274	0.0176	p = 0.12	−0.0565
PFOS at Age 5	Linear	Yes[b]	−0.0039	0.0198	p = 0.84	−0.0365

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOS), and sex, exact age at the 7-year-old examination, and booster type at age 5 years.
[b] Multi-PFAS model: adjusted for PFOS and PFOA, and sex, exact age at the 7-year-old examination, and booster type at age 5 years.

Interpretation of results in Table E-2:

- PFOS is a non-significant predictor in the single-PFAS model ($\beta = -0.0274$; $p = 0.12$).
- Effects of PFOS in the single-PFAS model are attenuated when $\log_2$(PFOA) is included in the model ($\beta = -0.0039$; $p = 0.84$).
- Nevertheless, these data can be used to estimate a BMDL for completeness and to allow comparisons across PFAS.

E.1.1.1.1.1 Selection of the Benchmark Response

The 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 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 dataset 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.

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome.

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., 2017b) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL "cutoff" level "to determine whether antibody concentrations could be considered protective," and Galazka and Kardymowicz (1989) mentions the same concentration. However, the 2018 WHO update (WHO, 2018) argues that:

> *"…the minimum amount of circulating antitoxin that in most cases ensures immunity to tetanus is assay specific. Within in vivo neutralization tests, modified ELISAs or bead-based immunofluorescence assays, concentrations at or exceeding 0.01 IU/mL are usually considered protective against disease, whereas antitoxin concentrations of at least 0.1–0.2 IU/mL are defined as positive when ELISA techniques are used for the assessment. Cases of tetanus have been documented, however, in persons with antitoxin concentrations above these thresholds. Hence, a "protective antibody concentration" may not be considered a guarantee of immunity under all circumstances."*

In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 or ½ SD change from the control mean may be selected (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 E-1 replicates a figure in the Technical Guidance (page 23) (U.S. EPA, 2012) to show that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of 1 SD results in a ~10% extra risk of being at risk of having an adverse effect.

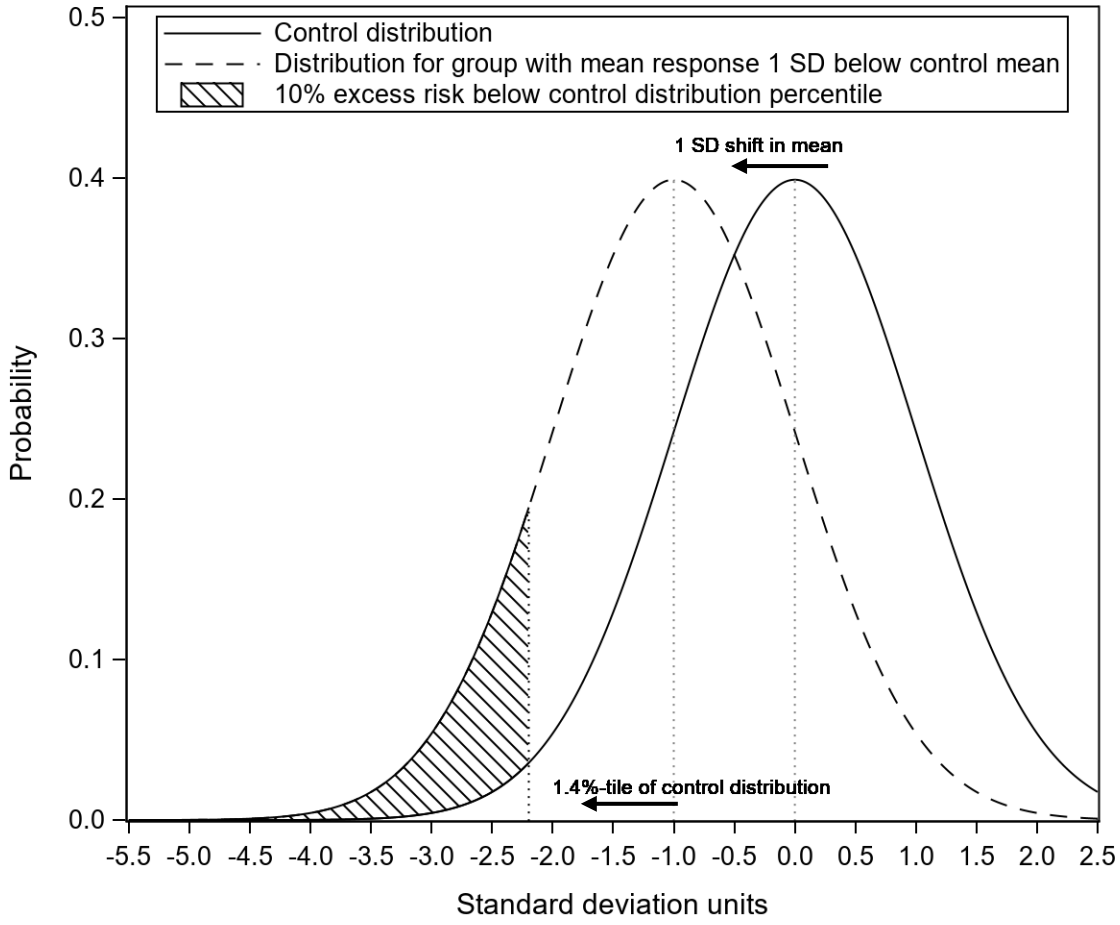


Figure E-1. Difference in Population Tail Probabilities Resulting from a One Standard Deviation Shift in the Mean from a Standard Normal Distribution, Illustrating the Theoretical Basis for a Baseline BMR of 1 SD

BMR = benchmark response; SD =standard deviation.

Statistically, the Technical Guidance additionally suggests that studies of developmental effects can support lower BMRs. Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

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 (CDC, 2011). The case-fatality rate can be more than 80% for early lifestage 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 immunotoxic 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 PFOS. 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 both a 1 SD change in the distribution of $\log_2$(tetanus antibody concentrations) and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations) (Table E-3). The SD of the $\log_2$(tetanus antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the 25th and 75th percentiles of the tetanus antibody concentrations at age 7 years in IU/mL were (0.65, 4.6). Log_2-tranforming these values provides the 25th and 75th percentiles in $\log_2$(IU/mL) as (−0.62, 2.20). Assuming that these $\log_2$-transformed values are reasonably represented by a normal distribution, the IQR (which is the difference between the 75th and 25th percentiles) is approximately 1.35 SDs (Rosner, 2015). Thus, SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (2.20–(−0.62))/1.35 = 2.09 $\log_2$(IU/mL).

While there was not a clear definition of the size 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 cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies at age 7 years in $\log_2$(IU/mL), EPA calculated that 2.8% of those values would be below the cutoff value of 0.1 IU/mL (i.e., −3.32 $\log_2$(IU/mL)). A BMR of ½ SD resulted in 7.9% of the values being below that cutoff, which is 5.1% extra risk. This demonstrates the generic guidance that a BMR of ½ SD can provide a reasonably good estimate of 5% extra risk. Figure E-2 shows an example of this.

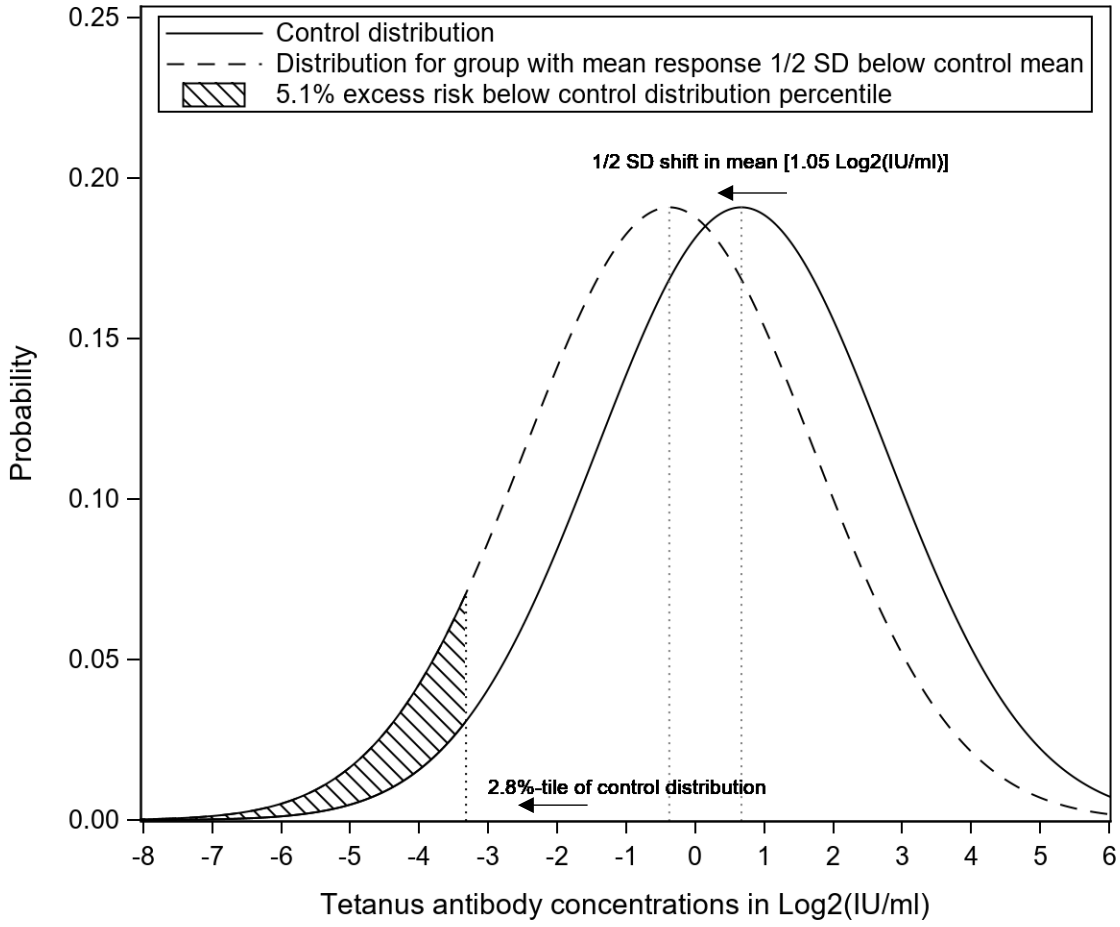


Figure E-2. Difference in Population Tail Probabilities Resulting from a ½ Standard Deviation Shift in the Mean from an Estimation of the Distribution of Log_2(Tetanus Antibody Concentrations at Age 7 Years)

IU = international units; SD =standard deviation.

Table E-3. BMDs and BMDLs for Effect of PFOS at Age 5 Years on Anti-Tetanus Antibody Concentrations at age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a BMR of ½ SD Change in Log_2(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_2(Tetanus Antibodies Concentration)

BMR	Estimated Without Control of PFOA		Estimated With Control of PFOA	
	BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
	$\beta = -0.274$ per ng/mL	$\beta_{LB} = -0.0565$ per ng/mL	$\beta = -0.0039$ per ng/mL	$\beta_{LB} = -0.0365$ per ng/mL
½ SD	38.1	18.5[a]	268	28.6
1 SD	76.2	37.0	536	57.3

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOS concentration measured at age 5 years was 3.3 ng/mL, the 5th percentile was 9.5 ng/mL, and the 10th percentile was 10.7 ng/mL (Grandjean and Bateson, 2021) so the

estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$ = 18.5 ng/mL) 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.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 7-fold higher than the $BMD_{½\ SD}$ estimate from the models with just PFOS, and the $BMDL_{½\ SD}$ estimates is 55% higher. 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 the 'better' estimate of the point estimate of the effect of PFOS considering potential confounding, the two $BMDL_{½\ SD}$ estimates are 55% different (18.5 ng/mL vs. 28.6 ng/mL). EPA advanced the derivation based on results that did not control for PFOA because this model appeared to fit PFOS better (p = 0.12 vs. 0.84) and there was moderate uncertainty due to potential confounding in the BMDL. However, confidence was diminished by the non-significant fit for PFOS (p = 0.12) and stronger potential confounding in the main effect—even though there was moderate confounding of the BMDL.

For immunotoxicity related to tetanus associated with PFOS exposure measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 18.5 ng/mL.

E.1.1.1.2 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Tetanus Antibody Concentrations at 5 Years of Age and PFOS Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOS 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 PFOA (as log_2(PFOA)), and without PFOA. Three model shapes of PFOS 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 with the linear model, the piecewise-linear model did not fit better than the linear model for either the PFOS exposure without adjustment for PFOA using a likelihood ratio test (p = 0.43; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOA (log_2(PFOA)) (p = 0.98).

Table E-4 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 obtained by EPA from the authors (Budtz-Jørgensen and Grandjean, 2022, 2018a).

Table E-4. Results of the Linear Analyses of PFOS Measured Perinatally and Tetanus Antibodies Measured at Age 5 Years from Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model and in a Multi-PFAS Model

Exposure	Model Shape	PFOA Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope ($β_{LB}$) ng/mL
Perinatal PFOS	Linear	No[a]	−0.0102	0.0095	p = 0.28	−0.0259
Perinatal PFOS	Linear	Yes[b]	0.0021	0.0107	p = 0.85	−0.0156

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOS), and sex, exact age at the 5-year-old examination, cohort, interaction terms between cohort and sex, and between cohort and age.
[b] Multi-PFAS model: adjusted for PFOS and PFOA, and sex, exact age at the 7-year-old examination cohort, and interaction terms between cohort and sex, and between cohort and age.

Interpretation of results in Table E-4:

- PFOS is a non-significant predictor in the single-PFAS model ($\beta = -0.0102$; $p = 0.28$).
- Effects are attenuated when $\log_2$(PFOA) are included in the model ($\beta = 0.0021$; $p = 0.85$).
- Nevertheless, these data can be used to estimate a BMDL for completeness and to allow comparisons across PFAS.

E.1.1.1.2.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 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 five-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017b) reported on 349 five-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately by the authors. EPA then pooled the summary statistics to describe the common SD. The 25th and 75th percentiles of the tetanus antibody concentrations in the earlier birth cohort at age 5 years in IU/mL were (0.10, 0.51). Log_2-tranforming these values provides the 25th and 75th percentiles in $\log_2$(IU/mL) as (−3.32, −0.97). Assuming that these $\log_2$-transformed values are similar to the normal distribution, 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 25th and 75th percentiles 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 25th and 75th percentiles 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).[8] To show the impact of the BMR on these results, Table E-5 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-5. BMDs and BMDLs for Effect of PFOS Measured Perinatally and Anti-Tetanus Antibody Concentrations at Age 5 Years (Budtz-Jørgensen and Grandjean, 2018a)

BMR	Estimated without control of PFOA		Estimated with control of PFOA	
	BMD (ng/mL) $\beta = -0.0102$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0259$ per ng/mL	BMD (ng/mL) $\beta = 0.00207$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0156$ per ng/mL
½ SD	75.9	29.9[a]	–[b]	49.8
1 SD	151.8	59.8	–	99.7

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD =standard deviation.
[a] Denotes the POD that corresponds to the analyses of PFOS concentrations perinatally and tetanus antibodies at age 5 years.
[b] Values cannot be determined.

The lowest perinatal maternal serum PFOS concentration measured was 9.4 ng/mL, the 5th percentile was 17.1 ng/mL, and the 10th percentile was 19.1 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDLs for a BMR of ½ SD ($BMDL_{½\ SD}$ = 29.9 ng/mL) 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. The $BMDL_{½\ SD}$ estimate from the single-PFAS models was 29.9 ng/mL. The BMDL estimates from the multi-PFAS models were about 67% higher than for the single-PFAS model.

Confidence is diminished by the low quality of the model fit for PFOS in either model compared with the PFOS 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 PFOS measured perinatally, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 29.9 ng/mL. Note that this result is based on a poorly fit PFOS regression parameter (β) estimated as −0.0102 per ng/mL (90% CI: −0.0259, 0.0055; p = 0.28) (Budtz-Jørgensen and Grandjean, 2018b).

For immunotoxicity related to tetanus associated with PFOS 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 29.9 ng/mL.

E.1.1.1.3 Timmerman et al. (2021)

Timmerman et al. (2021) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOS and log10-transformed anti-tetanus antibody concentrations measured at the same time as PFOS, controlling for time since vaccine booster/estimated time since vaccine booster, and duration of being breastfed (<6 months, 6–12 months, >1 year) and area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq) and including children with known tetanus-diphtheria booster date only. Estimates from the linear regression

[8] Pooled variance for tetanus in five-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).

models were subsequently back-transformed to express the percent difference in antibody concentrations at each ng/mL increase in serum PFOS concentrations in children, which was −3 (95% CI: −8, 3) (Table 4, Timmerman et al. (2021)). Using the equation provided below, EPA estimated the regression slope as −0.013 (95% CI: −0.036, 0.013).

$$Percent\ Difference = (10^{\beta} - 1) \times 100$$

Following the approach described previously for Budtz-Jørgensen and Grandjean (2018a), EPA derived BMDs and BMDLs were derived for both a one SD change in the distribution of $\log_{10}$ (tetanus antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_{10}$ (tetanus antibody concentrations) (Table E-6). The SD of the $\log_{10}$ (tetanus antibody concentrations) was estimated from the median (25th, 75th percentiles) of 0.92 (0.25, 2.20) tetanus antibody concentrations in IU/mL (Table 1 in Timmerman et al. (2021)). Log_{10}-transforming these values results in 25th and 75th percentiles in $\log_{10}$ (IU/mL) as −0.60 and 0.34, respectively. Assuming that these $\log_{10}$-transformed values are reasonably represented by a normal distribution, the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of tetanus antibodies in $\log_{10}$ (IU/mL) is (0.34 – (−0.60))/1.35 = 0.70 $\log_{10}$ (IU/mL).

Table E-6. BMDs and BMDLs for Effect of PFOS on Anti-Tetanus Antibody Concentrations (Timmermann et al., 2021) Using a BMR of ½ SD Change in Log_{10}(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_{10}(Tetanus Antibodies Concentration)

BMR	BMD (ng/mL) β = −0.013 per ng/mL	BMDL (ng/mL) β = −0.036 per ng/mL
½ SD	26.4	9.66
1 SD	52.9	19.3

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD =standard deviation.

As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies in $\log_{10}$ (IU/mL), a BMR of ½ SD resulted in 10.6% extra risk. This suggests that, in this case, a BMR of ½ SD may not be a reasonably good estimate of 5% extra risk.

Note that this BMDL is based on a poorly fit PFOS regression parameter (β) estimated as −0.013 (95% CI: −0.036, 0.013) (Timmermann et al., 2021).

For immunotoxicity related to tetanus associated with PFOS exposure measured at ages 5 to 10 years old, the POD estimated for comparison purposes was based on a BMR of ½ SD and a $\text{BMDL}_{½\ SD}$ of 9.7 ng/mL.

E.1.1.1.4 Summary of Modeling Results for Decreased Tetanus Antibody Concentrations

Table E-7 summarizes the PODs resulting from the modeling approaches for decreased tetanus antibody concentrations. The selected and comparison PODs were based on a BMR of ½ SD, resulting in BMDLs ranging from 9.7 to 29.9, with the selected POD of 18.5 also representing the median of the BMDLs.

Table E-7. BMDLs for Effect of PFOS on Anti-Tetanus Antibody Concentrations Using a BMR of ½ SD (Timmermann et al., 2021)

Study	Effect	$BMDL_{½\ SD}$ (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018a)	PFOS at age 5 years and anti-tetanus antibody concentrations at age 7 years	**18.5**	1.05 log_2 (IU/mL)
Budtz-Jørgensen and Grandjean (2018a)	PFOS perinatally and anti-tetanus antibody concentrations at age 7 years	29.9	0.78 log_2 (IU/mL)
Timmerman et al. (2021)	PFOS and anti-tetanus antibody concentrations at ages 7–12 years	9.66	0.35 log_{10} (IU/mL)

Notes: BMDL = benchmark dose lower limit; BMR = benchmark response; IU = international units; SD =standard deviation.

E.1.1.2 Modeling Results for Decreased Diphtheria Antibody Concentrations

E.1.1.2.1 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Diphtheria Antibody Concentrations at 7 Years of Age and PFOS Exposure Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOS 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 PFOA (as log_2(PFOA)), and without PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFOS, 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 PFOS exposure without adjustment for PFOA using a likelihood ratio test ($p = 0.30$; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOA (log_2(PFOA)) ($p = 0.34$). Table E-8 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These β and their SE were obtained by EPA from study authors (Budtz-Jørgensen and Grandjean, 2022, 2018a).

Table E-8. Results Specific to the Slope from the Linear Analyses of PFOS Measured at Age 5 Years and Log_2(Diphtheria Antibodies) Measured at Age 7 Years from Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOA Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope ($β_{LB}$) ng/mL
PFOS at Age 5	Linear	No[a]	−0.0322	0.0163	p = 0.05	−0.0591
PFOS at Age 5	Linear	Yes[b]	−0.0207	0.0184	p = 0.26	−0.0510

Notes: SE = standard error.

[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOS), and sex, exact age at the 7-year-old examination, and booster type at age 5 years.

[b] Multi-PFAS model: adjusted for PFOS and PFOA, and sex, exact age at the 7-year-old examination, and booster type at age 5 years.

Interpretation of results in Table E-8:

- PFOS is a significant predictor in the single-PFAS model ($\beta = -0.0322$; $p = 0.05$).
- Effects are attenuated when $\log_2$(PFOA) are included in the model ($\beta = -0.0207$; $p = 0.26$).
- The point estimate results for PFOS are potentially confounded by PFOA since there was a 36% reduction in the effect size for PFOS from −0.0322 to −0.0207 when controlling for PFOA.
- One explanation is that PFOA was a confounder of the PFOS effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.
- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which estimate is the best representation of any effect of PFOS.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. This is because there is only 36% difference in the BMD and 16% difference in the BMDL when PFOS is included in the model.

E.1.1.2.1.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which state that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 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. (2017b) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL 'cutoff' level; and Galazka et al. (1993) mentions the same concentration, but also 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 (1993) mentions a case-fatality rate of 5%–10%) and PFOS-related changes in anti-diphtheria antibody concentrations cannot be considered 'minimally adverse' given the historic lethality of diphtheria in the absence of vaccination. 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 immunotoxic effect.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 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 25th and 75th percentiles of the diphtheria antibody concentrations at age 7 years in IU/mL were (0.4, 1.6). Log_2-tranforming these values provides the 25th and 75th percentiles in $\log_2$(IU/mL) as (−1.32, 0.68). Assuming that these $\log_2$-transformed values are similar to the normal distribution, 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 E-9 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-9. BMDs and BMDLs for Effect of PFOS at Age 5 Years on Anti-Diphtheria Antibody Concentrations at Age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a

BMR of ½ SD Change in Log_2(Diphtheria Antibodies Concentration) and a BMR of 1 SD Log_2(Diphtheria Antibodies Concentration)

BMR	Estimated Without Control of PFOA		Estimated With Control of PFOA	
	BMD (ng/mL) β = −0.0322 per ng/mL	BMDL (ng/mL) β_{LB} = −0.0592 per ng/mL	BMD (ng/mL) β = −0.0207 per ng/mL	BMDL (ng/mL) β_{LB} = −0.0510 per ng/mL
½ SD	23.0	12.5[a]	35.8	14.5
1 SD	46.0	25.0	71.7	29.0

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD =standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOS concentration measured at age 5 years was 3.3 ng/mL, the 5th percentile was 9.5 ng/mL, and the 10th percentile was 10.7 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$ = 12.5 ng/mL) 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 PFOS well (p = 0.05).

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 56% higher than the $BMD_{½\ SD}$ estimate from the model with just PFOS, and the $BMDL_{½\ SD}$ is 16% higher. This 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 PFOS in light of potential confounding, the two $BMDL_{½\ SD}$ estimates that serve as the PODs are comparable (12.5 ng/mL vs. 14.5 ng/mL). EPA advanced POD based on results that did not controls for PFOA because this model appeared to fit PFOS data better (p = 0.05 vs. 0.26) and there was low uncertainty due to potential confounding in the BMDL. However, confidence was diminished by the potential confounding in the main effect—even though there was low confounding of the BMDL.

For immunotoxicity related to diphtheria, associated with PFOS measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 12.5 ng/mL.

E.1.1.2.2 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Diphtheria Antibody Concentrations at 5 Years of Age and PFOS Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOS 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 PFOA (as log_2(PFOA)), and without PFOA. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFOS, 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 with the linear model, the piecewise-linear model did not fit better than the linear model for either the PFOS exposure without adjustment for PFOA using a likelihood ratio test (p = 0.55; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOA (log_2(PFOA)) (p = 0.84). Table E-10 summarizes the results from Budtz-

Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These β and their SE were obtained by EPA from the study authors (Budtz-Jørgensen and Grandjean, 2022, 2018a).

Table E-10. Results of the Linear Analyses of PFOS Measured Perinatally and Diphtheria Antibodies Measured at age 5 Years from Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOA Adjusted	Slope (β) per ng/mL	SE (β)	Slope (β) Fit	Lower Bound Slope (β_{LB})
Perinatal PFOS	Linear	No[a]	−0.0310	0.0100	p = 0.002	−0.0475
Perinatal PFOS	Linear	Yes[b]	−0.0241	0.0113	p = 0.033	−0.0427

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOS), and sex, and exact age at the 5-year-old examination.
[b] Multi-PFAS model: adjusted for PFOS and PFOA and sex, and exact age at the 5-year-old examination.

Interpretation of results in Table E-10:

- PFOS is a significant predictor in the single-PFAS model (β = −0.0310; p = 0.002).
- Effects of PFOS are attenuated when PFOA is in the model (β = −0.0241; p = 0.033).
- Results for PFOS are potentially confounded by PFOA since there was a 22% change in the effect size for PFOS from −0.0310 to −0.0241 when controlling for PFOA.
- One explanation is that PFOA was a confounder of the PFOS effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.
- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which estimate is the best representation of any effect of PFOS.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. This is because there is only a 22% difference in the BMD and 11% difference in the BMDL when PFOS is included in the model.

E.1.1.2.2.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a

developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 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. (2017b) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately by the author. EPA then pooled the summary statistics to describe the common SD. The 25th and 75th percentiles of the diphtheria antibody concentrations in the earlier birth cohort at age 5 years in IU/mL were (0.05, 0.4). Log_2-tranforming these values provides the 25th and 75th percentiles in $\log_2$(IU/mL) as (−4.32, −1.32). Assuming that these $\log_2$-transformed values are similar to the normal distribution, 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 25th and 75th percentiles of the diphtheria antibody concentrations in the later birth cohort at age 5 years in IU/mL were (0.1, 0.3). Log_2-tranforming these values provides the 25th and 75th percentiles 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).[9] To show the impact of the BMR on these results, Table E-11 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-11. BMDs and BMDLs for Effect of PFOS Measured Perinatally and Anti-Diphtheria Antibody Concentrations at age 5 Years (Budtz-Jørgensen and Grandjean, 2018a)

BMR	Estimated Without Control of PFOA		Estimated With Control of PFOA	
	BMD (ng/mL) $\beta = -0.031$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0475$ per ng/mL	BMD (ng/mL) $\beta = -0.0241$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0427$per ng/mL
½ SD	30.6	20.0[a]	39.4	22.3
1 SD	61.3	40.0	78.9	44.5

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD =standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOS concentration measured perinatally was 9.4 ng/mL, the 5th percentile was 17.1 ng/mL, and the 10th percentile was 19.1 ng/mL (Grandjean and Bateson, 2021) so the estimated BMD for a BMR of ½ SD ($\text{BMDL}_{½\ \text{SD}}$ = 20.0 ng/mL) in the single-PFAS model is well within the observed range. No information was available to judge the fit of the model in the

[9] Pooled variance for diphtheria in 5-year-olds = [(502−1)(2.22)^2+(298−1)(1.17)^2]/[502+298−2] = 3.60. The pooled SD is the square root of 3.60 which is 1.90 $\log_2$(IU/mL).

range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFOS well (p = 0.002).

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 29% higher than the $BMD_{½\ SD}$ estimated from the model with just PFOS, and the $BMDL_{½\ SD}$ is 12% higher. This may, or may not, reflect control for any potential confounding of the regression effect estimates. The BMDLs that serve as the PODs are comparable (20.0 ng/mL vs. 22.3 ng/mL) and EPA advanced the derivation based on results that did not control for PFOA because this model appeared to fit PFOS well (p = 0.002 vs. 0.031) and there was low uncertainty due to potential confounding in the BMD and moderate uncertainty in the BMDL.

For immunotoxicity related to diphtheria, associated with PFOS measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 20.0 ng/mL.

E.1.1.2.3 Timmerman et al. (2021)

Timmerman et al. (2021) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOS against log10-transformed anti-diphtheria antibody concentrations measured at the same time as PFOS, controlling for time since vaccine booster/estimated time since vaccine booster, and duration of being breastfed (<6 months, 6–12 months, >1 year) and area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq) and including children with known tetanus-diphtheria booster date only. Estimates from the linear regression models were subsequently back-transformed to express the percent difference in antibody concentrations at each ng/mL increase in serum PFOS concentrations in children, which was −9 (95% CI: −16, 2) (Table 4, Timmerman et al. (2021)). Using the equation provided below, EPA estimated the regression slope as −0.04 (95% CI: −0.08, 0.01).

$$Percent\ Difference = (10^{\beta} - 1) \times 100$$

Following the description provided for Budtz-Jørgensen and Grandjean (2018a), EPA derived BMDs and BMDLs for both a one SD change in the distribution of log_{10} (diphtheria antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of log_{10} (diphtheria antibody concentrations) (Table E-12). The SD of the log_{10} (diphtheria antibody concentrations) was estimated from the distributional data presented in Table 1 as follows: the 25th and 75th percentiles of the diphtheria antibody concentrations in IU/mL were 0.02 and 0.28, respectively. Log_{10} -transforming these values provides the 25th and 75th percentiles in log_{10} (IU/mL) as (−1.7, −0.55). Assuming that these log_{10} -transformed values are reasonably represented by a normal distribution, the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of tetanus antibodies in log_{10}(IU/mL) is 0.85 log_{10}(IU/mL).

Table E-12. BMDs and BMDLs for Effect of PFOS on Anti-Diphtheria Antibody Concentrations (Timmermann et al., 2021) Using a BMR of ½ SD Change in Log_{10}(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_{10}(Tetanus Antibodies Concentration)

BMR	BMD (ng/mL) β = −0.11 per ng/mL	BMDL (ng/mL) β = −0.28 per ng/mL
½ SD	10.4	5.61
1 SD	20.7	11.2

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.

As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of diphtheria antibodies in $\log_{10}$ (IU/mL), EPA calculated that 57% of those values would be below the cutoff value of 0.1 IU/mL. A BMR of ½ SD resulted in 75% of the values being below that cutoff, which is 18% extra risk. This suggest that in this case a BMR of ½ SD may not be a reasonably good estimate of 5% extra risk.

Note that this result is based on a poorly fit PFOS regression parameter (β) estimated as −0.04 (95% CI: −0.08, 0.01) (Timmermann et al., 2021).

For immunotoxicity related to tetanus associated with PFOS exposure measured at ages 5 to 10 years old, the POD estimated for comparison purposes were based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 5.6 ng/mL.

E.1.1.2.4 Summary of Modeling Results for Decreased Diphtheria Antibody Concentrations

Table E-13 summarizes the PODs resulting from the modeling approaches for decreased tetanus antibody concentrations. The selected and comparison PODs were based on a BMR of ½ SD, resulting in BMDLs ranging from 5.6 ng/mL to 20.0 ng/mL with the selected POD of 12.5 also representing the median of the BMDLs. The comparison PODs are considered *low* confidence because they are based on a poorly fit PFOS regression parameters.

Table E-13. BMDLs for Effect of PFOS on Anti-Diphtheria Antibody Concentrations Using a BMR of ½ SD (Timmermann et al., 2021)

Study Name	Effect	BMDL (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018a)	PFOS at age 5 years on anti-diphtheria antibody concentrations at age 7 years	**12.5**	0.74 $\log_2$(IU/mL)
Budtz-Jørgensen and Grandjean (2018a)	PFOS perinatally on anti-diphtheria antibody concentrations at age 7 years	20.0	0.95 $\log_2$(IU/mL)
Timmerman et al. (2021)	PFOS and anti-diphtheria antibody concentrations at ages 7–12 years	5.6	0.48 $\log_{10}$(IU/mL)

Notes: BMDL = benchmark dose lower limit; IU = international units; SD = standard deviation.

E.1.1.3 Modeling Results for Decreased Rubella Antibody Concentrations

E.1.1.3.1 Granum et al. (2013)

Granum et al. (2013) investigated the association between prenatal exposure to perfluorinated compounds and vaccination responses and clinical health outcomes in early childhood in the BraMat subcohort of the Norwegian Mother and Child Cohort Study. A total of 56 mother-child pairs with maternal blood samples at delivery and blood samples from the children at 3 years of age were evaluated. Antibody titers specific to rubella were measured in 50 serum samples. Prenatal exposure to PFOS (mean = 5.6 ng/mL) was inversely associated with rubella antibody levels at age 3. Granum et al. (2013) fit multivariate linear regression models of maternal PFOS

against antibody concentrations in units of optical density (OD) adjusted for maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up. The estimated regression coefficient and 95% confidence interval was −0.08, 95% CI: −0.14, −0.02 (Table 4, Granum et al., 2013). The summary statistics for rubella antibody levels at the age of 3 in units of OD were median = 1.9; 25th, 75th percentiles: 1.5, 2.1. Study authors were contacted to provide these summary statistics in units of IU/mL (median = 60.6; 25th, 75th percentiles: 41.8, 80.2), and the corresponding regression coefficient and 95% confidence interval: −5.1, 95% CI: −9.0, −1.1 (Table E-14).

Table E-14. Levels of Rubella Vaccine-Induced Antibodies at the Age of 3 Years (Adapted from Table 3 in Granum et al. (2013))

Parameter	Optical Density (OD)	IU/mL[a]
25th percentile	1.5	41.8
Median	1.9	60.6
75th percentile	2.1	80.2
Min–Max	0.8–2.4	15.0–120.0
Mean	1.7	61.6
0.5 SD	0.22	14.3
1 SD	0.44	28.6
β (95% CI) for PFOS	−0.08 (−0.14, −0.02)	−5.1 (−9.0, −1.1)

Notes: IU = international units; OD = optical density; SD = standard deviation.
[a] Authors were contacted to provide summary statistics for rubella antibody levels in IU/mL (n = 50).

Following the technical guidance (U.S. EPA, 2012) and the approach described previously for Budtz-Jørgensen and Grandjean (2018, 5083631; see Section E.1.1.1.1) and accounting for the fact that here the outcome variable is not log-transformed, EPA derived BMDs and BMDLs for both a 1 and ½ SD change from the control mean in the distribution of rubella antibody concentrations. However, rubella differs from diphtheria and tetanus in that several levels for rubella antibody have been cited in the literature as "protective levels," representing a clinically significant cutoff for an adverse response. These levels vary depending on geography and study, ranging from 4 IU/mL in Finland (Davidkin et al., 2008), to 11 IU/mL in Iran (Honarvar et al., 2013), or 15 IU/mL in the United States (Tosh et al., 2009). However, 10 IU/mL appears to be the most widely accepted standard for rubella immunity. For example, Skenzdel et al. (1996) noted:

> *"…The Rubella Subcommittee of the National Committee for Clinical Laboratory Standards has proposed lowering the breakpoint to define rubella immunity from 15 to 10 IU/mL. This recommendation stems from epidemiologic studies on vaccinated persons with low levels of antibody and anecdotal reports. Additional support comes from Centers for Disease Control and Prevention studies and reports. The effectiveness of rubella vaccination is well documented and the 10 IU/mL antibody level is protective in the vast majority of persons… The Subcommittee, recognizing that sporadic and conflicting reports may suggest a relationship between antibody levels and protection against the rubella virus, did not advocate lowering the breakpoint <10 IU/mL"*

Charlton et al. (2016), provides further context:

> *"…the level of rubella IgG antibody is used as a surrogate marker for protection. In 1985, the Rubella Subcommittee of the National Committee on Clinical Laboratory Standards (NCCLS) set a level of >15 IU/ml for rubella IgG antibodies as the indicator of immunity. In light of further epidemiological investigations, and additional studies indicating that individuals with low levels of antibody (<15 IU/ml) produced a secondary immune response upon vaccine challenge rather than a primary immune response, these cut offs were revised by the Subcommittee from 15 IU/ml to 10 IU/ml in 1992. However, since 1992, the rubella cutoffs have not been assessed."*

As noted by Charlton et al. (2016) and the other literature cited above, the geographical variability, lack of consensus, and relatively dated assessment of this cutoff precludes its use as the basis of the BMR.

In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 or ½ SD change from the control mean may be selected (U.S. EPA, 2012). The SD of the rubella antibody concentrations in OD units was estimated from the distributional data provided in Table 3 in Granum et al. (2013): the 25th and 75th percentiles of the rubella antibody concentrations in OD units were 1.5 and 2.1, respectively. Assuming that these values are reasonably represented by a normal distribution, the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of rubella antibodies in OD is 0.44. The SD of rubella antibodies in IU/mL units was provided by study authors and was 28.6. Table E-15 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD. Note that the estimated BMD/BMDLs were the same regardless of the units (OD or IU/mL) used in the analysis.

As an additional check, EPA evaluated how much extra risk would have been associated with a BMR set at a 10 IU/mL cutoff value for rubella seropositivity, given the uncertainty in definitive cutoffs for rubella in OD or IU/mL units discussed above. Because rubella antibody levels were reported in OD units and IU/mL units, EPA investigated the extra risk using both units.

First, the extra risk was investigated using the distributional data in OD units and the BMR cutoff value of 0.990 or 0.927 OD, which were used to determine rubella seropositivity in Granum et al. (2013). Communications with the study authors confirmed that in Granum et al. (2013), two different OD cutoffs were used for rubella seropositivity in two different runs: >0.990 OD or >0.927 OD (Stølevik, 2012). Of the 50 samples, 47 samples were seropositive. The remaining three samples were equivocal (i.e., between 0.590–0.990 or 0.553–0.927 OD). None of the 50 samples were considered seronegative (i.e., <0.590 or <0.553 OD) for rubella. All participants were vaccinated for rubella, and Granum et al. (2013) noted that "[c]hildren not following the Norwegian Childhood Vaccination Program (n = 4) were excluded from the statistical analyses regarding vaccination responses."

Using these BMR cutoffs and the distribution of rubella antibodies in OD, EPA calculated that 1.4–2.0% of the values would be below the cutoffs. A BMR of ½ SD resulted in 4.6% or 6.1% of the values being below the cutoffs of 0.927 or 0.990 OD, respectively, which is ~4% extra risk. A BMR of 1 SD resulted in 12% or 15% of the values being below the cutoffs of 0.927 or 0.990 OD, respectively, which is ~12.7% extra risk. This suggests that in this case, BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk.

Then, using the distributional data of rubella antibodies in IU/mL and a cutoff of 10 IU/mL, which was considered as the protective antibody level for rubella, EPA calculated that 3.8% of the values would be below the cutoff. A BMR of ½ SD resulted in 10% of the values being below the cutoff, which is ~6.3% extra risk. A BMR of 1 SD resulted in 21.8% of the values being below the cutoff, which is ~18% extra risk. This further suggests that in this case, BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk.

Table E-15. BMDs and BMDLs for Effect of Maternal Serum PFOS on Anti-Rubella Antibody Concentrations in Children Using a BMR of ½ SD Change in Rubella Antibodies Concentration and a BMR of 1 SD Change in Rubella Antibodies Concentration (Granum et al., 2013)

BMR	BMD (ng/mL) β = −0.08 per ng/mL (For Units of OD) β = −5.1 per ng/mL (For Units of IU/mL)	BMDL (ng/mL) β = −0.14 per ng/mL (For Units of OD) β = −9.0 per ng/mL (For Units of IU/mL)
½ SD	2.8	1.6
1 SD	5.7	3.2

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; IU = international units; OD = optical density; SD = standard deviation.

For immunotoxicity related to Rubella associated with PFOS exposure measured at age three years old, the POD estimated for comparison purposes were based on a BMR of ½ SD and a BMDL½ SD of 1.6 ng/mL.

E.1.1.3.2 Zhang et al. (2023c)

Zhang et al. (2023c) investigated the association between exposure to PFAS and vaccination responses in children aged 12 to 19 years. A total of 819 children in the United States were evaluated from the 2003–2004 and 2009–2010 cycles of NHANES. Antibody titers specific to rubella, mumps, and measles were measured in serum, and rubella antibody levels were inversely associated with PFOS serum levels (mean = 12.44 ng/mL).

Zhang et al. (2023c) fit multivariate regression models of natural log (ln) transformed serum PFOS concentrations against ln-transformed anti-rubella antibody levels in children, adjusting for age, sex, race, income−poverty ratio, BMI, serum cotinine concentrations, survey cycle, and dietary intake of milk and milk products, eggs, and meat. Estimates from the linear regression models for the total population were then back-transformed to express the results as percent difference in rubella antibody concentrations per each 2.7-fold increase in serum PFAS concentration, which was −8.16 (95% CI: −13.67, −2.31, Table 2, Zhang et al. (2023c).

Using the equation provided below, EPA estimated the regression slope as −0.085 (95% CI: −0.15, −0.02).

$$Percent\ Difference = (2.71^{\beta} - 1) \times 100$$

Zhang et al. (2023c) also reported summary statistics of PFOS concentration in ng/mL (GM: 12.44; 25th, 75th percentiles: 7.35, 21.90) and of rubella antibody levels in IU/mL (GM: 45.21; 25th, 75th percentiles: 31.25, 64.52) for the total population of 819 participants. All participants had detectable levels of PFOS in serum.

As a sensitivity analysis, EPA also re-expressed the reported β coefficients in terms of per ng/mL, according to Dzierlenga et al. (2020). Then EPA used the re-expressed β and lower limit on the confidence interval to estimate BMD and BMDL.

EPA considered a similar approach to those described above for decreased tetanus antibody concentrations in Budtz-Jørgensen and Grandjean (2018, 5083631; see Section E.1.1.1.1), to estimate the BMD/BMDL associated with decreased rubella antibody concentrations in Zhang et al. (2023c). In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 or ½ SD change from the control mean may be selected (U.S. EPA, 2012). Table E-16 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

As an additional check, EPA evaluated how much extra risk would have been associated with a BMR set at a 10 IU/mL cutoff value for rubella seropositivity. EPA calculated that 0.25% of the values would be below the cutoff. A BMR of ½ SD resulted in 1.1% of the values being below the cutoff, which is ~0.8% extra risk. A BMR of 1 SD resulted in 3.5% of the values being below the cutoff, which is ~3.3% extra risk. The Benchmark Dose Technical Guidance (U.S. EPA, 2012) explains that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of 1 SD results in about 10% extra risk of having an adverse effect. However, the BMR cutoff value of 10 IU/mL in the observed distribution of rubella antibodies in ln(IU/mL) resulted in only 0.25% of the control population at risk of having an adverse effect, a value much smaller than 1.4% recommended by the technical guidance, suggesting that, in this case, a BMR of 1 SD or ½ SD may not be a reasonably good estimate of 10% and 5% extra risk, respectively.

This may be due to the way the study population was restricted to only seropositive adolescents. The 886 NHANES children with complete data had a rubella seropositivity rate of 96.39%. Participants without detectable antibodies were excluded and only the 819 children with detectable antibody serum levels to both measles and rubella (as a proxy for having measles−mumps−rubella (MMR) vaccination, to reduce confounding by vaccination and health consciousness) were included in the final study population. This makes it likely that the children in the study all had antibody rubella levels above the hypothesized clinical threshold of 10 IU/mL.

Table E-16. BMDs and BMDLs for Effect of PFOS on Anti-Rubella Antibody Concentrations in Adolescents (Zhang et al., 2023c) Using a BMR of ½ SD Change in Ln(Rubella Antibodies Concentration) and a BMR of 1 SD Change in Ln(Rubella Antibodies Concentration)

BMR	BMD (ln(ng/mL)) β = −0.085 per ln(ng/mL)	BMDL (ln(ng/mL)) β = −0.147 per ln(ng/mL)	BMD (ng/mL) β = −0.006 per ng/mL	BMDL (ng/mL) β = −0.011 per ng/mL
½ SD	3.2	1.8	41.9	24.3
1 SD	6.3	3.7	83.8	48.6

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.

For immunotoxicity related to rubella associated with PFOS exposure measured at ages 12 to 19 years old, the POD estimated for comparison purposes were based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 1.8 ln (ng/mL) or 24.3 ng/mL.

E.1.1.3.3 Summary of Modeling Results for Decreased Rubella Antibody Concentrations

Table E-17 presents the BMDs and BMDLs for all studies considered for POD derivation, with and without accounting for re-expression of the reported β coefficients in terms of per ng/mL when necessary.

Table E-17. BMDs and BMDLs in ng/mL for Effect of PFOS on Anti-Rubella Antibody Concentrations

Study	Exposure Mean	Reported β (95% CI) Units	Re-Expressed β (95% CI) Ln(IU/mL)/(ng/mL)	BMR = ½ SD		BMR = 1 SD	
				BMD	BMDL	BMD	BMDL
Granum et al. (2013)	5.6	−5.1 (−9.0, −1.1) (IU/mL)/ng/mL	NA	2.8	1.6	5.7	3.2
Zhang et al. (2023c)	12.4	−8.16 (−13.67, −2.31) (IU/mL)/ln(ng/mL)	NA	23.4	6.2	549.3	38.6
Zhang et al. (2023c)	12.4	−8.16 (−13.67, −2.31) (IU/mL)/ln(ng/mL)	−0.0006 (−0.0111, −0.0018)	41.9	24.3	83.9	48.6

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; CI = confidence interval; IU = international units; NA = not applicable; SD =standard deviation; SE = standard error.

Table E-18 summarizes the PODs resulting from the modeling approaches for decreased rubella antibody concentrations. The selected and comparison PODs were based on a BMR of ½ SD, resulting in BMDLs ranging from 1.6 ng/mL to 24.3 ng/mL with the selected POD of 1.6 ng/mL.

Table E-18. BMDLs for Effect of PFOS on Anti-Rubella Antibody Concentrations Using a BMR of 5%

Study Name	Effect	BMDL (ng/mL)
Granum et al. (2013)	PFOS prenatally on anti-rubella antibody concentrations at age three years	1.6
Zhang et al. (2023c)	PFOS and anti-rubella antibody concentrations at ages 12–19 years	24.3

Notes: BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.2 Modeling Results for Decreased Birthweight

Six *high* confidence studies (Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Darrow et al., 2013) reported decreased birth weight in infants whose mothers were exposed to PFOS. These candidate studies offer a variety of PFOS exposure measures across the fetal and neonatal window. All six studies reported their exposure metric in units of ng/mL and reported the β coefficients per ng/mL or ln(ng/mL), along with 95% confidence intervals, estimated from linear regression models. The logarithmic transformation of

exposure yields a negative value for small 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 confidence interval 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 $\bar{y}$ using an average birth weight from an external population as $\bar{y}$, an average exposure as $\bar{x}$ 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 3,261.6 ± 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., 2500 g, or 5.5 lb). The full natality data for the United States data on birth weight was used as it is more relevant for deriving toxicity values for the U.S. general public 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 cutoff value for clinical adversity. America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals (https://www.epa.gov/americaschildrenenvironment/data-tables-biomonitoring-perfluorochemicals-pfcs) provides in Table B6b the median blood serum levels of PFOS of 2.6 ng/mL in 2015–2016 in woman ages 16 to 49, using 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 four epidemiological studies.

E.1.2.1 Chu et al. (2020)

Chu et al. (2020) reported a β coefficient of −83.3 g (95%CI: −133.2, −33.4) per ln(ng/mL) increase for the association between birth weight and maternal PFOS serum concentrations (collected within 3 days of delivery) in a China cohort. The reported β coefficient can be re-expressed in terms of per ng/mL according to Dzierlenga et al. (2020). Given the reported study-specific median (7.2 ng/mL) and the 25th and 75th percentiles (4.4 and 11.9 ng/mL) of the exposure from Chu et al. (2020), EPA estimated the distribution of exposure by assuming the exposure follows a lognormal distribution with mean and standard deviation as:

$$\mu = ln(q_{50}) = ln(7.2) = 1.97 \qquad (1)$$

$$\sigma = ln(q_{75}/q_{25})/1.349 = ln(11.9/4.4)/1.349 = 0.75 \qquad (2)$$

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. Doing so results in a re-expressed β coefficient of −11.0 g (95% CI: −17.6, −4.4) per ng/mL.

Typically, for continuous data, the preferred definition of the 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., 2019a; Reyes and Mañalich, 2005; Hack et al., 1995). Given this clinical cutoff for adversity and that 8.27% of all live births in the United States in 2018 fell below this cutoff, the hybrid approach can be used to define the BMR. The hybrid approach harmonizes the definition of the BMR for continuous data with that for dichotomous data, and therefore is an advantageous approach[10]. Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cutoff 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 animal 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 2,500 g cutoff 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 in 2018 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 3,169.2 g. Given the median exposure of 2.6 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:

$$b = \overline{y} - m\overline{x} = 3261.6\ g - \left(-11.0\ g(\frac{ng}{mL})^{-1}\right) 2.6\frac{ng}{mL} = 3290.3\ g \quad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x, using 3,290.3 g for the *b* term and −11.0 for the *m* term. Doing so results in a value of 11.0 ng/mL:

$$x = (y - b)/m = (3169.2\ g - 3290.3\ g)/(-11.0\ g(\frac{ng}{mL})^{-1}) = 11.0\ ng/mL$$

To calculate the BMDL, the method is essentially the same except that the lower limit (LL) on the β coefficient (β_{LL}= −17.6) is used for the *m* term. However, Chu et al. (2020) reported a two-

[10] 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 cutoff 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%.

sided 95% confidence interval for the β coefficient, meaning that the LL of that confidence interval corresponds to a 97.5% one-sided LL. The BMDL is defined as the 95% LL of the BMD (i.e., corresponds to a two-sided 90% confidence interval), so the corresponding LL on the β coefficient needs to be calculated before calculating the BMDL. First, the standard error of the β coefficient can be calculated as:

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{-4.4\ g(\frac{ng}{mL})^{-1} - \left(-17.6\ g(\frac{ng}{mL})^{-1}\right)}{3.92} = 3.37\ g(\frac{ng}{mL})^{-1}$$

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

$$95\%\ one - sided\ LL = \beta - 1.645\big(SE(\beta)\big) = -11\ g(\frac{ng}{mL})^{-1} - 1.645\left(3.37\ g(\frac{ng}{mL})^{-1}\right)$$
$$= -16.5 g(\frac{ng}{mL})^{-1}$$

Using this value for the *m* term results in a BMDL value of 7.3 ng/mL maternal serum concentration.

E.1.2.2 Wikström et al. (2020)

Wikström et al. (2020) reported a β coefficient of −46.0 g (95%CI: −88.0, −3.0) per ln(ng/mL) for the association between birth weight and maternal PFOS serum concentrations (collected during 9 weeks to 10 weeks of pregnancy with a median of 10 weeks) in a Swedish cohort. Given the reported study-specific median (5.4 ng/mL) and the 25th and 75th percentiles (4.0, 7.6 ng/mL) of the exposure, EPA estimated the mean (1.68) and standard deviation (0.48) of the log normally distributed exposure. The re-expressed β coefficient is −8.4 g (95%CI: −16.0, −0.5) per ng/mL and the intercept *b* is 3,283.4 g. The 95% one-sided LL for the re-expressed β coefficient is −14.8 g per ng/mL. The values of the BMD and BMDL are 13.7 ng/mL and 7.7 ng/mL, respectively.

E.1.2.3 Sagiv et al. (2018)

Sagiv et al. (2018) reported a β coefficient of −17.9 g (95% CI: −40.9, 5.1) per IQR increase in PFOS (ng/mL), corresponding to a β coefficient of −1.1 g (95%CI: −2.6, 0.3) per ng/mL increase, for the association between birth weight and maternal PFOS serum concentrations (collected during 5 weeks to 19 weeks of pregnancy with a median of 9 weeks) in a U.S. cohort. The intercept *b* is 3,264.5 g based on the β coefficient of −1.1 g per ng/mL. A BMD of 85.2 ng/mL is calculated from Sagiv et al. (2018) 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 is used to calculate the corresponding 95% one-sided LL for the β coefficient from the LL on the 95% two-sided confidence interval of −2.6 g per ng/mL. Using the corresponding LL (−2.3 g per ng/mL), a BMDL of 41.0 ng/mL is calculated.

E.1.2.4 Starling et al. (2017)

Starling et al. (2017) reported a β coefficient of −13.8 g (95%CI: −53.8, 26.3) per ln(ng/mL) for the association between birth weight and maternal PFOS serum concentrations (collected during 20 to 34 weeks of pregnancy with a median of 27 weeks) in a U.S. cohort. Given the reported study-specific median (2.4 ng/mL) and the 25th and 75th percentiles (1.5, 3.7 ng/mL) of the exposure, EPA estimated the mean (0.88) and standard deviation (0.67) of the log normally distributed exposure. The re-expressed β coefficient is −5.5 g (95%CI: −21.4, 10.5) per ng/mL and the intercept *b* is 3,275.9 g. The 95% one-sided LL for the re-expressed β coefficient is −18.9 g per ng/mL. The values of the BMD and BMDL are 19.4 ng/mL and 5.7 ng/mL, respectively.

E.1.2.5 Darrow et al. (2013)

Darrow et al. (2013) reported a β coefficient of −49.0 g (95%CI: −90.0, −8.0) per ln(ng/mL) for the association between birth weight and maternal PFOS serum concentrations in a U.S. cohort. Given the reported study-specific median (13.9 ng/mL) and the 25th and 75th percentiles (9.5, 19.7 ng/mL) of the exposure, EPA estimated the mean (2.63) and standard deviation (0.54) of the log normally distributed exposure. The re-expressed β coefficient is −3.4 g (95%CI: −6.3, −0.6) per ng/mL and the intercept *b* is 3,270.5 g. The 95% one-sided LL for the re-expressed β coefficient is −5.8 g per ng/mL. The values of the BMD and BMDL are 29.6 ng/mL and 17.4 ng/mL, respectively.

E.1.2.6 Yao et al. (2021)

Yao et al. (2021) reported a β coefficient of −32.3 g (95%CI: −116.2, 51.6) per ln(ng/mL) for the association between birth weight and maternal PFOS serum concentrations (collected within 3 days of delivery) in a China cohort. Given the cohort-specific median (4.6 ng/mL) and the 25th and 75th percentiles (3.2, 5.9 ng/mL) of the exposure reported in Han et al. (2018b), EPA estimated the mean (1.52) and standard deviation (0.45) of the log normally distributed exposure. The re-expressed β coefficient is −6.9 g (95%CI: −25.0, 11.1) per ng/mL and the intercept *b* is 3,279.7 g. The 95% one-sided LL for the re-expressed β coefficient is −22.1 g per ng/mL. The values of the BMD and BMDL are 15.9 ng/mL and 5.0 ng/mL, respectively.

E.1.2.7 Summary of Modeling Results for Decreased Birthweight

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 cutoff 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 PFOS exposure in the U.S. 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 fells below the cutoff of 2,500 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:

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

$$b = \overline{y} - m\overline{x} = 3261.6 - (\beta_{re-expressed} * 3\frac{ng}{mL})$$

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\overline{x} = 0$). By using the median of serum PFOS concentrations (2.6 ng/mL) from ACE Biomonitoring on Perfluorochemicals as background exposure ($\overline{x}$), the tail probabilities using this alternative approach was study-specific and ranged from 9.05% to 9.78%. As such, the results from this alternative approach, presented under the column of "Alternative Tail Probability" in Table E-19, are very similar to the main results, presented under the column of "Exact Percentage" in Table E-19, when background exposure was not accounted for while estimating the tail probability.

Table E-19 presents the BMDs and BMDLs for all studies considered for POD derivation, with and without accounting for background exposure while estimating the percentage of the population falling below the cutoff value. The BMDLs across the studies ranged from 5.0 ng/mL to 57.6 ng/mL.

Table E-19. BMDs and BMDLs in ng/mL for Effect of PFOS on Decreased Birth Weight, by Using the Exact Percentage (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight, or Alternative Study-Specific Tail

Study	Exposure Median (25th–75th Percentiles)	Exposure Distribution (μ, σ)	Reported β (95% CI) units	Re-expressed β (95% CI) g/(ng/mL)	Intercept b	SE(β)	β_{LL}	Exact Percentage (P(0) = 8.27%)		Alternative Tail Probability [a]		
								BMD	BMDL	P(0)	BMD	BMDL
Chu et al. (2020)	7.2 (4.4–11.9)	(1.97, 0.75)	−83.3 (−133.2, −33.4) g/ln(ng/mL)	−11.0 (−17.6, −4.4)	3,290.3	3.37	−16.5	11.0	7.3	9.05%	12.8	8.5
Sagiv et al. (2018)	25.7 (18.9–34.9)	(3.25, 0.45)	−17.9 (−40.9, 5.1) g/IQR (ng/mL)	−1.1 (−2.6, 0.3)	3,264.5	0.73	−2.3	85.2	41.0	9.78%	119.8	57.6
Starling et al. (2017)	2.4 (1.5–3.7)	(0.88, 0.67)	−13.8 (−53.8, 26.3) g/ln(ng/mL)	−5.5 (−21.4, 10.5)	3,275.9	8.14	−18.9	19.4	5.7	9.45%	25.0	7.3
Wikström et al. (2020)	5.4 (4.0–7.6)	(1.68, 0.48)	−46.0 (−88.0, −3.0) g/ln(ng/mL)	−8.4 (−16.0, −0.5)	3,283.4	3.94	−14.8	13.7	7.7	9.24%	16.7	9.4
Darrow et al. (2013)	13.9 (9.5–19.7)	(2.63, 0.54)	−49.0 (−90.0, −8.0) g/ln(ng/mL)	−3.4 (−6.3, −0.6)	3,270.5	1.46	−5.8	29.6	17.4	9.60%	40.0	23.3
Yao et al. (2021)	4.6 (3.2–5.9)	(1.52, 0.45)	−32.3 (−116.2, 51.6) g/ln(ng/mL)	−6.9 (−25.0, 11.1)	3,279.7	9.22	−22.1	15.9	5.0	9.34%	19.9	6.3

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; CI = confidence interval; IQR = interquartile range; SE = standard error.
[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 the U.S. population.

ACE Biomonitoring on Perfluorochemicals also provides the median blood serum levels of PFOS among women ages 16 to 49 in 1999–2000 (23.8 ng/mL), in 2009–2010 (5.7 ng/m) and in 2013–2014 (3.0 ng/mL). EPA performed a sensitivity analysis by estimating BMD and BMDL using these values as background exposures. The results for each study considered for POD derivation, presented in Table E-20, demonstrate the robustness of EPA's approaches with alternative assumptions on background exposures.

Table E-20. BMDs and BMDLs for Effect of PFOS 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 Tail Probability

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)
Wikström et al. (2020)	2.6	3,283.4	13.7	7.7	9.24%	16.7	9.4
	3.0	3,286.7	14.1	7.9	9.14%	16.8	9.5
	5.7	3,309.2	16.8	9.4	8.53%	17.6	9.9
	23.8	3,460.4	34.9	19.6	5.20%	24.1	13.6
Chu et al. (2020)	2.6	3,290.3	11.0	7.3	9.05%	12.8	8.5
	3.0	3,294.7	11.4	7.6	8.93%	13.0	8.6
	5.7	3,324.4	14.1	9.4	8.14%	13.8	9.2
	23.8	3,523.6	32.2	21.4	4.16%	20.9	13.9
Darrow et al. (2013)	2.6	3,270.5	29.6	17.4	9.60%	39.7	23.3
	3.0	3,271.9	30.0	17.6	9.56%	39.8	23.4
	5.7	3,281.1	32.7	19.2	9.30%	40.5	23.8
	23.8	3,343.1	50.8	29.9	7.67%	46.0	27.1
Sagiv et al. (2018)	2.6	3,264.5	85.2	41.0	9.78%	119.8	57.6
	3.0	3,265.0	85.6	41.2	9.76%	119.9	57.7
	5.7	3,268.0	88.3	42.5	9.68%	120.7	58.1
	23.8	3,288.3	106.4	51.2	9.10%	125.8	60.5
Starling et al. (2017)	2.6	3,275.9	19.4	5.7	9.45%	25.0	7.3
	3.0	3,278.1	19.8	5.8	9.39%	25.1	7.3
	5.7	3,293.0	22.5	6.6	8.97%	25.9	7.5
	23.8	3,392.4	40.6	11.8	6.54%	31.8	9.3
Yao et al. (2021)	2.6	3,279.7	15.9	5.0	9.34%	19.9	6.3
	3.0	3,282.5	16.3	5.1	9.26%	20.0	6.3
	5.7	3,301.2	19.0	6.0	8.75%	20.8	6.5
	23.8	3,427.0	37.1	11.7	5.83%	27.0	8.5

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit;.
[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.

For decreased birth weight associated with PFOS exposure, the POD selected from the available epidemiologic literature is 7.7 ng/mL maternal serum concentration, based on birth weight data

from Wikström et al. (2020). Of the six individual studies, Sagiv et al. (2018) and Wikström et al. (2020) assessed maternal PFOS serum concentrations primarily or exclusively in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Therefore, the PODs from these two studies were considered further for POD selection. The POD from Wikström et al. (2020) was ultimately selected as the reported PFOS exposure concentrations were more representative of current U.S. exposure levels compared with the levels reported in Sagiv et al. (2018), and it was the lowest POD from these two studies.

E.1.3 Modeling Results for Liver Toxicity

This updated review indicated that PFOS is associated with increases in the liver enzyme ALT (See Toxicity Assessment, (U.S. EPA, 2024)). Three *medium* confidence studies were selected as candidates for POD derivation. One of the largest studies of PFOS and ALT in adults is Gallo et al. (2012) conducted in 47,092 adults from the C8 Study Project (for detailed descriptions of the study and findings see Toxicity Assessment, (U.S. EPA, 2024) and Appendix D). Two additional studies (Nian et al., 2019; Lin et al., 2010) were considered by EPA for POD derivation because they reported significant association in general populations in the United States and a high exposed population China, respectively. In an NHANES adult population, Lin et al. (2010) observed elevated ALT levels per log-unit increase in PFOS in the models adjusted for age, gender, and race/ethnicity, but not in the fully adjusted models or in the models additionally adjusted for PFOA, PFHxS, and PFNA. While this is a large nationally representative population, several methodological limitations preclude its use for POD derivation. Limitations include lack of clarity about base of logarithmic transformation applied to PFOS concentrations in regression models, and the choice to model ALT as an untransformed variable, a departure from the typically lognormality assumed in most of the ALT literature.

Nian et al. (2019) examined 1,605 adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) part of the Isomers of C8 Health Project and observed significant increases in ln-transformed ALT per each ln-unit increase in PFOS, as well significant increases in odds ratios of elevated ALT. Median serum PFOS concentrations were 24.22 ng/mL.

E.1.3.1 Nian et al. (2019)

No-observed-adverse-effect concentration/lowest-observed-adverse-effect concentration (NOAEC/LOAEC) method. Significant positive linear trends were observed for branched PFOS with ORs of elevated ALT across quartiles of exposure (p-value = 0.04). However, categorical data, which can be used to develop NOAECs, were not available for total PFOS from the peer-reviewed publication.

Hybrid method. The previously described hybrid method was implemented using data from Nian et al. (2019). The regression model adjusted for age, sex, career, income, education, drink, smoke, giblet and seafood consumption, exercise, and BMI. The percentage change in ln ALT for ln-unit increase in PFOS was 4.1 (95% CI: 0.6, 7.7) (Table 3, Nian et al. (2019). The reported regression coefficient β, which is also referred to as *m*, was calculated from the reported percent change expressed as $(e^{\beta}-1)*100$, resulting in a slope of 0.04 (95% CI: 0.01, 0.07) ln ALT (IU/L) per ln ng/mL PFOS. The estimated BMDs and BMDLs are presented in Table E-21.

For increased ALT associated with PFOS exposure, the POD is based on the data Nian et al. (2019), a BMR of 5% and a $BMDL_5$ of 15.1 ng/mL.

Table E-21. BMD and BMDL for Effect of PFOS (ng/mL) on Increased ALT in Nian et al. (2019), for 5% and 10% Extra Risk

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
BMR = 5%, P(0) Empirical						
BMD	36.82	**25.93**	41.00	24.89	19.58	10.97
BMDL	22.29	**15.12**	23.49	14.57	11.73	6.84
BMR = 5%, P(0) Lognormal						
BMD	69.49	43.37	68.30	40.87	34.44	20.81
BMDL	32.30	20.42	31.64	19.46	16.32	9.94
BMR = 10%, P(0) Empirical						
BMD	206.25	134.66	225.92	126.14	105.81	57.11
BMDL	60.98	39.58	63.63	37.58	31.43	17.93
BMR = 10%, P(0) Lognormal						
BMD	352.86	206.31	347.61	190.43	171.58	97.41
BMDL	83.44	50.78	81.84	47.80	41.68	24.50

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.3.2 Gallo et al. (2012)

Gallo et al. (2012) evaluated the relationship between PFOS and ALT using two general types of analyses. In the first, subjects were divided into deciles of PFOS exposure, and linear regression models were used to compare mean ALT levels by each non-reference quantile versus mean ALT level in the lowest decile. In the second type of analysis, a logistic regression evaluated ORs for having an ALT level above a certain cutoff for each non-reference deciles compared with the lowest (reference) deciles. The cutoff values used to define elevated ALT levels in this study were 45 IU/L for men and 34 IU/L for women, clinically based value recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002), and were approximately the 90th percentile of all ALT values in this study.

E.1.3.2.1 Elevated ALT

E.1.3.2.1.1 Hybrid Method

The hybrid method used the regression slope from the linear regression model of ln-transformed ALT and ln PFOS concentrations adjusted for age, sex, alcohol consumption, socioeconomic status, fasting status, race, month of blood sample collection, smoking status, body mass index, physical activity, and insulin resistance. The reported regression coefficient β, which is also referred to as *m*, was 0.02 (95% CI: 0.014, 0.026) of ln ALT (IU/L) per ln ng/mL PFOS (Table 2, Gallo et al. (2012), model 3).

Using a normal approximation, the standard error of the regression coefficient is estimated as

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{0.026 - 0.014}{3.92} = 0.0025$$

Elevated ALT is a biomarker of acute liver disease. For the following analyses, the adverse effect level of ALT for liver disease was chosen to be C = 42 IU/L for males and C = 30 IU/L for females, based on the sex-specific upper reference limits found in Valenti et al. (2021). These are slightly lower and more health protective than the cutoff values used in the original study (45 IU/L for men and 34 IU/L for women). These cutoffs are also slightly higher than the American College of Gastroenterology (ACG) cutoffs, which considers that "true healthy normal ALT level ranges from 29 to 33 IU/L for males, 19 to 25 IU/L for females" (Kwo et al., 2017). They are the most updated clinical consensus cutoffs, which update the American Association for the Study of Liver Diseases (AASLD) journal Clinical Liver Disease recommended values of 30 IU/L for males, and 19 IU/L for females (Ducatman et al., 2023; Kasarala and Tillmann, 2016). Valenti et al. (2021) determined the updated values using the same approach at the same center but using an updated standardized method.

These analyses were for the periods 1999–2018, 2003–2018, and 2017–2018, separately for males and females ages 18 and over, assuming that the Gallo regression model coefficient developed for the C8 Health Project data in Ohio starting in 2005 and 2006 can be applied to the alternative NHANES periods. These analyses used the NHANES-recommended regression model adjustment to correct the 2017–2018 ALT data to match the earlier laboratory method. EPA used the NHANES PFOS data for each NHANES period including data adjustments to stored biospecimen data collected in 1999–2000 and 2013–2014 that were publicly released in April 2022. NHANES survey weights were applied.

Using the NHANES data for each period and sex, EPA estimated the mean and standard deviation of ln ALT and the estimated mean ln PFOS (Table E-22). The unrounded values were used in the calculations:

Table E-22. NHANES Mean and Standard Deviation of Ln(ALT) (ln IU/L) and Mean PFOS (Ln ng/mL)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Mean ln ALT (ln IU/L) ($\overline{y}$)	3.28	2.96	3.28	2.96	3.29	2.96
Standard Deviation ln ALT (ln IU/L) (S)	0.46	0.41	0.46	0.41	0.48	0.42
Mean ln PFOS (ln ng/mL) ($\overline{x}$)	2.40	1.96	2.37	1.93	1.74	1.26

Notes: ALT = alanine transaminase; IU = international units; NHANES = National Health and Nutrition Examination Survey.

For the BMD analyses, the response of interest is elevated ALT, defined as ALT greater than or equal to an adverse effect threshold C IU/L defined as 42 IU/C for males and 30 IU/L for females. EPA estimated P(0), the prevalence of population with elevated ALT using two approaches. First, the empirical estimate of P(0), "P(0) Empirical," was calculated as the proportion of the population with ALT greater than or equal to C, using the NHANES survey weights. Second, the lognormal estimate of P(0), "P(0) Lognormal," was calculated assuming that ALT is lognormally distributed using the equation:

$$P(0)\ Lognormal = 1 - \Phi\left\{\frac{\ln(C) - mean(\ln ALT)}{sd(\ln ALT)}\right\}$$

where Φ is the normal cumulative distribution function.

The selected BMR is an extra risk of either 5% or 10%. The extra risk of high ALT is given by the equation

$$Extra\ Risk = \frac{P(d) - P(0)}{1 - P(0)}$$

where P(d) is the probability of ALT greater than or equal to C (IU/L) for a given PFOS dose d. Thus

$$P(d) = \{1 - P(0)\} \times Extra\ Risk + P(0)$$

The values of C, *P*(0) Empirical, *P*(d) Empirical, *P*(d) Lognormal for Extra Risk 5% or 10%, and *P*(d) Lognormal for Extra Risk 5% or 10% are shown in Table E-23.

Table E-23. Prevalence of Elevated ALT

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Adverse effect level C (IU/L)	42	30	42	30	42	30
P(0) Empirical	0.14	0.13	0.15	0.13	0.16	0.13
P(d) Empirical, Extra Risk 5%	0.19	0.17	0.19	0.17	0.20	0.17
P(d) Empirical, Extra Risk 10%	0.23	0.21	0.23	0.21	0.24	0.22
P(0) Lognormal	0.16	0.14	0.16	0.14	0.17	0.15
P(d) Lognormal, Extra Risk 5%	0.20	0.18	0.20	0.18	0.22	0.19
P(d) Lognormal, Extra Risk 10%	0.24	0.23	0.24	0.23	0.26	0.23

Notes: ALT = alanine transaminase; IU = international units.

The mean ln ALT *y* for a ln PFOS dose x is given by the equation

$$y = mx + b$$

where m is the slope, β, (from the Gallo regression model) and b is the intercept. The intercept b is the mean ln ALT for a population exposed to 1 ng/mL PFOS. For the U.S. population, the mean ln ALT is $\bar{y}$ (tabulated above) and the mean ln PFOS is $\bar{x}$ (tabulated above) so the intercept is given by the equation

$$b = \bar{y} - m\bar{x}$$

For a given group and dose, the probability of ALT greater than or equal to C is

$$P(d) = P(ALT \geq C) = P(\ln ALT \geq \ln C) = 1 - \Phi\left(\frac{\ln C - y}{S}\right)$$

where Φ is the normal cumulative distribution function. Thus, the mean ln ALT, y, is the solution of the last equation, i.e., $y = ln\ C - S \times \Phi^{-1}\{1 - P(d)\}$, where Φ^{-1} is the inverse of the normal cumulative distribution function.

The ln PFOS benchmark dose (ln BMD) is the corresponding dose x such that $y = mx + b$. Thus

$$\ln BMD = \frac{y - b}{m}$$

This gives the PFOS BMD as exp(ln BMD).

For the BMDL, the lower bound of the dose is calculated, so that in the last equation, instead of m the 95th upper limit for β is used, which is given by

$$\beta 95 = 95th\ Upper\ limit\ for\ \beta = \beta + 1.645 \times se(\beta)$$

Thus

$$\ln BMDL = \frac{y - b}{\beta 95}$$

This gives the PFOS BMDL as exp(ln BMDL) (Table E-24). Note that β95 is different from the upper bound of the 95% confidence interval, since that number is the 97.5th percentile.

Table E-24. BMD and BMDL for Effect of PFOS (ng/mL) on Increased ALT in Gallo et al. (2012)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
BMR = 5%, P(0) Empirical						
BMD	124.39	**95.88**	158.42	91.30	67.81	34.50
BMDL	76.39	**56.79**	92.20	54.25	41.23	21.81
BMR = 5%, P(0) Lognormal						
BMD	445.63	269.46	441.62	247.28	210.92	124.77
BMDL	211.73	129.65	209.13	120.26	102.08	60.90
BMR = 10%, P(0) Empirical						
BMD	3,964.56	2,624.95	4,884.94	2,380.02	2011.20	948.37
BMDL	1,213.59	799.02	1,426.49	733.99	618.43	307.81
BMR = 10%, P(0) Lognormal						
BMD	11,660.73	6,185.61	11,609.86	5,444.59	5,311.44	2,772.69
BMDL	2,873.18	1,584.68	2,848.49	1,421.63	1,343.43	725.26

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

For increased ALT associated with PFOS exposure, the POD is based on the data Gallo et al. (2012), a BMR of 5% and a $BMDL_5$ of 56.79 ng/mL.

E.1.3.2.1.2 NOAEC/LOAEC Method

The results of the logistic regression analysis of elevated ALT across deciles of PFOS are presented in Table E-25. The mean, median and ranges of PFOS concentrations in each decile were not provided with the OR results in the publication. EPA obtained these from author correspondence, and they are illustrated in Table E-25. The NOAEC is bolded and is the mean PFOS serum concentration in the highest decile of PFOS that did not show a statistically significant OR of elevated ALT, which in this case is the 2nd decile, compared with the reference category (the lowest decile of PFOS). The NOAEC based on the elevated ALT data from Gallo et al. (2012) is 10.6 ng/mL.

Table E-25. Odds Ratios for Elevated ALT by Decile of PFOS Serum Concentrations (ng/mL) from Gallo et al. (2012)

Decile	Minimum (ng/mL)	Maximum (ng/mL)	Median (ng/mL)	Mean (ng/mL)	OR	95% CI	Participants Without Elevated ALT	Participants With Elevated ALT	Total (N)
0	0.25	8.8	6.4	5.751386	1	reference	4,119	427	4,546
1	8.9	12.2	**10.7**	**10.63289**	**1.09**	**0.94, 1.26**	4,264	446	4,710
2	12.3	14.9	13.6	13.60556	1.19	1.03, 1.37	4,113	459	4,572
3	15	17.5	16.3	16.26427	1.26	1.09, 1.45	4,104	500	4,604
4	17.6	20.2	18.9	18.88567	1.40	1.22, 1.62	4,115	545	4,660
5	20.3	23.3	21.7	21.74935	1.39	1.21, 1.60	4,181	571	4,752
6	23.4	27	25.1	25.11534	1.31	1.14, 1.52	4,099	561	4,660
7	27.1	32	29.3	29.38941	1.42	1.23, 1.64	4,071	586	4,657
8	32.1	40.4	35.6	35.76743	1.40	1.21, 1.62	4,068	547	4,615
9	40.5	585.2	49.7	56.12528	1.54	1.33, 1.78	4,124	552	4,676

Notes: ALT = alanine transaminase; CI = confidence interval; NOAEC = no-observed-adverse-effect concentration. The NOAEC is bolded.

E.1.3.2.1.3 BMD Method

EPA applied BMDS to calculate a BMD. In addition, EPA performed a sensitivity analysis using the generalized least-squares for trend (glst) method (Greenland and Longnecker, 1992), which assumes a linear relationship between exposure and log-transformed ORs, and accounts for covariance between estimates. These analyses were performed in STATA v17.0 (StataCorp, 2021). Through author correspondence, EPA obtained the number of participants with and without elevated ALT for each decile of PFOS (Table E-25).

Applying BMDS v3.3rc10 using a BMR of 10% and 5% the data for all 10 deciles did not result in any viable models. Applying BMDS v3.3rc10 to the data for all first five deciles did result in viable models. The data associated with the first five deciles was also run using a no intercept approach in which the lowest dose was subtracted out, subsequently referred to as an adjusted dose. The results of this modeling using both the mean and median doses are summarized in Table E-26, Table E-27, Table E-28, Table E-29. This modeling approach results in BMD and BMDL values higher than the maximum dose included in the modeled dataset. The BMD and BMDL values were inside the range of mean exposure values when considering all 10 deciles.

Table E-26. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted Mean PFOS Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	–[b]	–	–	–	–	–	–	–	–
Gamma	0.92	15,296.47	−0.11	−0.11	0.16	28.37	25.58	22.69	20.63
Log-Logistic	0.91	15,296.50	−0.11	−0.11	0.17	27.68	22.17	22.50	20.19
Weibull	**0.98**	**15,294.50**	**−0.11**	**−0.11**	**0.17**	**27.47**	**23.26**	**22.46**	**20.46**
Logistic	0.52	15,296.80	0.67	0.67	0.83	43.97	33.33	25.48	19.53
Log-Probit	0.94	15,296.44	−0.10	−0.10	0.14	29.51	22.98	22.98	20.39
Probit	0.51	15,296.87	0.69	0.69	0.83	45.41	34.13	25.66	19.47
Quantal Linear	0.45	15,297.26	0.80	0.80	0.82	54.66	38.95	26.61	18.96

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD Computation failed.

Table E-27. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Mean PFOS Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	–[b]	–	–	–	–	–	–	–	–
Gamma	0.95	15,296.40	−0.09	−0.09	0.12	24.22	18.67	17.44	15.03
Log-Logistic	0.95	15,296.41	−0.09	−0.09	0.14	23.67	16.76	17.30	14.58
Weibull	**0.94**	**15,296.42**	**−0.09**	**−0.09**	**0.14**	**23.39**	**17.63**	**17.25**	**14.87**
Logistic	0.52	15,296.80	0.67	0.67	0.83	41.00	30.25	23.47	17.42
Log-Probit	0.97	15,296.36	−0.07	−0.07	0.10	26.47	17.71	17.96	14.79
Probit	0.51	15,296.87	0.69	0.69	0.83	42.78	31.38	23.92	17.64
Quantal Linear	0.45	15,297.26	0.80	0.80	0.82	54.66	38.95	26.61	18.96

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD Computation failed.

Table E-28. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted, Median PFOS Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	– [b]	–	–	–	–	–	–	–	–
Gamma	0.93	15,296.46	−0.10	−0.10	0.16	28.47	25.68	22.71	20.60
Log-Logistic	0.92	15,296.49	−0.10	−0.10	0.17	27.80	22.17	22.53	20.20
Weibull	**0.98**	**15,294.49**	**−0.10**	**−0.10**	**0.17**	**27.60**	**23.80**	**22.49**	**20.44**
Logistic	0.59	15,296.40	0.59	0.59	0.79	42.06	32.11	24.42	18.86
Log-Probit	0.94	15,296.43	−0.10	−0.10	0.14	29.59	22.97	23.01	20.40
Probit	0.58	15,296.47	0.61	0.61	0.79	43.34	32.79	24.53	18.75
Quantal Linear	0.52	15,296.83	0.72	0.72	0.79	51.43	36.76	25.04	17.89

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD Computation failed.

Table E-29. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Median PFOS Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	–[b]	–	–	–	–	–	–	–	–
Gamma	0.96	15,296.38	−0.08	−0.08	0.12	23.95	18.49	16.91	14.37
Log-Logistic	0.95	15,296.40	−0.08	−0.08	0.13	23.44	16.17	16.78	13.96
Weibull	0.95	15,296.40	−0.08	−0.08	0.13	23.14	16.75	16.73	14.27
Logistic	0.59	15,296.40	0.59	0.59	0.79	38.74	28.66	22.18	16.50
Log-Probit	**0.98**	**15,296.34**	**−0.06**	**−0.06**	**0.09**	**26.43**	**17.13**	**17.48**	14.18
Probit	0.58	15,296.47	0.61	0.61	0.79	40.40	29.72	22.58	16.70
Quantal Linear	0.52	15,296.83	0.72	0.72	0.79	51.43	36.75	25.04	17.89

Notes: AIC = Akaike information criterion; ALT = alanine transaminase
; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD Computation failed.

E.1.3.3 Summary of Modeling Results for Liver Toxicity

Table E-30. BMDs and BMDLs in ng/mL for Effect of PFOS on Serum Ln(ALT) in Females

Study	Exposure Median (25th–75th Percentiles)	Reported β (95% CI) Units	Re-Expressed β (95% CI) Ln(IU/L)/(ng/mL)	SE(β)	β_{UL}	Exact Percentage, P(0) = 13.0%			
						BMR = 5%		BMR = 10%	
						BMD	BMDL	BMD	BMDL
Gallo et al. (2012)	20.3 (13.7–29.4)	0.02 (0.014, 0.026) ln(IU/L)/ln(ng/mL)	NA	0.0030612	0.025	95.88	56.79	2,624	799.02
Nian et al. (2019)	25.7 (18.9–34.9)	0.0401818 (0.00598, 0.0741794) ln(IU/L)/ln(ng/mL)	0.00158 (0.00023527, 0.00292)	0.0006842	0.00235	44.4	30.69	86.28	51.15
Nian et al. (2019)	25.7 (18.9–34.9)	0.0401818 (0.00598, 0.0741794) ln(IU/L)/ln(ng/mL)	NA	0.017397806	0.07	25.93	15.12	134.66	39.58

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; CI = confidence interval; IU = international units; NA = not applicable; SE = standard error; TBD = to be determined.

Table E-31 summarizes the PODs resulting from the modeling approaches for increased ALT. The selected PODs were based on a BMR of 5%, resulting in BMDLs ranging from 15.12 ng/mL to 56.79 ng/mL, with a selected POD of 15.12 ng/mL.

Table E-31. BMDLs for Effect of PFOS on Serum ALT Using a BMR of 5%

Study Name	BMDL (ng/mL)
Gallo et al. (2012)	56.79
Nian et al. (2019)	**15.12**

Notes: ALT = alanine transaminase; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.4 Modeling Results for Increased Cholesterol

This updated review indicated that there was as association between increases in PFOS and increases in total cholesterol (TC) in adults. Three *medium* confidence studies were considered for POD derivation (Dong et al., 2019; Lin et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019) investigated an NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Lin et al. (2019) collected data from prediabetic adults from the Diabetes Prevention Program (DPP) and DPP Outcomes Study at baseline (1996–1999).

E.1.4.1 Dong et al. (2019)

Using data from NHANES (2003–2014) on 8,948 adults, Dong et al. (2019) calculated a BMD for PFOS and TC using a hybrid model (Crump, 1995). The cutoff for adverse response (i.e., elevated TC) was set at the upper 5th percentile of TC values in the lowest PFOS exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 44.2 ng/mL and 24.1 ng/mL, respectively. Key variables or other results such as the cutoff point used to define elevated TC or model fit parameters were not provided.

Although the hybrid approach has several advantages (Crump, 1995), few details were provided in Dong et al. (2019) on several important aspects of this approach or on other key issues, including the definition of the unexposed reference group, the distribution of PFOS or TC values in this group, model fit (e.g., the fit of linear vs. non-linear models), the impact of potential confounders, or the potential role of reverse causality.

EPA re-analyzed the data using the regression models from the Dong et al. (2019) study, together with updated NHANES data, applied to a modified hybrid model to develop BMD and BMDL estimates for various time periods and assumptions. The BMD values for a BMR of 5% ranged from 15.84 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 36.20 ng/mL for the period 2017–2018, for all adults. The BMDL values for a BMR of 5% ranged from 9.34 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 21.35 ng/mL for the period 2017–2018, for all adults. The BMD values for a BMR of 10% ranged from 35.79 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 55.71 ng/mL for the period 2017–2018, for all adults. The BMDL values for a BMR of 10% ranged from 21.11 ng/mL for the period 1999–2018, excluding

adults taking cholesterol medications, up to 32.86 ng/mL for the period 2017–2018, for all adults.

An important caveat is that these calculations assume that Dong's regression model is still applicable, or at least a good approximation, for all the time periods, for all adults and for adults taking cholesterol medications, and for the recently updated NHANES data.

Dong et al. (2019) reported a regression coefficient β, which is also referred to as *m*, of 0.4 mg/dL TC per ng/mL PFOS (95% CI: 0.06, 0.6). From correspondence with the author, EPA obtained an updated estimated coefficient of 0.35 (95% CI: 0.06, 0.64) mg/dL TC per ng/mL PFOS, which EPA used for these analyses. The regression model applies to all adults 20 to 80 years old and was adjusted for age, gender, race, poverty income ratio, body mass index, waist circumference, physical activity level, diabetes status, smoking status, and number of alcoholic drinks per day. Using a normal approximation, the standard error of the regression coefficient is estimated as

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{0.64 - 0.06}{3.92} = 0.148 \frac{\mathrm{m}g}{dL} \left(\frac{ng}{mL}\right)^{-1}$$

These analyses were for the periods 1999–2008, 2003–2014, 2003–2018, and 2017–2018, assuming that regression model coefficient developed for the period 2003–2014 in the Dong et al. (2019) study can be applied to the alternative NHANES periods. These analyses used the NHANES-recommended reference method data for TC. EPA used the NHANES PFOS data for each NHANES period including data adjustments to stored biospecimen data collected in 1999–2000 and 2013–2014 that were publicly released in April 2022. Alternative analyses were for all adults ages 20 and over, and for adults ages 20 and over that reported not taking prescribed cholesterol medications. NHANES survey weights were applied.

EPA estimated the distribution of TC assuming a normal distribution and also estimated the mean PFOS. The means and standard deviations for each group are shown in Table E-32.

Table E-32. NHANES Mean and Standard Deviation of Total Cholesterol (mg/dL) and Mean PFOS (ng/mL)

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?	No		No		No		No	
Mean TC ($\overline{y}$)	196.17	197.89	196.36	198.01	194.86	196.96	189.01	192.12
Standard Deviation TC (*S*)	41.99	41.47	41.84	41.39	41.80	41.28	40.57	39.67
Mean PFOS ($\overline{x}$)	13.73	13.73	15.64	15.64	13.21	13.21	6.13	6.13

Notes: NHANES = National Health and Nutrition Examination Survey; TC = total cholesterol.

For the BMD analyses, the response of interest is having elevated serum cholesterol, defined as greater than or equal to 240 mg/dL. The baseline probability of such a response is P(0), estimated as 11.5%, for adults aged 20 and older in 2015–2018, as reported by the CDC Health, United States, 2019 Data Finder (NCHS, 2019).

The selected BMR is an extra risk of either 5% or 10%. The extra risk of high serum cholesterol is given by the equation

$$Extra\ Risk = \frac{P(d) - P(0)}{1 - P(0)}$$

where P(d) is the probability of serum cholesterol greater than or equal to 240 mg/dL for a given PFOS dose d. Thus

$$P(d) = \{1 - P(0)\} \times Extra\ Risk + P(0)$$

$$P(d) = \{1 - 0.115\} \times Extra\ Risk + 0.115$$

P(d) = 0.1593 for 5% extra risk and P(d) = 0.2035 for 10% extra risk.

The mean serum cholesterol *y* for a PFOS dose x is given by the equation

$$y = mx + b$$

where m is the slope, β, (from the Dong regression model) and b is the intercept. The intercept b is the mean serum cholesterol for an unexposed population. For the U.S. population, the mean TC is $\bar{y}$ (tabulated above) and the mean PFOS is $\bar{x}$ (tabulated above) so the intercept is given by the equation

$$b = \bar{y} - m\bar{x}$$

For a given group and dose, the probability of serum cholesterol greater than or equal to 240 mg/dL is

$$P(d) = P(TC \geq 240) = 1 - \Phi\left(\frac{240 - y}{S}\right)$$

where Φ is the normal cumulative distribution function. Thus, the mean serum cholesterol y is the solution of the last equation, i.e., $y = 240 - S \times \Phi^{-1}\{1 - P(d)\}$, where Φ^{-1} is the inverse of the normal cumulative distribution function.

The BMD is the corresponding dose x such that $y = mx + b$. Thus

$$BMD = \frac{y - b}{m}$$

For the BMDL, the lower bound of the dose is calculated, so that in the last equation, instead of m the 95th upper limit for β is used, which is given by

$$\beta 95 = 95th\ Upper\ limit\ for\ \beta = \beta + 1.645 \times se(\beta)$$

Thus

$$BMDL = \frac{y - b}{\beta 95}$$

Note that β95 is different from the upper bound of the 95% confidence interval, since that number is the 97.5th percentile. The estimated BMDs and BMDLs are presented in Table E-33:

Table E-33. BMDs and BMDLs for Effect of PFOS on Increased Cholesterol in Dong et al. (2019)

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?	No		No		No		No	
BMR = 5%								
BMD (ng/mL)	19.28	15.84	21.08	17.63	23.07	18.54	36.20	29.86
BMDL (ng/mL)	11.37	**9.34**	12.44	10.40	13.61	10.93	21.35	17.61
BMR = 10%								
BMD (ng/mL)	39.48	35.79	41.21	37.54	43.18	38.39	55.71	48.95
BMDL (ng/mL)	23.29	21.11	24.31	22.14	25.47	22.65	32.86	28.87

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

Given the potential impact of taking cholesterol medication on the true association between PFOS and increased TC, the results based on the data excluding such possibility is considered higher confidence. As illustrated in

Table E-33 there was a decline over time in PFOS levels based on NHANES data, suggesting that reliance on distributional data based on the most recent NHANES cycle available (2017–2018) might be more reflective of recent exposure levels. However, given the chronic nature of both exposure and increased TC development, a higher confidence might be the given to estimates based on the largest period available (1999–2018).

For increased cholesterol associated with PFOS exposure, the POD is based on the data Dong et al. (2019) excluding people taking cholesterol medication, the longest period available, a BMR of 5% and a $BMDL_5$ of 9.3 ng/mL.

E.1.4.2 Steenland et al. (2009)

The above hybrid approach was also applied to Steenland et al. (2009) using log-transformed values. In Table 4, Steenland et al. (2009) reported a linear regression coefficient for change in ln-transformed TC per ln(PFOS): 0.02660 with a standard deviation of 0.00140. The NHANES data used in this approached is summarized in Table E-34 and BMD/BMDL values are presented in Table E-35.

Table E-34. NHANES Mean and Standard Deviation of Ln(TC) (Ln(mg/dL)) and Mean Ln(PFOS) (Ln(ng/mL))

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?	No		No		No		No	
Mean ln(TC) ($\bar{y}$)	5.26	5.27	5.26	5.27	5.25	5.26	5.22	5.24
Standard Deviation ln(TC) (S)	0.21	0.21	0.21	0.21	0.21	0.21	0.22	0.21
Mean ln(PFOS) ($\bar{x}$)	2.17	2.17	2.36	2.36	2.14	2.14	1.50	1.50

Notes: NHANES = National Health and Nutrition Examination Survey; TC = total cholesterol.

Table E-35. BMDs and BMDLs for Effect of PFOS on Increased Cholesterol in Steenland et al. (2009)

Time Period	**1999**–2018	**1999**–2018	**2003**–2014	**2003**–2014	**2003**–2018	**2003**–2018	**2017**–2018	**2017**–2018
Taking prescribed cholesterol medication?	No		No		No		No	
BMR = 5%								
BMD (ng/mL)	14.16	11.58	16.77	13.48	17.21	13.23	26.36	18.88
BMDL (ng/mL)	11.46	**9.52**	13.39	10.95	13.72	10.77	20.31	**14.94**
BMR = 10%								
BMD (ng/mL)	54.05	43.02	63.79	50.20	66.14	49.34	102.98	69.54
BMDL (ng/mL)	39.33	31.88	45.81	36.75	47.36	36.17	71.18	49.59

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

Mean serum TC

EPA also conducted dose-response modeling using mean serum TC reported across PFOS deciles from Table 3 in Steenland et al. (2009). The associated standard error terms were found through author correspondence. BMDS 3.3rc10 was used to fit the dose-response data using all deciles, no viable models were identified. To further investigate, BMDS 3.3rc10 was used to fit the dose-response data in the lowest five deciles and regression coefficients for the mean change of ln-transformed serum TC (Table 3 in Steenland et al. (2009)), summarized in Table E-36. BMRs of a change in the mean equal to ½ and 1 SDs from the control mean were chosen. The BMD modeling results are summarized in Table E-37.

Table E-36. Regression Results for Serum Total Cholesterol by Deciles of Serum PFOS from Steenland et al. (2009)

Decile	Dose (ng/mL)	N	Regression Coefficient[a] (SD)
1	6.37	4,629	0.00 (0.192)
2	10.60	4,629	0.01 (0.192)
3	13.65	4,629	0.01 (0.192)
4	16.19	4,629	0.03 (0.192)
5	18.79	4,629	0.03 (0.192)

Notes: SD = standard deviation.
[a] Regression coefficient, change in the natural log of total cholesterol

Table E-37. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol in Steenland et al. (2009)

Model[a]	Goodness of Fit		Scaled Residual			BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)	$BMD_{0.5SD}$ (ng/mL)	$BMDL_{0.5SD}$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{1SD}	Dose Group Near $BMD_{0.5SD}$	Control Dose Group				
Exponential 3	<0.0001	−10,350.92	0.00	−1.16	−1.54	0.76	0.00	25.38	24.66
Exponential 5	–	–	–	–	–	–	–	–	–
Hill	–	–	–	–	–	–	–	–	–
Polynomial Degree 3	0.00	−10,588.86	−0.78	−0.78	0.00	45.95	33.33	31.36	26.15
Polynomial Degree 2	0.00	−10,588.82	−0.71	–	–	47.85	39.78	–	–
Power	0.00	−10,588.89	−0.75	−0.75	0.02	48.56	47.46	32.31	29.22
Linear	0.01	−10,589.87	−0.23	−0.23	0.51	74.49	62.75	37.24	31.37

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{1SD} = dose level corresponding to a change in the mean equal to one standard deviation from the control mean; $BMDL_{1SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean. $BMD_{0.5SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

[a] No viable models. No model was selected.

[b] BMD Computation failed

E.1.4.2.1 Elevated TC

In additional to modeling the regression coefficients, dichotomous models using BMDS 3.3rc10 were used to fit the ORs of elevated TC from Steenland et al. (2009) as shown in Table E-38. Sample sizes, mean PFOS concentrations in each quartile and prevalence of elevated TC in each exposure group were obtained from Dr. Kyle Steenland. A BMR of 10% and 5% extra risk were both included. The BMD modeling results are summarized in Table E-39.

Table E-38. Odds Ratios for Elevated Serum Total Cholesterol by Quartiles of Serum PFOS from Steenland et al. (2009)

Quartile	Dose (ng/mL)	N	Incidence	OR	95% CI
1	6.6	11,534	1,479	1	Ref
2	16.4	11,587	1,634	1.14	1.05, 1.23
3	23.8	11,441	1,795	1.28	1.19, 1.39
4	50.55	11,400	2,158	1.51	1.40, 1.64

Notes: CI = confidence interval; OR = odds ratio.

Table E-39. Summary of Benchmark Dose Modeling Results for Elevated Total Cholesterol in Steenland et al. (2009)

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	–[b]	–	3.56×10^{-6}	–	–	–	–	31.08	26.59
Gamma	0.53	39,272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.21
Log-Logistic	**0.57**	**39,272.40**	**−0.24**	**−0.24**	**−0.05**	**63.18**	**55.91**	**29.93**	**26.39**
Multistage Degree 3	0.01	39,282.00	−0.58	−0.58	−1.57	62.48	0.00	40.96	40.29
Multistage Degree 2	0.53	39,272.57	−0.28	−0.28	−0.14	63.00	55.88	30.67	27.20
Multistage Degree 1	0.53	39,272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.20
Weibull	0.53	39,272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.21
Logistic	0.27	39,274.11	−0.42	−0.42	−0.62	62.30	56.70	34.49	31.47
Log-Probit	0.35	39,274.11	−0.10	−0.10	0.16	66.02	57.02	29.71	14.27
Probit	0.31	39,273.81	−0.40	−0.40	−0.55	62.43	56.61	33.93	30.84
Quantal Linear	0.53	39,272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.21

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD Computation failed

Given the potential impact of taking cholesterol medication on the true association between PFOS and increased TC, the results based on the data excluding such possibility is considered higher confidence. As illustrated in Table E-34 there was a dramatic decline over time in PFOS levels based on NHANES data, suggesting that reliance on distributional data based on the most recent NHANES cycle available (2017–2018) might be more reflective or current impacts. However, given the chronic nature of both exposure and increased TC development, a higher confidence might be the given to estimates based on the largest period available (1999–2018).

For increased cholesterol associated with PFOS exposure, the POD is based on the data from Steenland et al. (2009) excluding people taking cholesterol medication, the longest period available, a BMR of 5% and a $BMDL_5$ of 9.52 ng/mL. A comparison BMDL of 14.9 ng/mL based on the most recent period available supports the selected POD.

E.1.4.3 Lin et al. (2019)

Lin et al. (2019) collected data from prediabetic adults from the DPP and DPP Outcomes Study at baseline (1996–1999). This study included 888 prediabetic adults who were recruited from 27 medical centers in the United States. Median PFOS levels at baseline were comparable to those from NHANES 1999–2000, 27.2 (25th, 75th percentiles: 18.0 ng/mL, 40.4 ng/mL). The study presented both cross-sectional and prospective analyses. The cross-sectional analyses evaluated associations between baseline PFAS and baseline lipid levels. The prospective analysis evaluated whether baseline PFAS levels predicted higher risk of incident hypercholesterolemia and hypertriglyceridemia, but in the placebo and the lifestyle intervention groups, separately.

EPA conducted dose-response modeling using mean serum TC reported across PFOS quartiles from Table S5 in Lin et al. (2019). For its POD calculations, EPA used the results from the cross-sectional analysis because they were presented in a format that was more amendable to dose-response analysis.

BMDS 3.3rc10 was used to fit the dose-response data for the adjusted mean difference in lipid levels (mg/dL) per quartile of baseline plasma PFOS concentrations (ng/mL), summarized in Table E-40. BMRs of a change in the mean equal to 0.5 SD and 1 SD from the control mean were used. The BMD modeling results are summarized in Table E-41. However, the PODs derived from this study are considered lower confidence since they are based on a poorly fir PFOS association (adjusted mean difference = 2.53, 95% CI: −0.10, 5.16).

Table E-40. Adjusted Mean Differences in Serum Total Cholesterol by Quartiles of Serum PFOS (ng/mL) from Lin et al. (2019)

Dose (ng/mL)	N	Adjusted Mean Difference TC (95% CI) (mg/dL)	Mean TC[a,b]
12.8	212	Ref	0.00 ± 35.48
21.7	224	1.13 (−5.50, 7.77)	1.13 ± 35.33
32.7	230	5.05 (−1.55, 11.66)	5.05 ± 35.39
53	222	5.13 (−1.58, 11.86)	5.13 ± 35.70

Notes: CI = confidence interval; TC = total cholesterol.
[a] Mean ± standard deviation.
[b] Adjusted mean difference in lipid levels (mg/dL) per quartile of baseline plasma PFOS concentration (ng/mL)

Table E-41. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol Lin et al. (2019)

Model[a]	Goodness of Fit		Scaled Residual			BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)	$BMD_{0.5SD}$ (ng/mL)	$BMDL_{0.5SD}$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{1SD}	Dose Group Near $BMD_{0.5SD}$	Control Dose Group				
Exponential 3	0.23	8,863.69	−0.21	−0.21	−0.60	108.34	61.19	88.53	57.34
Exponential 5	–[b]	–	–	–	–	–	–	–	–
Hill	–	–	–	–	–	–	–	–	–
Polynomial Degree 3	0.65	8,861.12	−0.35	−0.35	−0.21	261.96	86.09	130.98	66.43
Polynomial Degree 2	0.65	8,861.12	−0.34	–	–	262.61	100.07	–	–
Power	0.65	8,861.12	−0.34	−0.34	−0.21	262.62	58.47	131.31	66.54
Linear	**0.65**	**8,861.12**	**−0.34**	**−0.34**	**−0.21**	**262.62**	**133.07**	**131.31**	**66.54**

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{1SD} = dose level corresponding to a change in the mean equal to one standard deviation from the control mean; $BMDL_{1SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean. $BMD_{0.5SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.
[a] Selected model in bold.
[b] BMD Computation failed

E.1.4.4 Summary of Modeling Results for Increased Cholesterol

Table E-42. BMDs and BMDLs in ng/mL for Effect of PFOS on Serum Total Cholesterol

Study	Exposure Mean (SD)	Reported β (95% CI) Units	Re-Expressed β (95% CI) Ln(mg/dL)/(ng/mL)	SE(β)	$β_{UL}$	Exact Percentage, P(0) = 11.5%			
						BMR = 5%		BMR = 10%	
						BMD	BMDL	BMD	BMDL
Steenland et al. (2009)	22.4 (14.8)	0.0266 (0.0243, 0.0289) ln(mg/dL)/ln(ng/mL)	0.00137 (0.00125, 0.00149)	0.0000605	0.00147	7.58	7.07	33.04	30.08
Steenland et al. (2009)	22.4 (14.8)	0.0266 (0.0243, 0.0289) ln(mg/dL)/ln(ng/mL)	NA	3.02415E−08	0.03	11.58	9.52	43.02	31.88
Dong et al. (2019)	15.6 (17.8)	0.35 (95% CI: 0.06, 0.64) mg/dL/ng/mL	NA	0.15	0.59	15.84	9.34	38.39	22.65

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; CI = confidence interval; NA = not applicable; SD =standard deviation; SE = standard error.

Table E-43 summarizes the PODs resulting from the modeling approaches for increased cholesterol. The selected and comparison PODs were based on a BMR of 5%, resulting in BMDLs ranging from 9.3 ng/mL to 66.5 ng/mL with the selected POD of 9.35 also representing the median of the BMDLs. The comparison POD based on the data from Lin et al. (2019) is considered *low* confidence because it is based on a poorly fit PFOS regression parameter.

Table E-43. BMDLs for Effect of PFOS on Serum Total Cholesterol Using a BMR of 5%

Study Name	Effect	BMDL (ng/mL)
Dong et al. (2019)	Exclude those prescribed cholesterol medication, 1999–2018	**9.34**
Steenland et al. (2009)	Exclude those prescribed cholesterol medication	9.52
Lin et al. (2019)	Diabetic adults	66.5

Notes: BMDL = benchmark dose lower limit; BMR = benchmark response

E.2 Toxicology Studies

E.2.1 Butenhoff et al. (2012)/Thomford (2002)

EPA conducted dose-response modeling of the Butenhoff et al. (2012)/Thomford (2002) study using the BMDS 3.2 program. This study addresses incidence of adenomas and/or carcinomas in the liver and pancreas in male rats and the liver and thyroid in female rats, and individual cell necrosis in the liver in female Sprague-Dawley Crl:CD(SD)IGS BR rats.

E.2.1.1 Hepatocellular Adenomas in Males

Increased incidence of hepatocellular adenomas was observed in male rats. Dichotomous models were used to fit dose-response data. Multistage models were used consistent with the longstanding practice of EPA to prefer multistage models to fit tumor dose-response data and a BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The dose and response data used for the modeling are listed in Table E-44. The area under the curve (AUC) normalized per day (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for modeling cancer endpoints (see the Toxicity Assessment, (U.S. EPA, 2024)). BMD analysis was conducted using both the number of animals at the start of the study and the number of animals alive at the time of first tumor.

Table E-44. Dose-Response Modeling Data for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (ppm)	Internal Dose (mg/L)	Number per Group at Start of Study	Number per Group at Time of First Tumor[a]	Incidence
0	0.0	50	41	0
0.5	1.4	50	42	3
2	5.9	50	47	3
5	14.3	50	44	1
20	57.8	50	43	7

Notes:
[a] The time of first occurrence of this tumor was day 512 in males.

BMD modeling results for hepatocellular adenomas following exposure to PFOS for the number of animals at the start of the study and the number of animals alive at the time of first tumor are summarized in Table E-45 and Figure E-3 and Figure E-4. The best fitting model was the Multistage Degree 4 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 4 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level $BMDL_{10}$ from the selected Multistage Degree 4 model for the number of animals at the start of the study is 29.3 mg/L and for the number of animals alive at the time of first tumor is 25.6 mg/L. The number of animals alive at the time of first tumor ensures the potency is not underestimated by mortality of animals prior to tumor occurrence. The relatively small difference in the two $BMDL_{10}$ values supports using these values and the selected value is based on the number of animals alive at the time of first tumor, 25.6 mg/L.

Table E-45. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

	Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
		p-value	AIC	Dose Group Near BMD	Control Dose Group			
Animals at the start of the study	**Multistage Degree 4**	**0.260**	**105.2**	**0.004**	**–1.35**	**56.6**	**29.3**	EPA selected the Multistage Degree 4 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 4 model had the lowest AIC.
	Multistage Degree 3	0.254	105.2	0.017	–1.34	56.3	29.1	
	Multistage Degree 2	0.235	105.4	0.065	–1.32	55.9	28.5	
	Multistage Degree 1	0.192	105.7	0.204	–1.19	54.5	27.6	
Animals alive at the time of first tumor	**Multistage Degree 4**	**0.281**	**100.9**	**0.005**	**–1.31**	**54.2**	**25.6**	EPA selected the Multistage Degree 4 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 4 model had the lowest AIC.
	Multistage Degree 3	0.275	101.0	0.018	–1.31	53.2	25.4	
	Multistage Degree 2	0.252	101.2	0.071	–1.29	51.4	24.9	
	Multistage Degree 1	0.196	101.6	0.238	–1.16	46.8	23.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

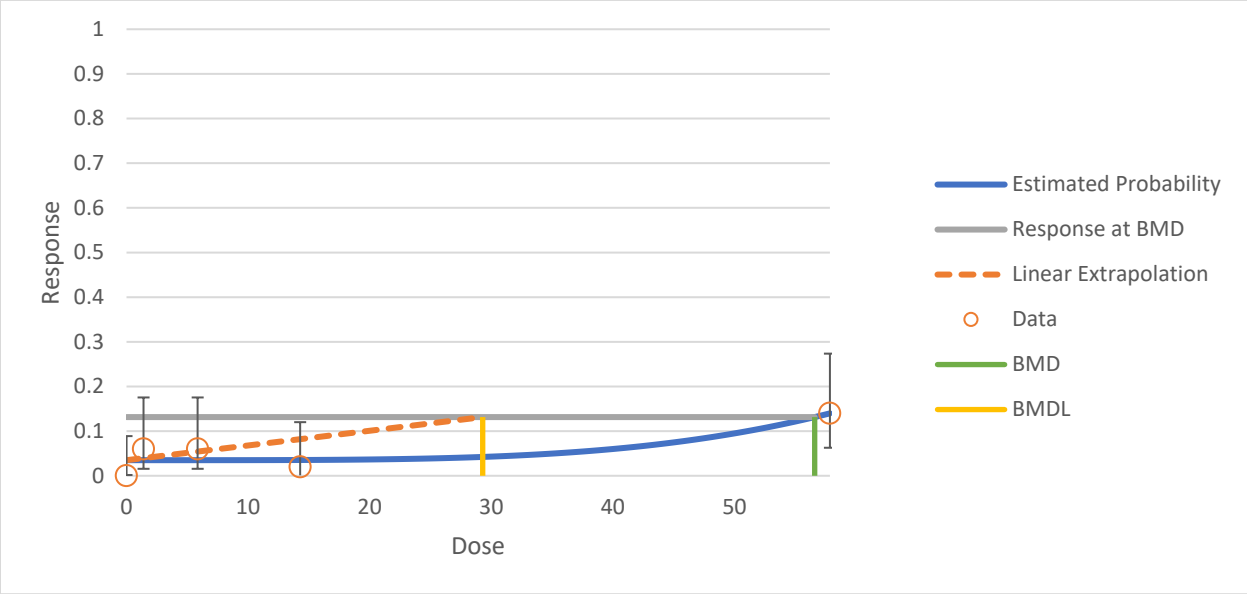


Figure E-3. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

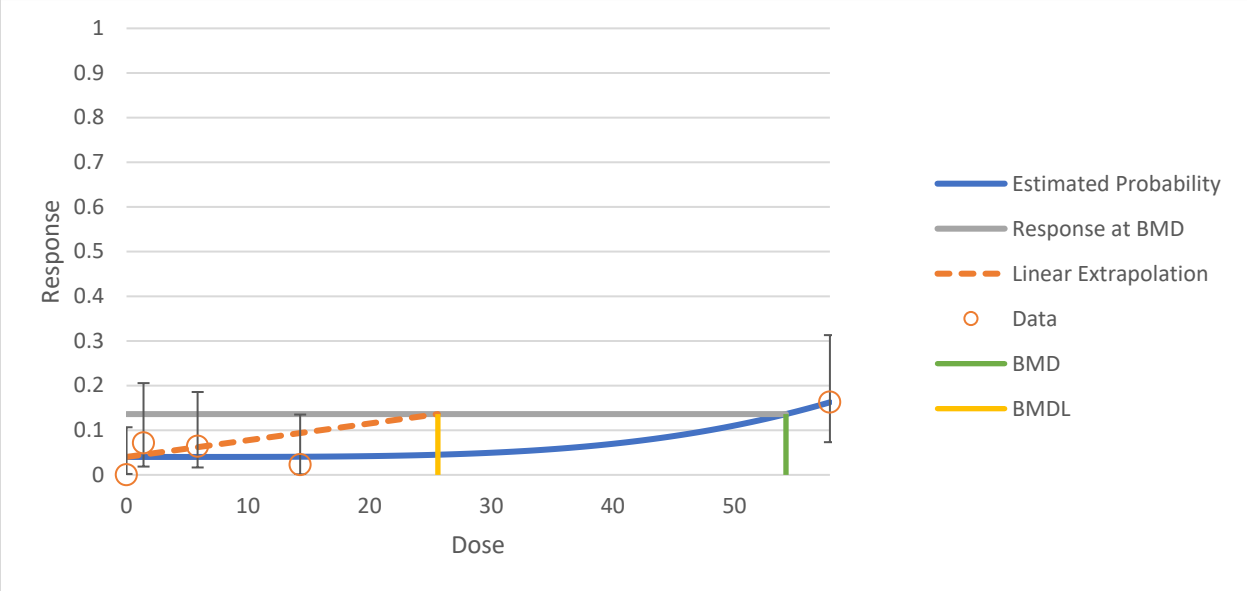


Figure E-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.1.2 Pancreas Islet Cell Carcinomas in Males

Increased incidence of islet cell carcinomas was observed in male rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The dose and response data used for the modeling are listed in Table E-46. The AUC_{avg}, equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for modeling cancer endpoints (see the Toxicity Assessment, (U.S. EPA, 2024)). BMD analysis was conducted using both the number of animals at the start of the study and the number of animals alive at the time of first tumor.

Table E-46. Dose-Response Modeling Data for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (ppm)	Internal Dose (mg/L)	Number per Group at Start of Study	Number per Group at Time of First Tumor[a]	Incidence
0	0.0	50	38	1
0.5	1.4	50	41	2
2	5.9	50	44	2
5	14.3	50	44	5
20	57.8	50	40	5

Notes:
[a] The time of first occurrence of this tumor was day 542 in males.

The BMD modeling results for incidence of islet cell carcinomas following exposure to PFOS for the number of animals at the start of the study and the number of animals alive at the time of first tumor are summarized in Table E-47 and Figure E-5 and Figure E-6. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the higher degree Multistage models estimated parameters at the zero boundary and reduced to the Multistage Degree 1 model. The $BMDL_{10}$ from the selected Multistage Degree 1 model for the number of animals at the start of the study is 29.7 mg/L and for the number of animals alive at the time of first tumor is 26.1 mg/L. The number of animals alive at the time of first tumor ensures the potency is not underestimated by mortality of animals prior to tumor occurrence. The relatively small difference in the two $BMDL_{10}$ values supports using these values and the selected value is based on the number of animals alive at the time of first tumor, 26.1 mg/L.

Table E-47. Summary of Benchmark Dose Modeling Results for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 4	0.526	114.5	−0.434	−0.633	67.6	29.7	EPA selected the Multistage Degree 1

	Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
		p-value	AIC	Dose Group Near BMD	Control Dose Group			
Animals at the start of the study	Multistage Degree 3	0.526	114.5	−0.434	−0.633	67.6	29.7	model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and higher degree models reduced to the Multistage Degree 1 model.
	Multistage Degree 2	0.526	114.5	−0.434	−0.633	67.6	29.7	
	Multistage Degree 1	**0.526**	**114.5**	**−0.434**	**−0.633**	**67.6**	**29.7**	
Animals alive at the time of first tumor	Multistage Degree 4	0.554	111.2	−0.417	−0.590	58.5	26.1	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and higher degree models reduced to the Multistage Degree 1 model.
	Multistage Degree 3	0.554	111.2	−0.417	−0.590	58.5	26.1	
	Multistage Degree 2	0.554	111.2	−0.417	−0.590	58.5	26.1	
	Multistage Degree 1	**0.554**	**111.2**	**−0.417**	**−0.590**	**58.5**	**26.1**	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

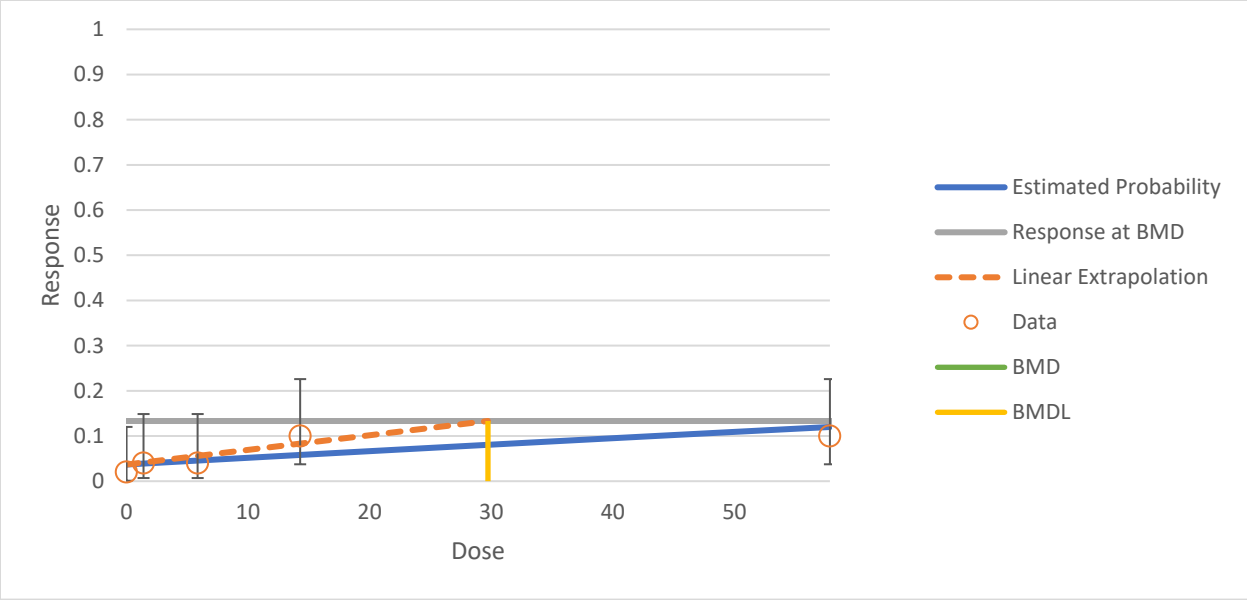


Figure E-5. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

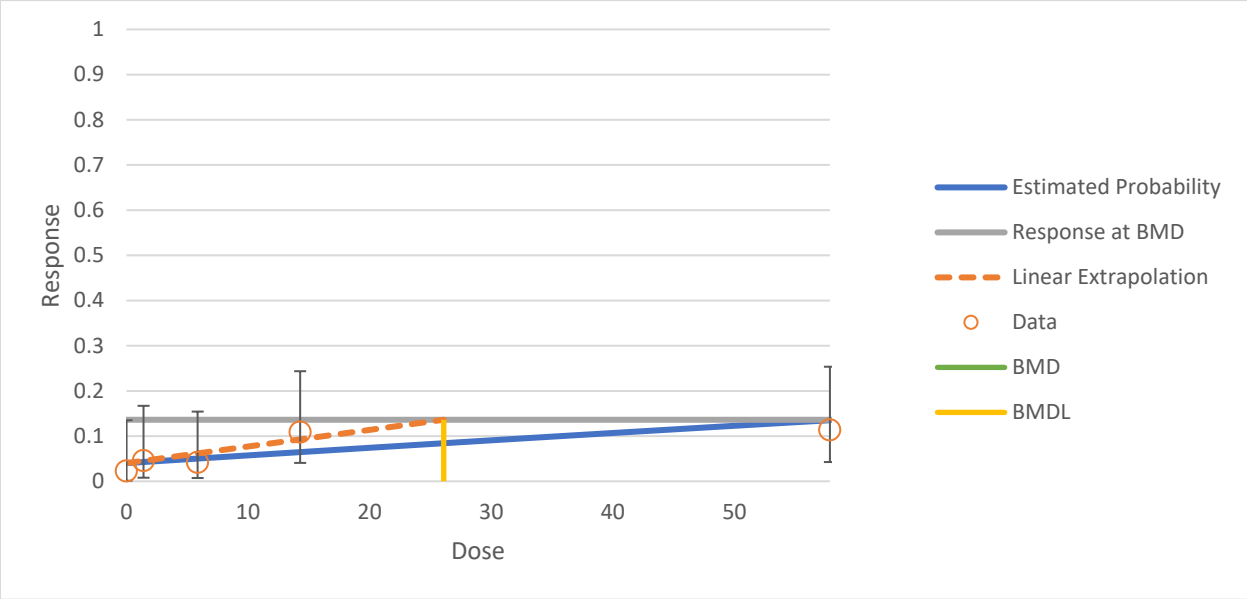


Figure E-6. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.1.3 Pancreas Combined Islet Cell Adenomas and Carcinomas in Males

Increased incidence of combined islet cell adenomas and carcinomas was observed in male rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The dose and response data used for the modeling are listed in Table E-48. The $AUC_{avg,}$ equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for modeling cancer endpoints (see Toxicity Assessment, (U.S. EPA, 2024)). BMD analysis was conducted using both the number of animals at the start of the study and the number of animals alive at the time of first tumor.

Table E-48. Dose-Response Modeling Data for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (ppm)	Internal Dose (mg/L)	Number per Group at Start of Study	Number per Group at Time of First Tumor[a]	Incidence
0	0.0	50	44	5
0.5	1.4	50	45	5
2	5.9	50	48	6
5	14.3	50	46	8
20	57.8	50	44	9

Notes:
[a] The time of first occurrence of this tumor was day 465 in males.

The BMD modeling results for combined incidence of islet cell adenomas and carcinomas following exposure to PFOS for the number of animals at the start of the study and the number of animals alive at the time of first tumor are summarized in Table E-49 and Figure E-7 and Figure E-8. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the benchmark dose lower limits (BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the higher degree Multistage models estimated parameters at the zero boundary and reduced to the Multistage Degree 1 model. The $BMDL_{10}$ from the selected Multistage Degree 1 model for the number of animals at the start of the study is 25.1 mg/L and for the number of animals alive at the time of first tumor is 21.7 mg/L. The number of animals alive at the time of first tumor ensures the potency is not underestimated by mortality of animals prior to tumor occurrence. The relatively small difference in the two $BMDL_{10}$ values supports using these values and the selected value is based on the number of animals alive at the time of first tumor, 21.7 mg/L.

Table E-49. Summary of Benchmark Dose Modeling Results for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 4	0.909	197.34	−0.191	−0.214	63.8	25.1	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and higher degree models reduced to the Multistage Degree 1 model.
Multistage Degree 3	0.909	197.34	−0.191	−0.214	63.8	25.1	
Multistage Degree 2	0.909	197.34	−0.191	−0.214	63.8	25.1	
Multistage Degree 1	**0.909**	**197.34**	**−0.191**	**−0.214**	**63.8**	**25.1**	
Multistage Degree 4	0.938	190.0	−0.162	−0.130	53.6	21.7	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and higher degree models reduced to the Multistage Degree 1 model.
Multistage Degree 3	0.938	190.0	−0.162	−0.130	53.6	21.7	
Multistage Degree 2	0.938	190.0	−0.162	−0.130	53.6	21.7	
Multistage Degree 1	**0.938**	**190.0**	**−0.162**	**−0.130**	53.6	21.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

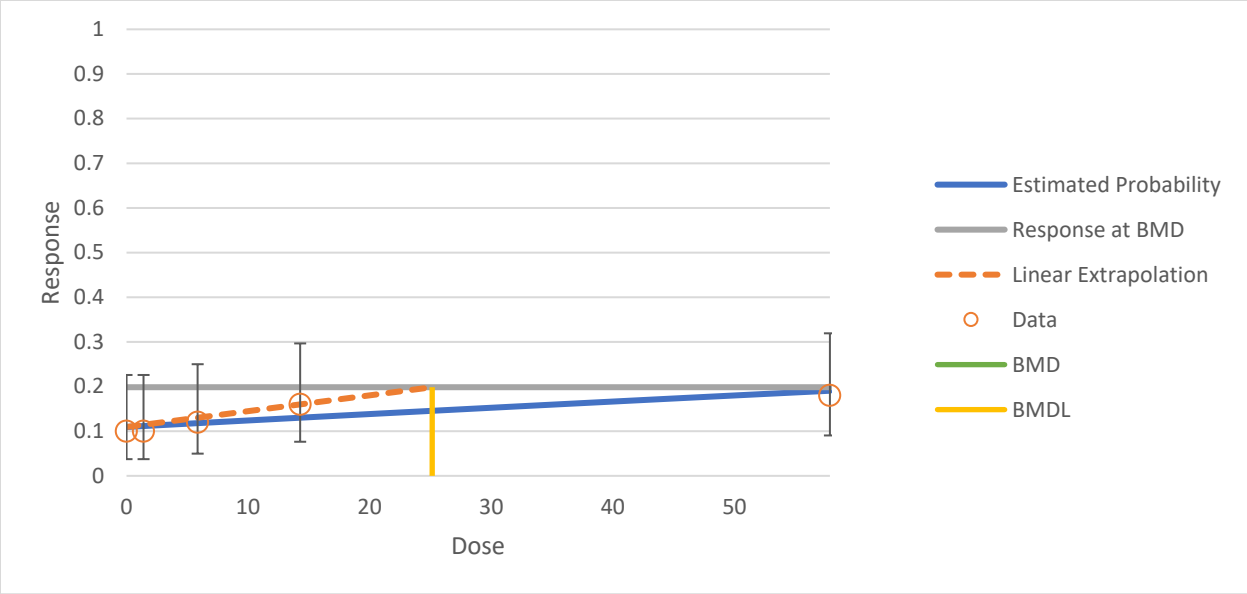


Figure E-7. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

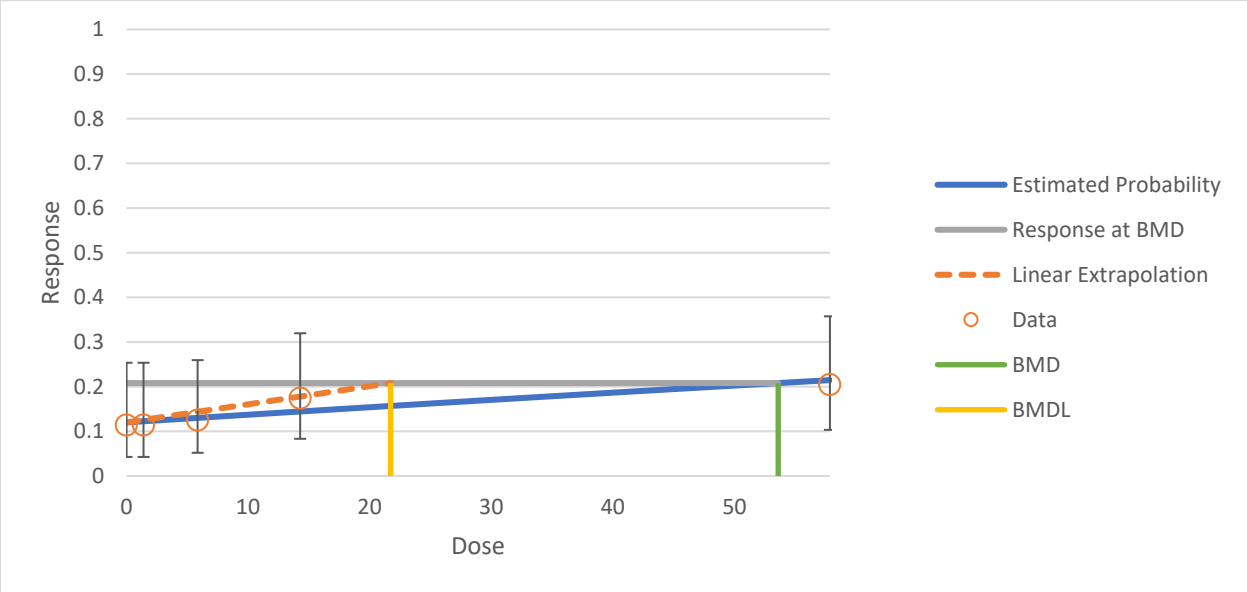


Figure E-8. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.1.4 Hepatocellular Adenomas in Females

Increased incidence of hepatocellular adenomas was observed in female rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-50. The AUC_{avg}, equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for modeling cancer endpoints (See Toxicity Assessment, (U.S. EPA, 2024)). BMD analysis was conducted using both the number of animals at the start of the study and the number of animals alive at the time of first tumor.

Table E-50. Dose-Response Modeling Data for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (ppm)	Internal Dose (mg/L)	Number per Group at Start of Study	Number per Group at Time of First Tumor[a]	Incidence
0	0.0	50	28	0
0.5	1.6	50	26	1
2	6.6	49	15	1
5	16.1	50	28	1
20	65.2	50	31	5

Notes:
[a] The time of first occurrence of this tumor was day 653 in females.

The BMD modeling results for hepatocellular adenomas following exposure to PFOS for the number of animals at the start of the study and the number of animals alive at the time of first tumor are summarized in Table E-51 and Figure E-9 and Figure E-10. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 1 model for the number of animals at the start of the study is 37.2 mg/L and for the number of animals alive at the time of first tumor is 21.8 mg/L. The number of animals alive at the time of first tumor ensures the potency is not underestimated by mortality of animals prior to tumor occurrence. The relatively small difference in the two $BMDL_{10}$ values supports using these values and the selected value is based on the number of animals alive at the time of first tumor, 21.8 mg/L.

Table E-51. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 4[b]	0.601	69.2	0.00105	−0.668	68.3	37.4	EPA selected the Multistage Degree 1

	Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
		p-value	AIC	Dose Group Near BMD	Control Dose Group			
Animals at the start of the study	Multistage Degree 3[b]	0.598	69.3	0.00722	−0.665	69.0	37.4	model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), the Multistage Degree 1 model had the lowest AIC.
	Multistage Degree 2	0.586	69.3	0.02918	−0.655	70.5	37.3	
	Multistage Degree 1	**0.761**	**67.3**	**0.08232**	**−0.608**	**73.0**	**37.2**	
Animals alive at the time of first tumor	Multistage Degree 4	0.449	59.8	0.0024	−0.719	46.7	21.8	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest AIC (the Degree 2 model estimated parameters at the zero boundary and reduced to the Degree 1 model).
	Multistage Degree 3	0.447	59.8	0.0094	−0.713	45.4	21.8	
	Multistage Degree 2[c]	0.654	57.8	0.0228	−0.701	43.9	21.8	
	Multistage Degree 1	**0.654**	**57.8**	**0.0228**	**−0.701**	**43.9**	**21.8**	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

[b] Degree 3 and 4 models estimated parameters at the zero boundary and reduced to the Multistage Degree 2 model.

[c] Degree 2 model estimated parameters at the zero boundary and reduced to the Multistage Degree 1 model.

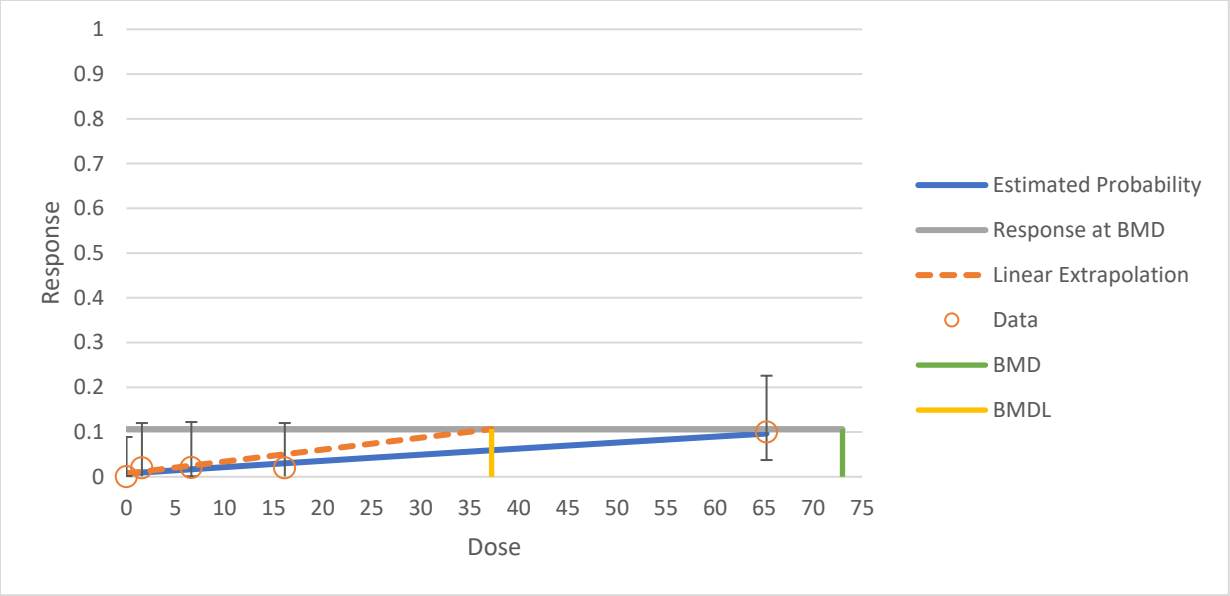


Figure E-9. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

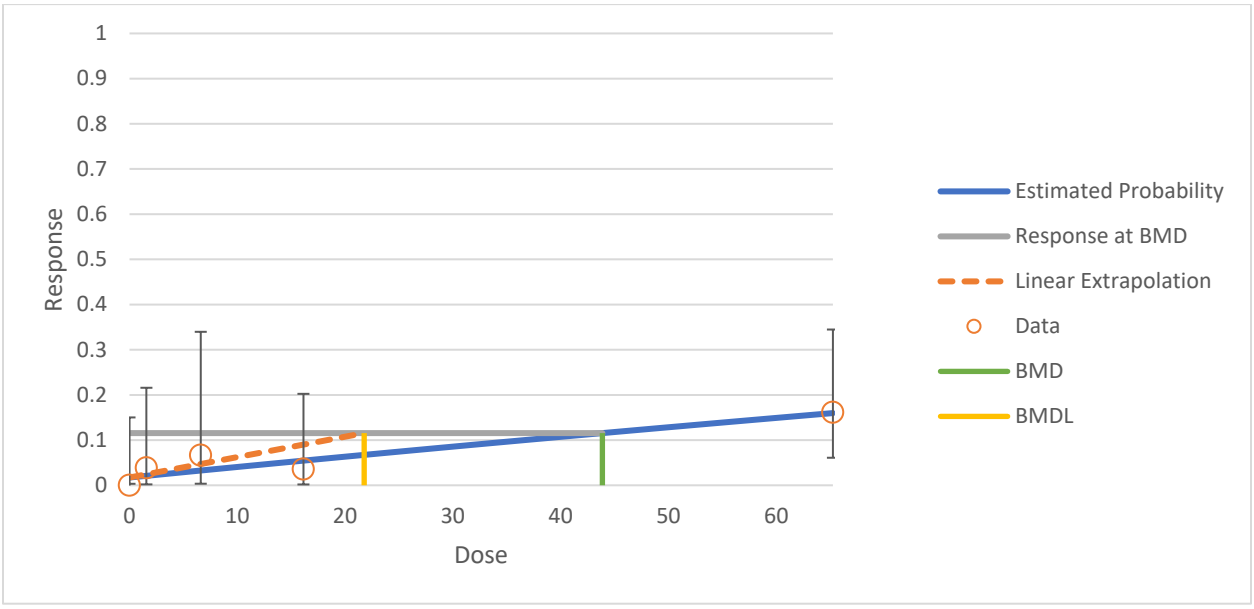


Figure E-10. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.1.5 Hepatocellular Combined Adenomas and Carcinomas in Females

Increased incidence of hepatocellular adenomas and carcinomas was observed in female rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The dose and response data used for the modeling are listed in Table E-52. The $AUC_{avg,}$ equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for modeling cancer endpoints (See Toxicity Assessment, (U.S. EPA, 2024)). BMD analysis was conducted using both the number of animals at the start of the study and the number of animals alive at the time of first tumor.

Table E-52. Dose-Response Modeling Data for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (ppm)	Internal Dose (mg/L)	Number per Group at Start of Study	Number per Group at Time of First Tumor[a]	Incidence
0	0.0	50	28	0
0.5	1.6	50	29	1
2	6.6	49	16	1
5	16.1	50	31	1
20	65.2	50	32	6

Notes:
[a] The time of first occurrence of this tumor was day 653 in females.

The BMD modeling results for hepatocellular adenomas following exposure to PFOS for the number of animals at the start of the study and the number of animals alive at the time of first tumor are summarized in Table E-53 and Figure E-11 and Figure E-12. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 1 model for the number of animals at the start of the study is 32.7 mg/L and for the number of animals alive at the time of first tumor is 19.8 mg/L. The number of animals alive at the time of first tumor ensures the potency is not underestimated by mortality of animals prior to tumor occurrence. The relatively small difference in the two $BMDL_{10}$ values supports using these values and the selected value is based on the number of animals alive at the time of first tumor, 19.8 mg/L.

Table E-53. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

	Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
		p-value	AIC	Dose Group Near BMD	Control Dose Group			
Animals at the start of the study	Multistage Degree 4	0.600	73.4	0.0021	−0.668	61.8	33.2	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit
	Multistage Degree 3	0.597	73.4	0.0081	−0.667	61.2	33.2	

	Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
		p-value	AIC	Dose Group Near BMD	Control Dose Group			
	Multistage Degree 2	0.581	73.5	0.0331	−0.663	60.6	33.0	(p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), the Multistage Degree 1 model had the lowest AIC.
	Multistage Degree 1	**0.723**	**71.6**	**0.1462**	**−0.565**	**60.3**	**32.7**	
Animals alive at the time of first tumor	Multistage Degree 4	0.466	63.8	0.0029	−0.716	47.5	20.0	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest AIC.
	Multistage Degree 3	0.461	63.8	0.0109	−0.711	45.2	20.0	
	Multistage Degree 2[b]	0.449	63.8	0.0415	−0.694	41.7	19.9	
	Multistage Degree 1	**0.643**	**61.8**	**−0.613**	**−0.630**	**37.2**	**19.8**	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.
[b] Degree 2 model estimated parameters at the zero boundary and reduced to the Multistage Degree 1 model.

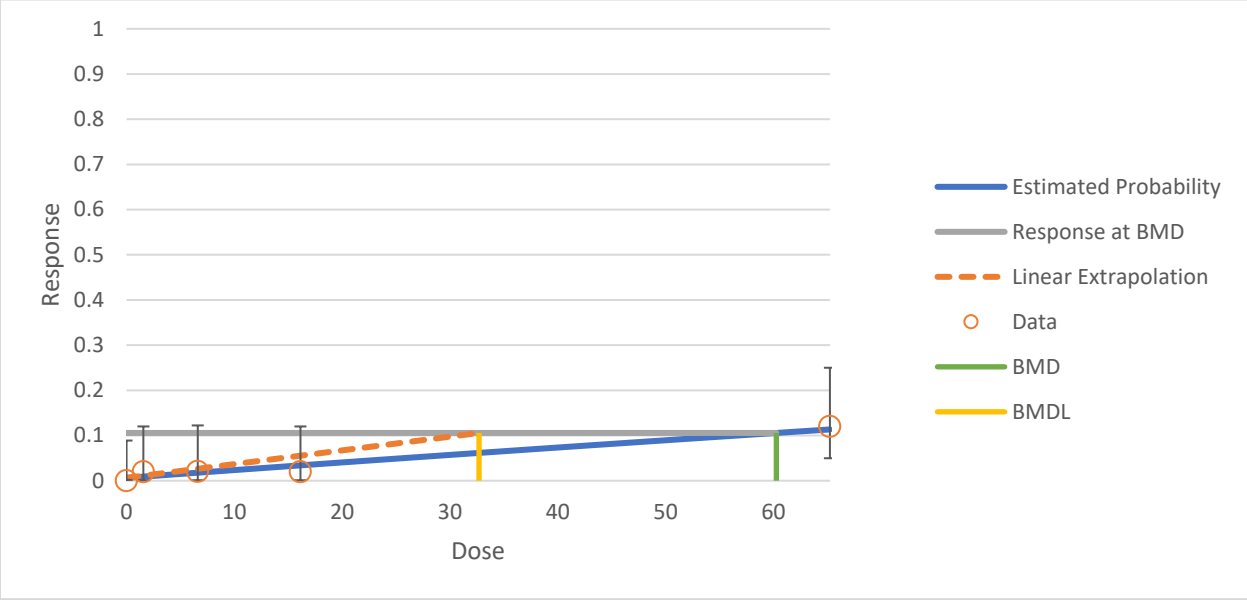


Figure E-11. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

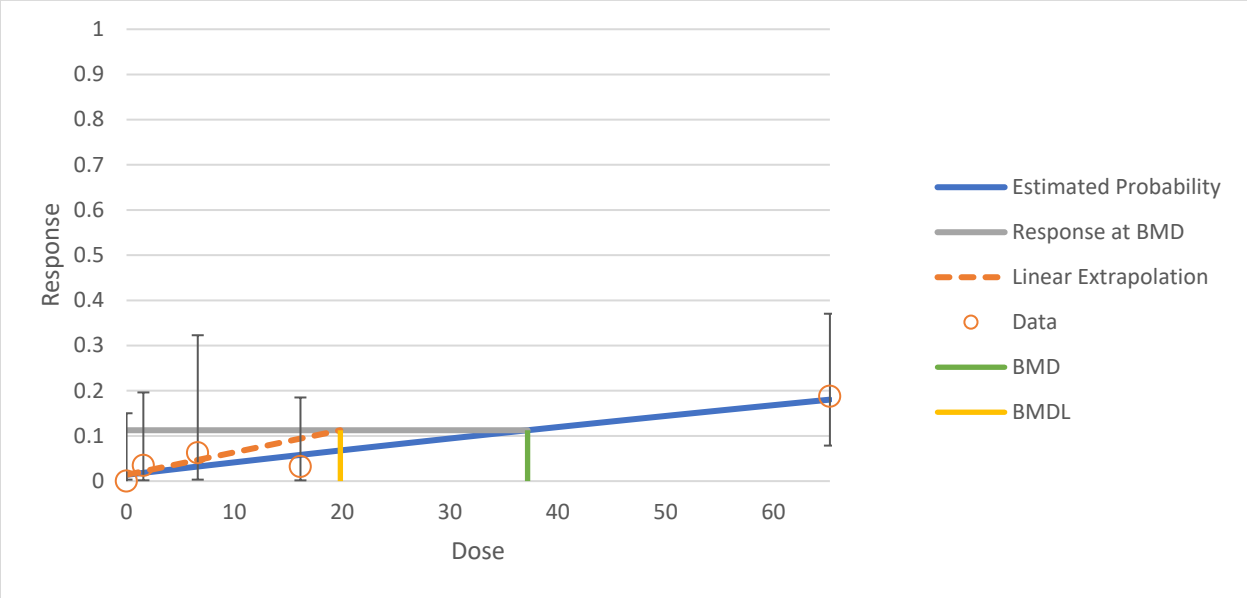


Figure E-12. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.1.6 Individual Cell Necrosis in the Liver in Females

Increased incidence of individual cell necrosis in the liver was observed in female Sprague-Dawley Crl:CD(SD)IGS BR rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-54. As described in the Toxicity Assessment (U.S. EPA, 2024), the average concentration over the final week of study $C_{last7,avg}$, was selected for all non-developmental studies to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human pharmacokinetic (PK) model.

Table E-54. Dose-Response Modeling Data for Individual Cell Necrosis in the Liver in Female Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	50	3
0.029	1.8	50	4
0.120	7.4	50	4
0.299	18.0	50	5
1.251	72.5	50	9

BMD modeling results for individual cell necrosis in the liver are summarized in Table E-55 and Figure E-13. The Log-Logistic model was selected based on adequate p-values (greater than 0.1) and had the lowest AIC among adequately fitting with BMD/BMDL ratios less than 3. The $BMDL_{10}$ from the selected Log-Logistic model is 27.0 mg/L.

Table E-55. Summary of Benchmark Dose Modeling Results for Individual Cell Necrosis in the Liver in Female Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	0.947	164.2	0.003	−0.201	57.1	9.4	EPA selected the Log-Logistic model. All models had adequate fit (p-values greater than 0.1). The Dichotomous Hill and Log-Probit were the only models that did not have BMD/BMDL ratio <3. Of the remaining models, the Log-Logistic model had the lowest AIC.
Gamma	0.990	162.2	−0.024	−0.239	59.2	29.0	
Log-Logistic	**0.990**	**162.2**	**−0.017**	**−0.226**	**58.5**	**27.0**	
Multistage Degree 4	0.990	162.2	−0.024	−0.239	59.2	29.0	
Multistage Degree 3	0.990	162.2	−0.024	−0.239	59.2	29.0	
Multistage Degree 2	0.990	162.2	−0.024	−0.239	59.2	29.0	
Multistage Degree 1	0.990	162.2	−0.024	−0.239	59.2	29.0	
Weibull	0.990	162.2	−0.024	−0.239	59.2	29.0	
Logistic	0.981	162.3	−0.040	−0.334	64.2	41.8	
Log-Probit	0.938	164.2	0.022	−0.208	57.0	0.6	
Probit	0.983	162.3	−0.041	−0.322	63.5	39.9	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

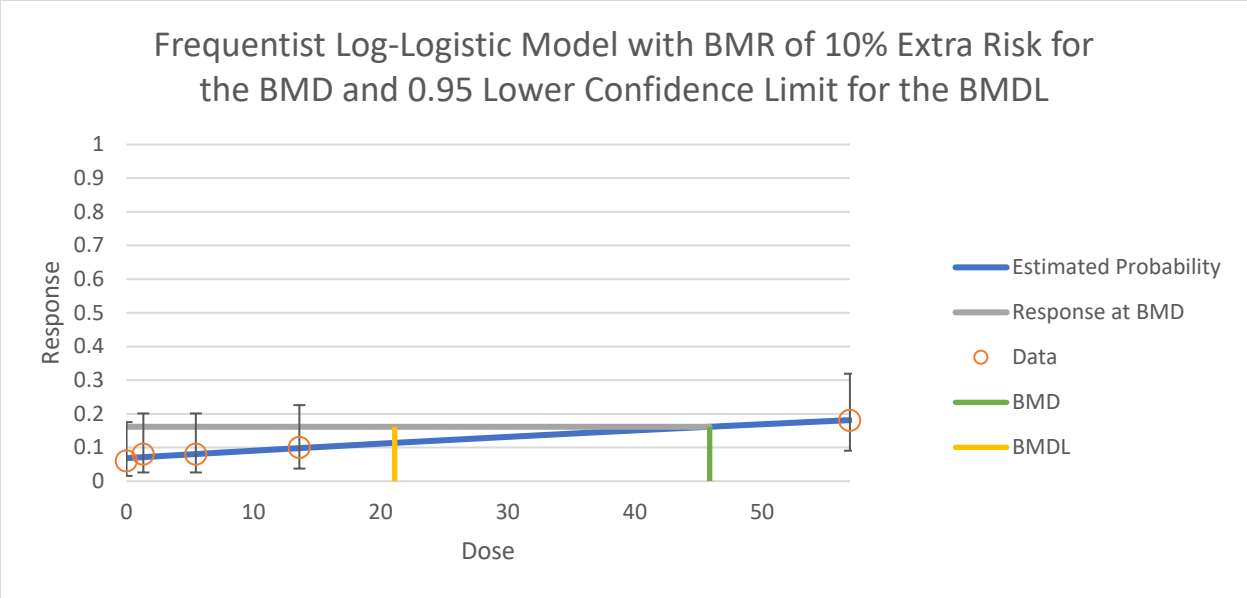


Figure E-13. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Individual Cell Necrosis in the Liver in Female Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS (Butenhoff et al., 2012; Thomford, 2002)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.2 Lee et al. (2015)

EPA conducted dose-response modeling of the Lee et al. (2015) study using the BMDS 3.2 program. This study addresses fetal body weight in F_1 male and female CD-1 mice.

E.2.2.1 Fetal Body Weight

Decreased mean response of fetal body weight was observed in F_1 male and female CD-1 mice. Continuous models were used to fit dose-response data. BMR of a 5% change was chosen and a change in the mean equal to 0.5 standard deviations from the control mean was also modeled for comparison purposes. The doses and response data used for the modeling are listed in Table E-56. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased fetal body weight, the $C_{avg,pup,gest}$ metric was selected because pups were exposed during gestation only.

Table E-56. Dose-Response Modeling Data for Fetal Body Weight in F_1 Male and Female CD-1 Mice Following Exposure to PFOS (Lee et al., 2015)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g)[a]
0	0	10	1.7 ± 0.2
0.5	0.9	10	1.5 ± 0.1
2	3.5	10	1.3 ± 0.1
8	14.0	10	1.1 ± 0.2

Notes:
[a] Data are presented as mean ± standard deviation.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.3 Luebker et al. (2005b)

EPA conducted dose-response modeling of the Luebker et al. (2005b) study using the BMDS 3.2 program. This study addresses pup body weight relative to the litter at LD 1 and LD 5 in F_1 male and female Sprague-Dawley rats.

E.2.3.1 Pup Body Weight Relative to Litter at LD 5

Decreased mean response of pup body weight relative to the litter at LD 5 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was selected and a BMR of a 0.5 standard deviation change from the mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-57. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased pup weight at LD 5, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation.

Table E-57. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (LD 5) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005b)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g)[a]
0	0	17	9.8 ± 2.1[b]
0.4	15.9	17	8.6 ± 1.9
0.8	31.9	17	8.5 ± 2.8
1	39.8	17	8.1 ± 2.5
1.2	47.8	17	7.5 ± 2.7
1.6	63.7	17	7.2 ± 2.7
2	79.6	17	7.3 ± 7.3

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

The dose-response data for the highest dose group was removed prior to modeling as the variance surrounding the mean response for this group was large and dropping this dose group ensured adequate model fit in the low-dose range (U.S. EPA, 2012). Figure E-14 shows the best viable model (Polynomial Degree 6) when the highest dose group is included in modeling for visual comparison of fit. The BMD modeling results for pup body weight relative to the litter at LD 5 are summarized in Table E-58 and Figure E-15. The Exponential 5 model was selected as it had the lowest BMDL among the viable models (the Hill model was questionable in this run). The $BMDL_5$ from the selected Exponential 5 model is 2.3 mg/L.

Table E-58. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter (LD 5) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Constant Variance) (Luebker et al., 2005b)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	BMD_5 (mg/L)	$BMDL_5$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.5}$	Dose Group Near BMD_5	Control Dose Group					
Exponential 2	0.951	474.7	0.4	−0.6	0.3	28.2	17.6	10.8	7.3	EPA selected the Exponential 5 model. All viable models had adequate fit (p-values greater than 0.1), and the Exponential 5 model was selected as it had the lowest BMDL among the viable models.
Exponential 3	0.951	474.7	0.4	−0.6	0.3	28.2	17.6	10.8	7.3	
Exponential 4	0.881	476.6	0.4	−0.5	0.2	25.5	6.9	9.5	2.3	
Exponential 5	**0.881**	**476.6**	0.4	**−0.5**	**0.2**	25.6	6.9	**9.5**	**2.3**	
Hill	0.882	476.6	0.4	−0.5	0.2	25.0	3.9	9.2	1.1	
Polynomial Degree 5	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	8.7	
Polynomial Degree 4	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	9.2	
Polynomial Degree 3	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	8.7	
Polynomial Degree 2	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	8.7	
Power	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	8.7	
Linear	0.941	474.8	0.3	−0.6	0.4	30.6	20.5	12.2	8.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.5SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviations from the control mean; BMD_5 = dose level corresponding to a 5% change in the mean from the control mean; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the mean from the control mean.

[a] Selected model in bold

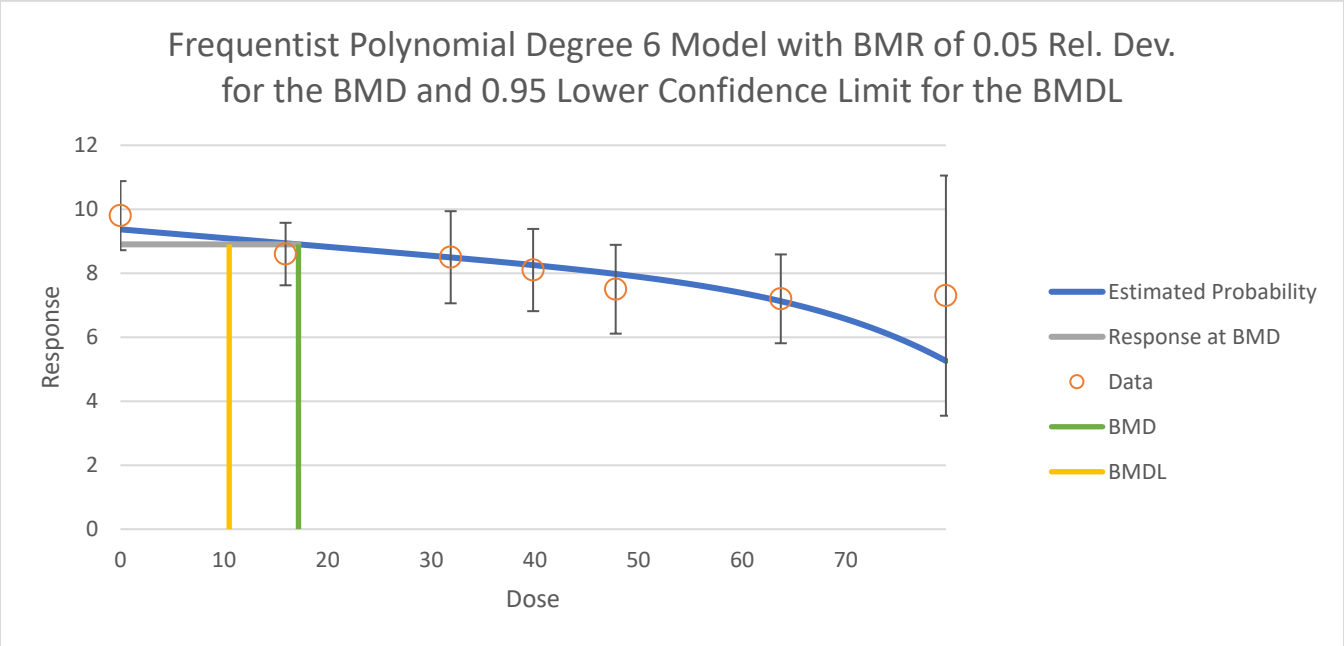


Figure E-14. Plot of Mean Response by Dose (Including Highest Dose) with Fitted Curve for the Polynomial 6 Model for Pup Body Weight Relative to the Litter at LD 5 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005b)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

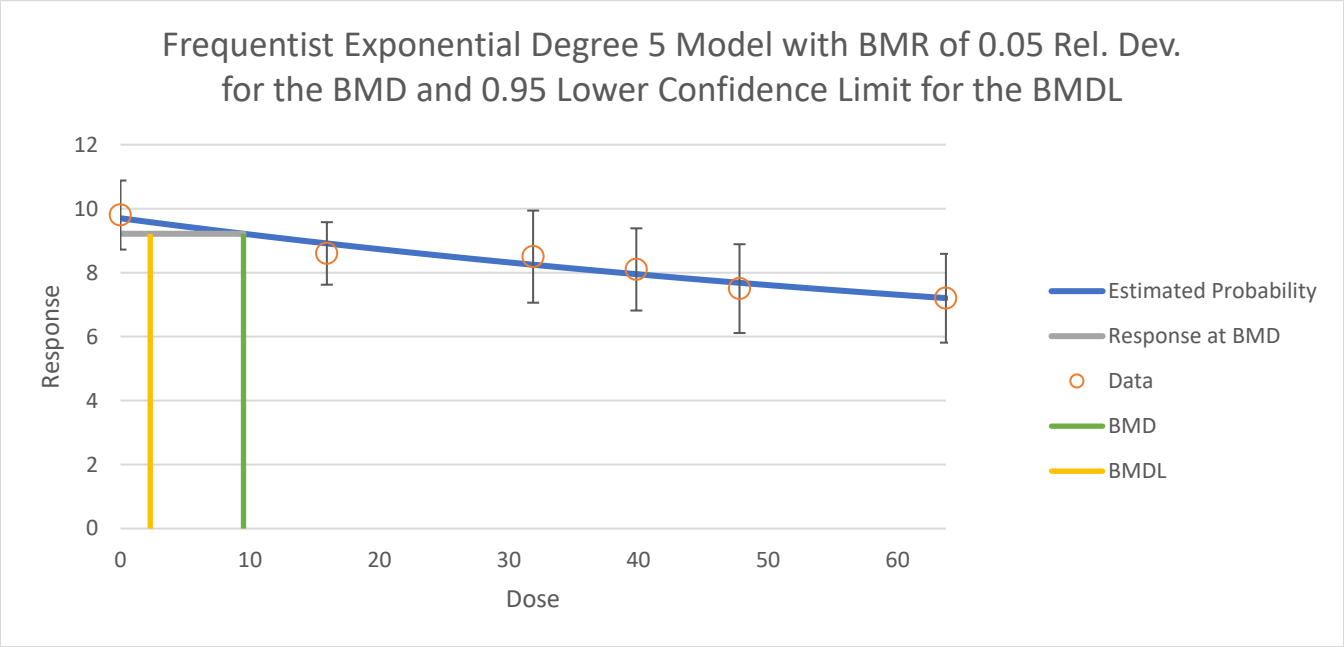


Figure E-15. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 5 Model for Pup Body Weight Relative to the Litter at LD 5 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005b)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.3.2 Pup Body Weight Relative to Litter at LD 1

Decreased mean response of pup body weight relative to the litter at LD 1 (i.e., day of birth) was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was selected and a BMR of a 0.5 standard deviation change from the mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-59. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased pup weight at LD 1, the $C_{avg,pup,gest}$ metric was selected because pups were exposed during gestation only.

Table E-59. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (LD 1) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005b)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g)[a]
0	0	17	6.4 ± 0.8[b]
0.4	15.4	17	6.0 ± 1.2
0.8	30.8	17	6.0 ± 1.2
1	38.5	17	5.9 ± 1.6
1.2	46.1	17	5.7 ± 1.2
1.6	61.5	17	5.4 ± 1.0
2	76.9	17	5.4 ± 1.0

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

The BMD modeling results for pup body weight relative to the litter at LD 1 are summarized in Table E-60 and Figure E-16. The Exponential 3 model was selected as it had the lowest AIC among the viable models. The $BMDL_5$ from the selected Exponential 3 model is 14.7 mg/L.

Table E-60. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter (LD 1) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Nonconstant Variance) (Luebker et al., 2005b)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	BMD_5 (mg/L)	$BMDL_5$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.5}$	Dose Group Near BMD_5	Control Dose Group					
Exponential 2	0.950	375.8	0.3	–0.5	0.2	40.5	23.8	22.6	14.7	EPA selected the Exponential 3 model. All models had adequate fit (p-values greater than 0.1), and the Exponential 3 model was selected as it had the lowest AIC among the viable models.
Exponential 3	**0.950**	**375.8**	0.3	**–0.5**	**0.2**	40.5	23.8	**22.6**	**14.7**	
Exponential 4	0.888	377.8	0.3	–0.5	0.2	40.3	0	22.5	0	
Exponential 5	0.887	377.8	0.3	–0.5	0.2	40.4	0	22.6	0	
Hill	0.974	378.8	0.4	0.4	–0.2	11.0	0	8.6	0	
Polynomial Degree 6	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	17.0	
Polynomial Degree 5	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.4	
Polynomial Degree 4	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.3	
Polynomial Degree 3	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.3	
Polynomial Degree 2	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.3	
Power	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.3	
Linear	0.946	375.8	–0.1	0.3	0.2	42.7	26.3	24.0	16.3	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.5SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviations from the control mean; BMD_5 = dose level corresponding to a 5% change in the mean from the control mean; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the mean from the control mean.

[a] Selected model in bold

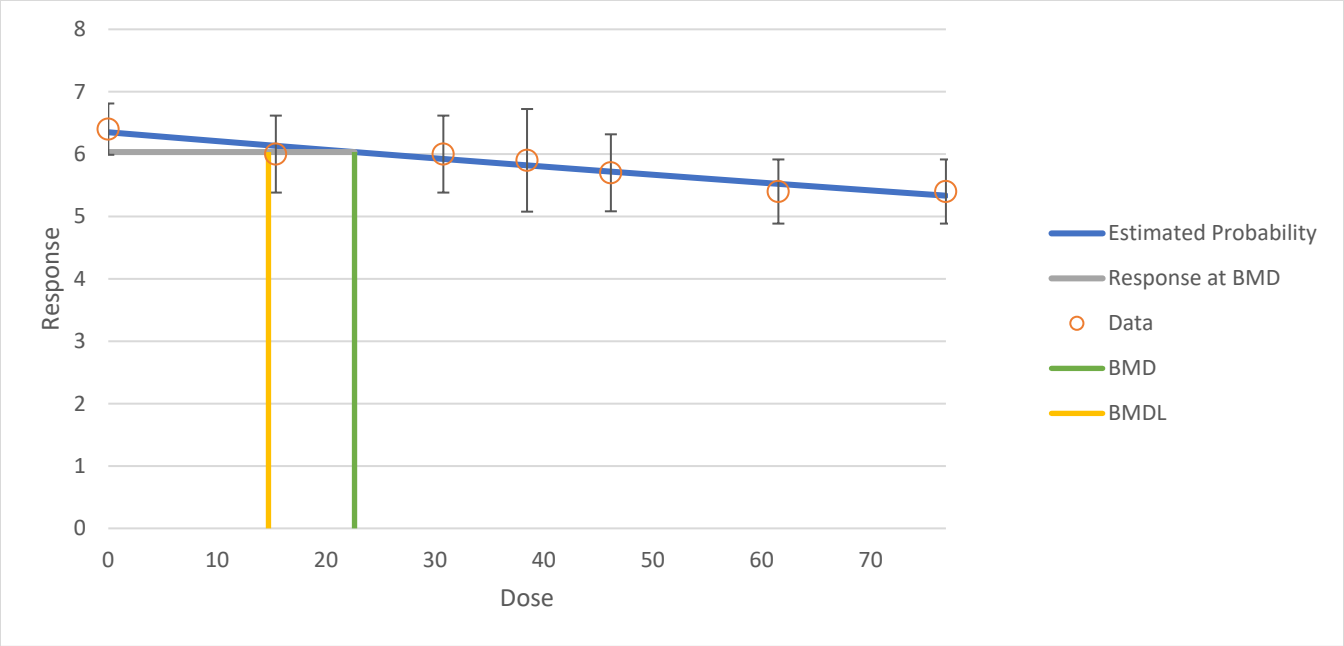


Figure E-16. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 3 Model for Pup Body Weight Relative to the Litter at LD 1 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005b)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.4 NTP (2019)

EPA conducted dose-response modeling of the NTP (2019) study using the BMDS 3.2 program. This study addresses extramedullary hematopoiesis in the spleen in male and female Sprague-Dawley rats.

E.2.4.1 Extramedullary Hematopoiesis in the Spleen in Male Sprague-Dawley Rats

Increased incidence of extramedullary hematopoiesis in the spleen was observed in male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-61. As described in the Toxicity Assessment (U.S. EPA, 2024), the $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model.

Table E-61. Dose-Response Modeling Data for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	10	1

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0.312	10.2	10	1
0.625	20.4	10	2
1.25	40.8	10	7
2.5	81.6	10	8
5	162.7	10	10

The BMD modeling results for extramedullary hematopoiesis in the spleen are summarized in Table E-62 and Figure E-17. The best fitting model was the Logistic model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Logistic model had the lowest AIC. The $BMDL_{10}$ from the selected Logistic model is 9.6 mg/L.

Table E-62. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	0.646	53.0	−0.3	0.2	15.7	7.1	EPA selected the Logistic model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Logistic model had the lowest AIC.
Gamma	0.594	53.2	−0.3	0.2	13.8	4.6	
Log-Logistic	0.646	53.0	−0.3	0.2	15.7	7.1	
Multistage Degree 5	0.487	53.7	−0.5	0.3	10.9	4.2	
Multistage Degree 4	0.487	53.7	−0.5	0.3	10.9	4.2	
Multistage Degree 3	0.487	53.7	−0.5	0.3	10.9	4.3	
Multistage Degree 2	0.487	53.7	−0.5	0.3	10.9	4.3	
Multistage Degree 1	0.475	53.4	−1.0	0.6	5.4	3.7	
Weibull	0.549	53.4	−0.4	0.3	12.1	4.4	
Logistic	**0.558**	**52.2**	**−0.6**	**−0.1**	**14.0**	**9.6**	
Log-Probit	0.676	52.8	−0.4	0.2	16.0	7.5	
Probit	0.558	52.3	−0.6	0.0	13.4	9.5	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

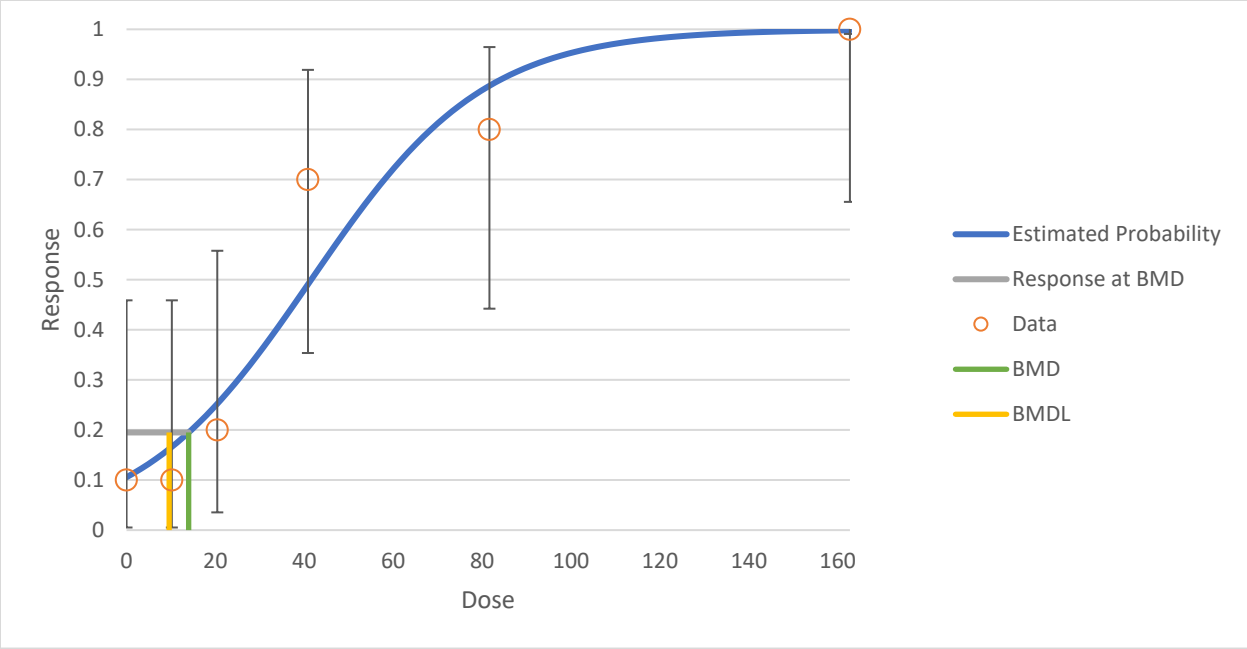


Figure E-17. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Logistic Model for Extramedullary Hematopoiesis in the Spleen in Male Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.4.2 Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats

Increased incidence of extramedullary hematopoiesis in the spleen was observed in female Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-63. As described in the Toxicity Assessment (U.S. EPA, 2024), the $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model.

Table E-63. Dose-Response Modeling Data for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	10	2
0.312	10.0	10	3
0.625	20.0	10	3
1.25	40.0	10	8
2.5	80.0	10	10
5	159.6	10	10

The BMD modeling results for extramedullary hematopoiesis in the spleen are summarized in Table E-64 and Figure E-18. The Multistage Degree 1 model was selected based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the lowest BMDL. The $BMDL_{10}$ from the selected Multistage Degree 1 model is 2.3 mg/L.

Table E-64. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	0.849	52.8	0.2	−0.5	26.4	9.1	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest BMDL.
Gamma	0.966	50.7	0.0	−0.4	21.8	5.7	
Log-Logistic	0.956	50.8	0.2	−0.4	25.7	9.1	
Multistage Degree 5	0.989	50.6	−0.2	−0.1	16.1	3.4	
Multistage Degree 4	0.981	50.6	−0.2	−0.1	16.5	3.4	
Multistage Degree 3	0.959	50.8	−0.3	−0.2	16.5	3.5	
Multistage Degree 2	0.948	49.2	0.3	0.1	11.5	3.6	
Multistage Degree 1	**0.448**	**53.0**	**0.6**	**0.6**	**3.5**	**2.3**	
Weibull	0.990	48.7	−0.2	−0.2	18.0	5.0	
Logistic	0.877	49.8	0.3	0.5	7.6	5.1	
Log-Probit	0.963	50.8	0.1	−0.4	22.5	8.8	
Probit	0.888	49.7	0.2	0.5	7.2	5.0	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

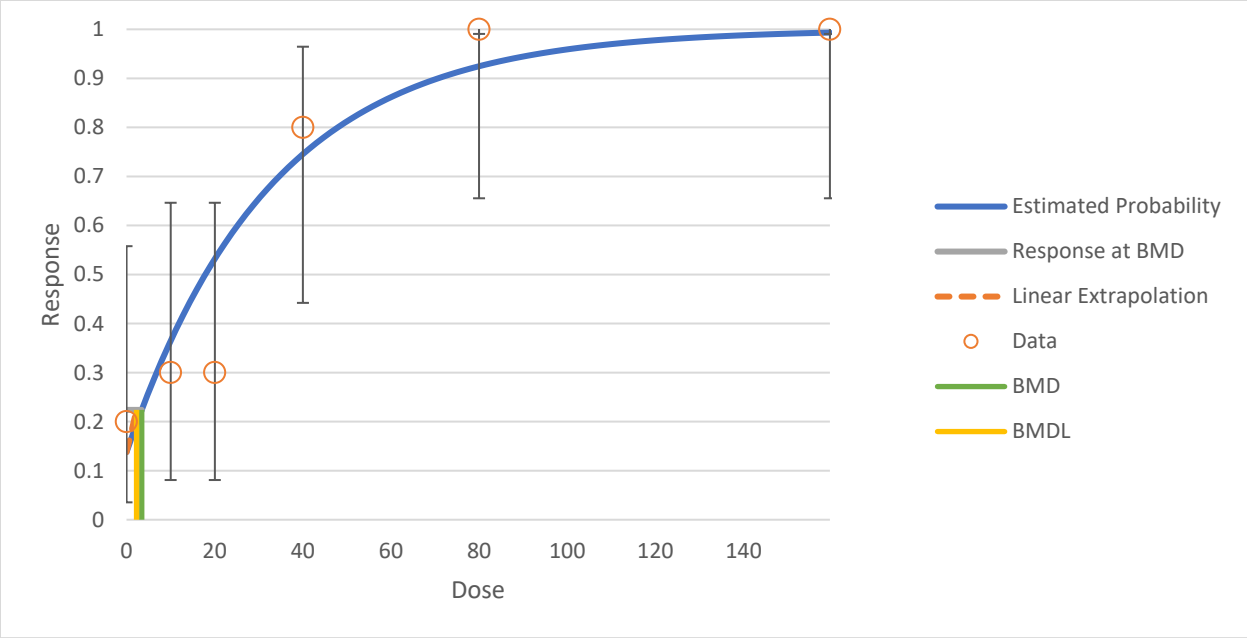


Figure E-18. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS (NTP, 2019)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.5 Zhong et al. (2016)

EPA conducted dose-response modeling of the Zhong et al. (2016) study using the BMDS 3.2 program. This study addresses plaque-forming cell (PFC) response of splenic cells in F_1 male C57BL/6 mice at PNW 4.

E.2.5.1 Plaque-Forming Cell Response of Splenic Cells in F_1 Male C57BL/6 Mice

Decreased mean response of PFC response of splenic cells was observed in F_1 male C57BL/6 mice at PNW 4. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-65. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased PFC response at PNW 4, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation.

Table E-65. Dose-Response Modeling Data for PFC Response of Splenic Cells in F_1 Male C57BL/6 Mice at PNW 4 Following Exposure to PFOS (Zhong et al., 2016)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (# Cells per 10^6 Spleen Cells)[a]
0	0.0	12	465.7 ± 78.5[b]
0.1	1.7	12	423.0 ± 60.4
1	16.8	12	398.7 ± 72.5
5	84.1	12	340.1 ± 54.4

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

BMD modeling results for PFC response of splenic cells are summarized in Table E-66 and Figure E-19. The best fitting model was the Hill model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Hill model had the lowest BMDL. The $BMDL_{1SD}$ from the selected Hill model is 1.8 mg/L.

Table E-66. Summary of Benchmark Dose Modeling Results for Plaque-Forming Cell Response of Splenic Cells in F_1 Male C57BL/6 Mice at PNW 4 Following Exposure to PFOS (Constant Variance) (Zhong et al., 2016)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Exponential 2	0.181	545.3	0.2	1.4	51.3	34.4	EPA selected the Hill model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Hill model had the lowest BMDL.
Exponential 3	0.181	545.3	0.2	1.4	51.3	34.4	
Exponential 4	0.174	545.7	0.2	0.9	22.3	6.6	
Exponential 5	0.174	545.7	0.2	0.9	22.2	6.6	
Hill	**0.190**	**545.6**	**0.3**	**0.8**	**20.6**	**1.8**	
Polynomial Degree 3	0.161	545.5	0.2	1.4	55.1	38.9	
Polynomial Degree 2	0.161	545.5	0.2	1.4	55.1	38.9	
Power	0.161	545.5	0.2	1.4	55.1	38.9	
Linear	0.161	545.5	0.2	1.4	55.1	38.9	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{1SD} = dose level corresponding to a change in the mean equal to 1 standard deviation from the control mean; $BMDL_{1SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 1 standard deviation from the control mean.
[a] Selected model in bold.

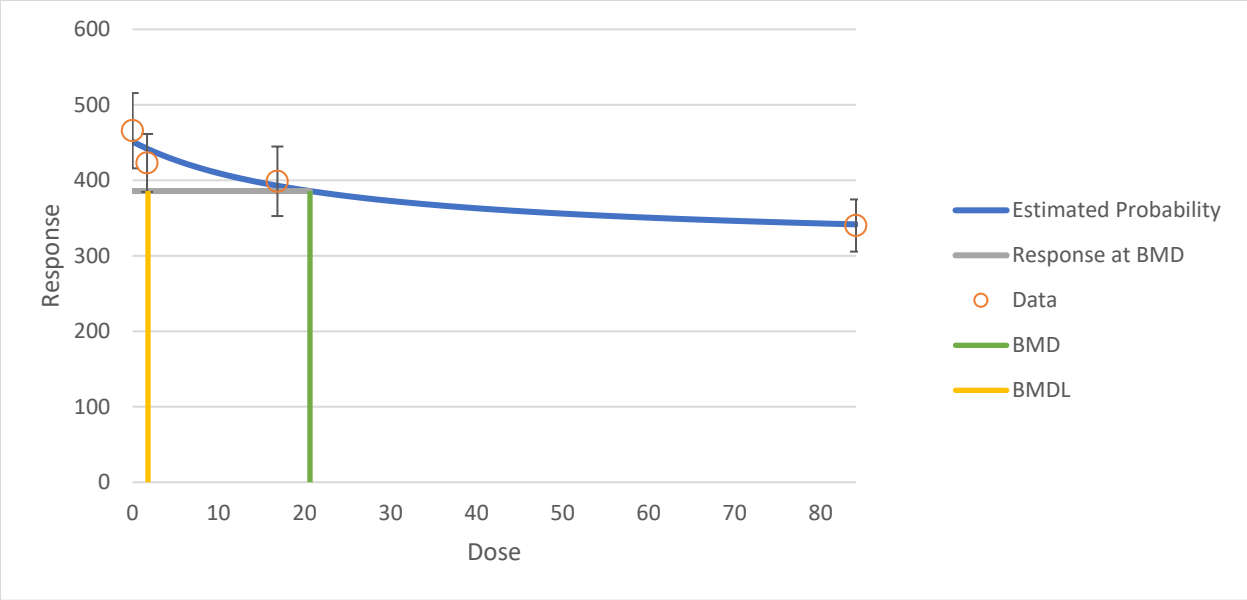


Figure E-19. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for PFC Response of Splenic Cells in F_1 Male C57BL/6 Mice at PNW 4 Following Exposure to PFOS (Zhong et al., 2016)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6 Lau et al. (2003)

EPA conducted dose-response modeling of the Lau et al. (2003) study using the BMDS 3.2 program. This study addresses offspring survival in F_1 male and female Sprague-Dawley rats at PND 5 and PND 22.

E.2.6.1 Pup Survival at PND 5

Decreased mean response of number of surviving offspring at PND 5 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.1 and 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table E-67. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased pup survival at PND 5, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation. The $C_{avg,pup,gest,lact}$ was selected for this model.

Table E-67. Dose-Response Modeling Data for Pup Survival at PND 5 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Lau et al., 2003)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (Percent Survival per Litter)[a]
0	0	18	90 ± 8.9[b]
1	13.0	12	86 ± 27.7
2	25.9	9	79 ± 20.1
3	38.9	17	45 ± 37.1
5	64.9	17	4 ± 10.3

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.6.2 Pup Survival at PND 22

Decreased mean response of number of surviving offspring at PND 22 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.1 and 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table E-68. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the Toxicity Assessment (U.S. EPA, 2024) for additional details. For decreased pup survival at PND 22, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation. The $C_{avg,pup,gest,lact}$ was selected for this model.

Table E-68. Dose-Response Modeling Data for Pup Survival at PND 22 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Lau et al., 2003)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (Percent Survival per Litter)[a]
0	0	18	78 ± 14.8[b]
1	17.3	12	74 ± 28.8
2	34.6	9	61 ± 40.2
3	51.9	17	34 ± 33

5	86.4	17	2 ± 8.7

Notes: PND =postnatal day.
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.7 Luebker et al. (2005a)

EPA conducted dose-response modeling of the Luebker et al. (2005a) study using the BMDS 3.2 program. This study addresses pup body weight relative to the litter observed on LD 1 (i.e., day of birth) in F_1 male and female Sprague-Dawley rats.

E.2.7.1 Pup Body Weight Relative to Litter at LD 1

Decreased mean response of pup body weight relative to the litter at LD 1 (i.e., day of birth) was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was selected and a BMR of a 0.5 standard deviation change from the mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-69. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See the toxicity assessment (U.S. EPA, 2024) for additional details. For decreased pup weight at LD 1, the $C_{avg,pup,gest}$ metric was selected because pups were exposed during gestation only.

Table E-69. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (LD 1) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005a)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g)[a]
0	0	23	6.6 ± 0.6[b]
0.1	3.8	25	6.6 ± 0.5
0.4	15.3	22	6.4 ± 0.7
1.6	61.6	20	5.7 ± 0.5
3.2	131.0	20	5.3 ± 0.4

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

The BMD modeling results for pup body weight relative to the litter at LD 1 are summarized in Table E-70 and Figure E-20. The Exponential 4 model was selected as it had the lowest AIC among the viable models. The $BMDL_5$ from the selected Exponential 4 model is 11.3 mg/L.

Table E-70. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter at LD 1 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Nonconstant Variance) (Luebker et al., 2005a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	BMD_5 (mg/L)	$BMDL_5$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.5}$	Dose Group Near BMD_5	Control Dose Group					
Exponential 2	0.259	184.4	0.04	0.04	0.3	27.1	21.5	29.5	25.3	EPA selected the Exponential 4 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 4 model had the lowest AIC.
Exponential 3	0.259	184.4	0.04	0.04	0.3	27.1	21.5	29.5	25.3	
Exponential 4	**0.675**	**183.1**	0.36	**0.36**	**−0.4**	15.9	9.9	**17.7**	**11.3**	
Exponential 5	0.969	184.4	−0.29	−0.29	0.1	24.1	10.6	25.9	12.1	
Hill	0.926	184.4	−0.29	−0.29	0.1	23.7	9.9	25.3	11.3	
Polynomial Degree 4	0.164	185.5	0.03	0.03	0.4	30.0	24.2	32.5	28.4	
Polynomial Degree 3	0.164	185.5	0.03	0.03	0.4	30.0	24.2	32.5	28.4	
Polynomial Degree 2	0.164	185.5	0.03	0.03	0.4	30.0	24.2	32.5	28.4	
Power	0.164	185.5	0.03	0.03	0.4	30.0	24.2	32.5	28.4	
Linear	0.164	185.5	0.03	0.03	0.4	30.0	24.2	32.5	28.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.5SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviations from the control mean; BMD_5 = dose level corresponding to a 5% change in the mean from the control mean; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the mean from the control mean.

[a] Selected model in bold

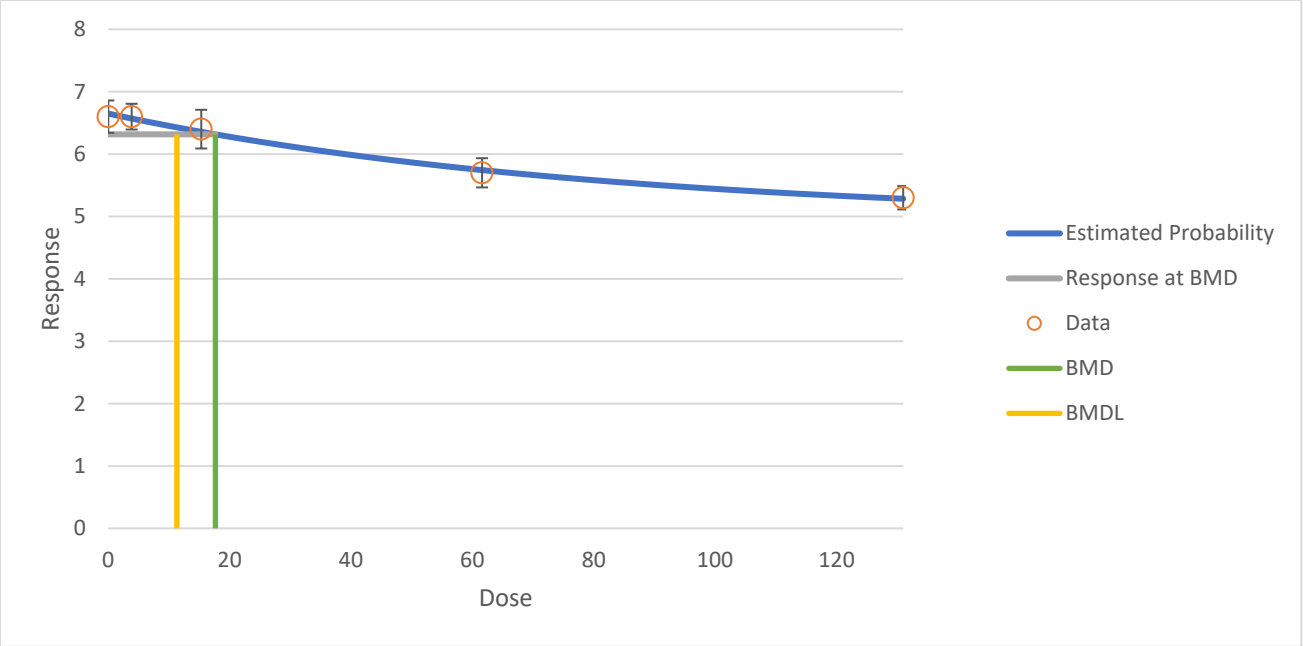


Figure E-20. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Pup Body Weight Relative to the Litter at LD 1 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (Luebker et al., 2005a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

Appendix F. Pharmacokinetic Modeling

For the animal pharmacokinetic model, model predictions from Wambaugh et al. (2013) were evaluated by comparing each predicted final serum concentration to the serum value in the supporting animal studies (training data set) and to animal studies published since the publication of Wambaugh et al. (2013) (test data set). The predictions to these two data sets were generally similar to the experimental values. There were no systematic differences between the experimental data and the model predictions across species, strain, or sex, and median model outputs uniformly appeared to be biologically plausible despite the uncertainty reflected in some of the 95th percentile confidence intervals (CIs). The application of the model outputs in the derivation of a human RfD can be found in the main perfluorooctane sulfonic acid (PFOS) document.

F.1 Comparison of Fits to Training Datasets Used in Wambaugh et al. (2013)

The following figures show comparisons of the model predicted serum concentrations to the data used for model training. Fits are also presented in supplemental material of Wambaugh et al. (2013).

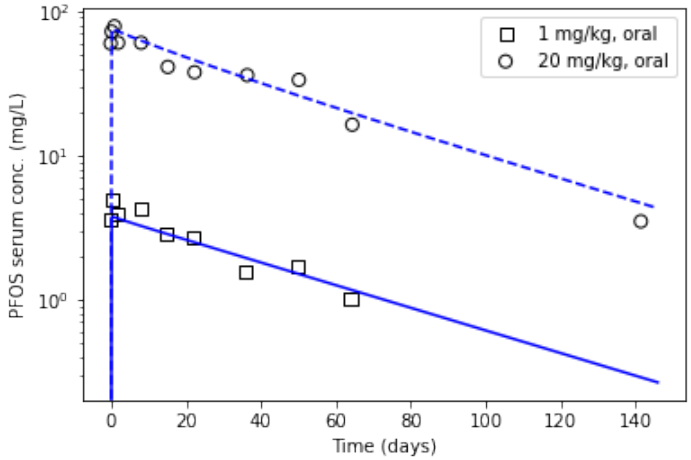


Figure F-1. Experimentally Observed Serum Concentrations (Chang et al., 2012) and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Female CD1 Mice

One mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

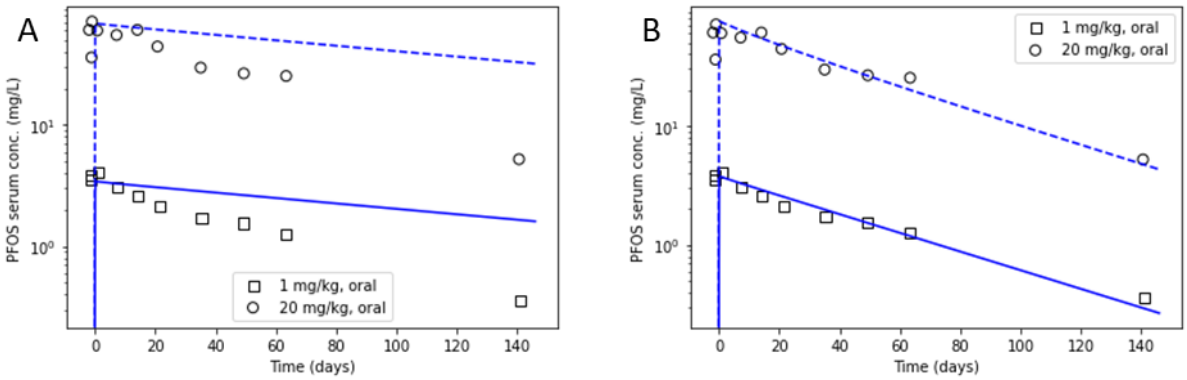


Figure F-2. Experimentally Observed Serum Concentrations (Chang et al., 2012) and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Male CD1 Mice

A) Fits to observed male data using male-specific model. B) Fits to observed male data using female-specific model parameters. One mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

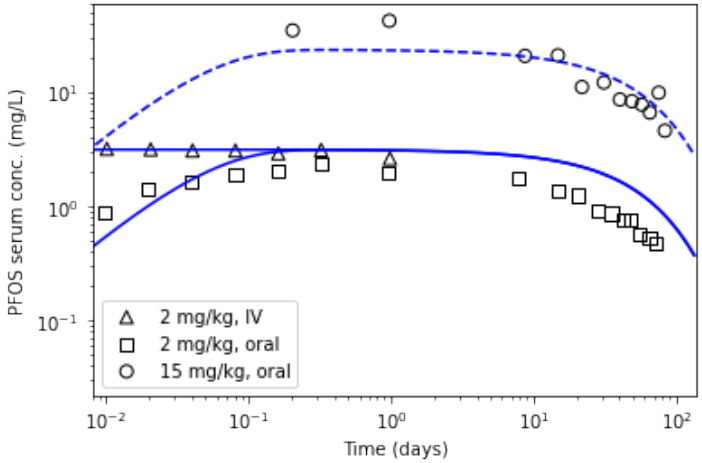


Figure F-3. Experimentally Observed Serum Concentrations (Chang et al., 2012) and Median Prediction for a Single IV Dose of 2 mg/kg or a Single Oral Dose of 2 or 15 mg/kg PFOS to Male Sprague-Dawley Rats

Two mg/kg intravenous (IV) dose represented by the upward triangles and solid line; 2 mg/kg oral dose represented by the squares and solid line; 15 mg/kg oral dose represented by the circles and dashed line.

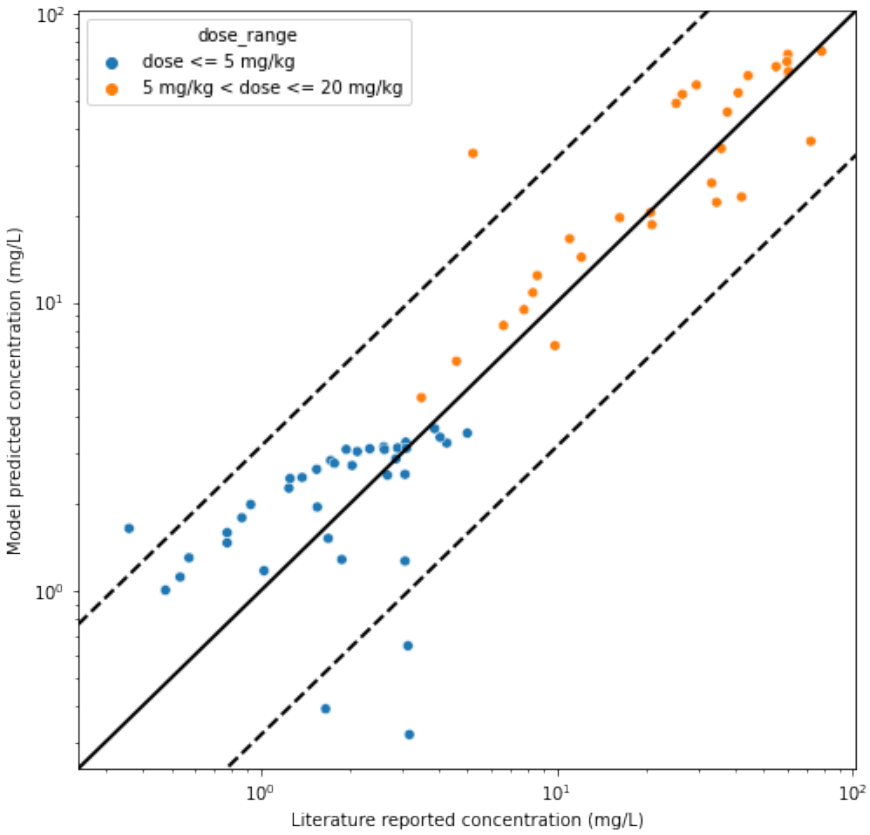
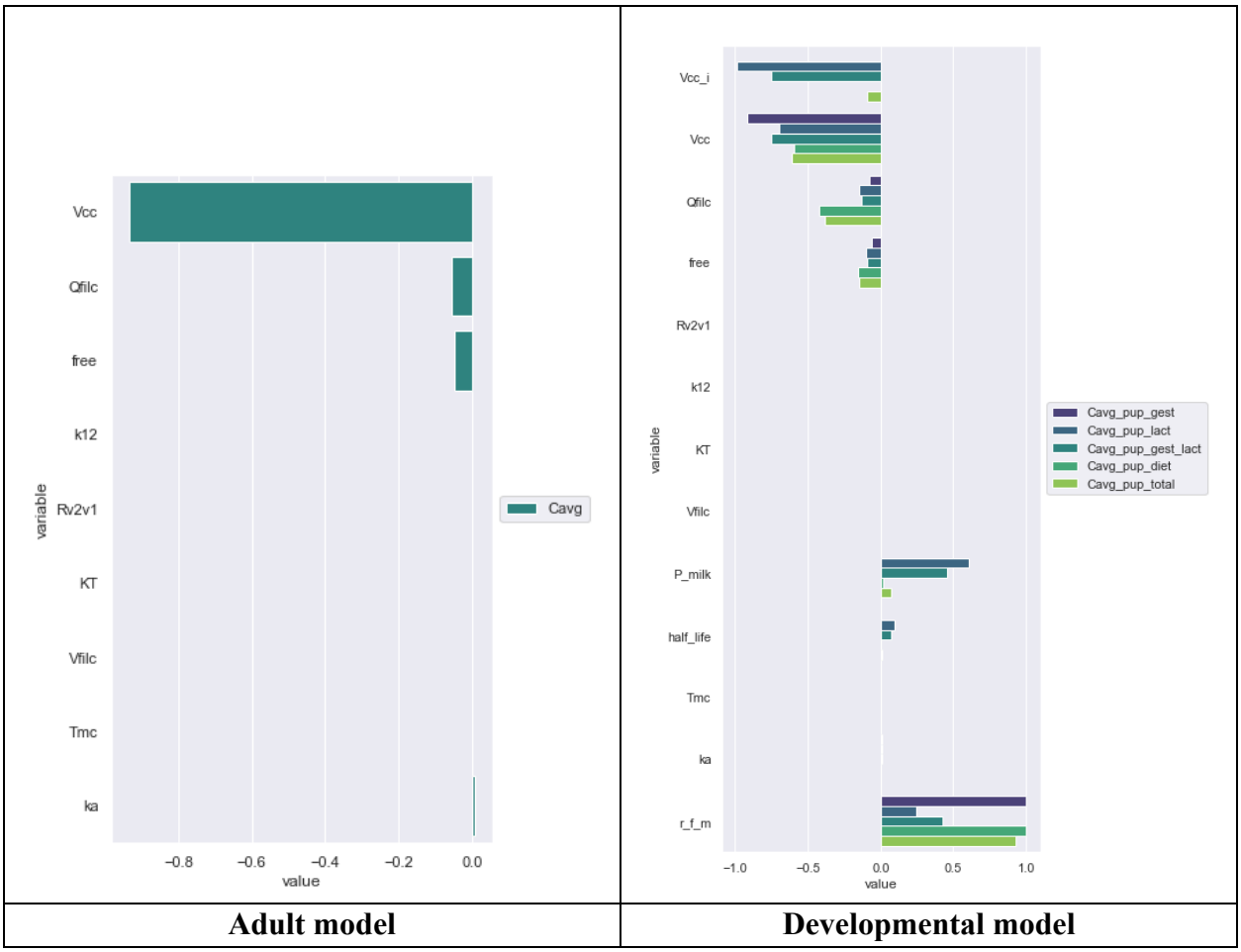


Figure F-4. Model Prediction Summary for PFOS Training Data

Model predictions on the training data result in a mean squared log error (MSLE) of 0.174. Dashed lines represent +/- one-half $\log_{10}$.

A local, one-at-a-time sensitivity analysis was conducted to examine how parameter sensitivity varied across the adult and developmental models (Figure F-5). For each parameter/dose metric pair, sensitivity coefficients were calculated to describe the relative change in a dose metric relative to the proportional change in a parameter value. A sensitivity coefficient of 1 describes the situation where a 1% increase in a parameter resulted in a 1% increase in the dose metric.



Adult model | **Developmental model**

Figure F-5. PFOS Sensitivity Coefficients of the Adult Model and Developmental Model

As demonstrated in Figure F-5, the volume of distribution (Vd) represents the most sensitive parameter for average concentrations in the adult animal. Because of the long half-life and high degree of plasma protein binding, renal resorption parameters that impact the effective half-life of PFOS are not as sensitive when compared to PFOA which has a shorter net half-life. Comparatively, the four one-compartment parameters for the infant (volume of distribution, half-life, serum:milk partition coefficient, and fetal:maternal ratio) are all sensitive to the gestational/lactational dose metrics. However, once the pup transitions to the adult model (Wambaugh model), PFOS transfer during gestation/lactation does not impact the average concentration during the post-weaning phase ($C_{avg\text{-}pup\text{-}diet}$). This is because the steady state concentration for the pup exposed to PFOS in the diet during growth is much larger than the steady state concentration during the 21 days of lactational exposure.

F.2 Visual Inspection of Test Datasets not Used for Initial Fitting

The following figures show a comparison between model predictions and data from more recently published studies that were not part of the Wambaugh et al. (2013) parameterization.

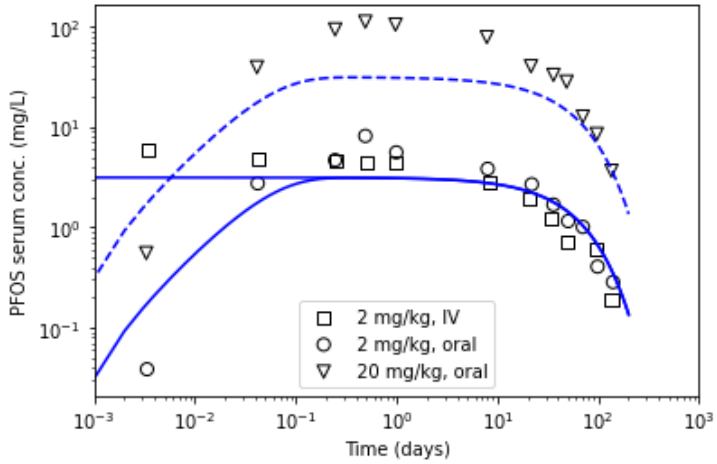


Figure F-6. mentally Observed Serum Concentrations (Huang et al., 2021) and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Male Sprague-Dawley Rats

Two mg/kg intravenous (IV) dose represented by the squares and solid line; 2 mg/kg oral dose represented by the circles and solid line; 20 mg/kg oral dose represented by the downward triangles and dashed line.

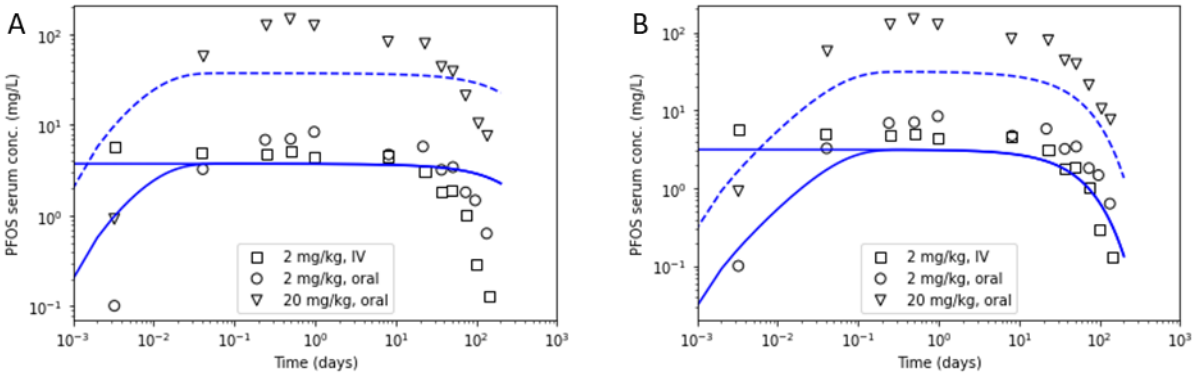


Figure F-7. Experimentally Observed Serum Concentrations (Huang et al., 2021) and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Female Sprague-Dawley Rats

A) Fits to observed female data using female-specific model parameters. B) Fits to observed female data using male-specific model parameters.
Two mg/kg intravenous (IV) dose represented by the squares and solid line; 2 mg/kg oral dose represented by the circles and solid line; 20 mg/kg oral dose represented by the downward triangles and dashed line.

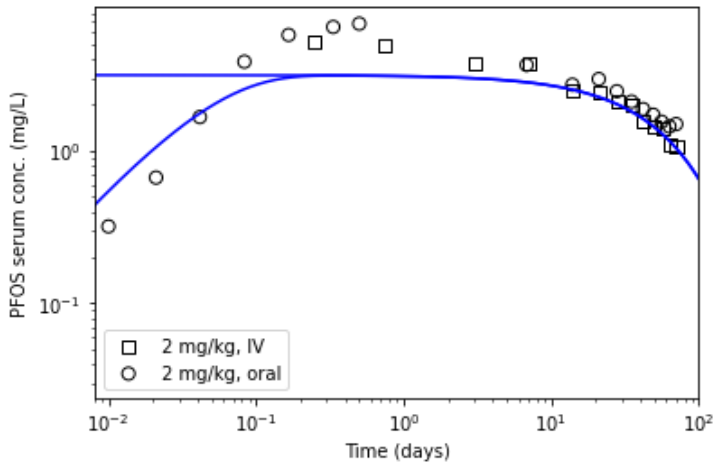


Figure F-8. Experimentally Observed Serum Concentrations (Kim et al., 2016b) and Median Prediction for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 mg/kg PFOS to Male Sprague-Dawley Rats

Two mg/kg intravenous (IV) dose represented by the squares; 2 mg/kg oral dose represented by the circles.

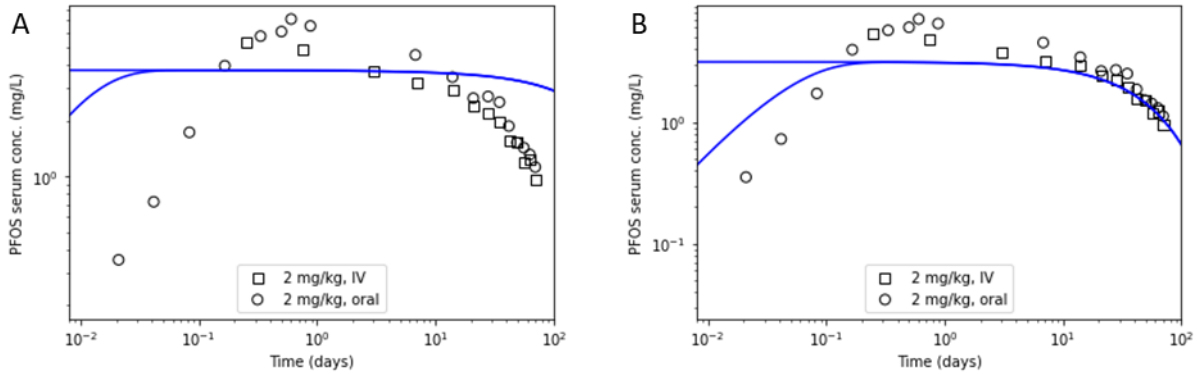


Figure F-9.Experimentally Observed Serum Concentrations (Kim et al., 2016b) and Median Prediction for a Single IV Dose of 2 mg/kg an Oral Dose of 2 mg/kg PFOS to Female Sprague-Dawley Rats

A) Fits to observed female data using female-specific model parameters. B) Fits to observed female data using male-specific model parameters.
Two mg/kg intravenous (IV) dose represented by the squares; 2 mg/kg oral dose represented by the circles.

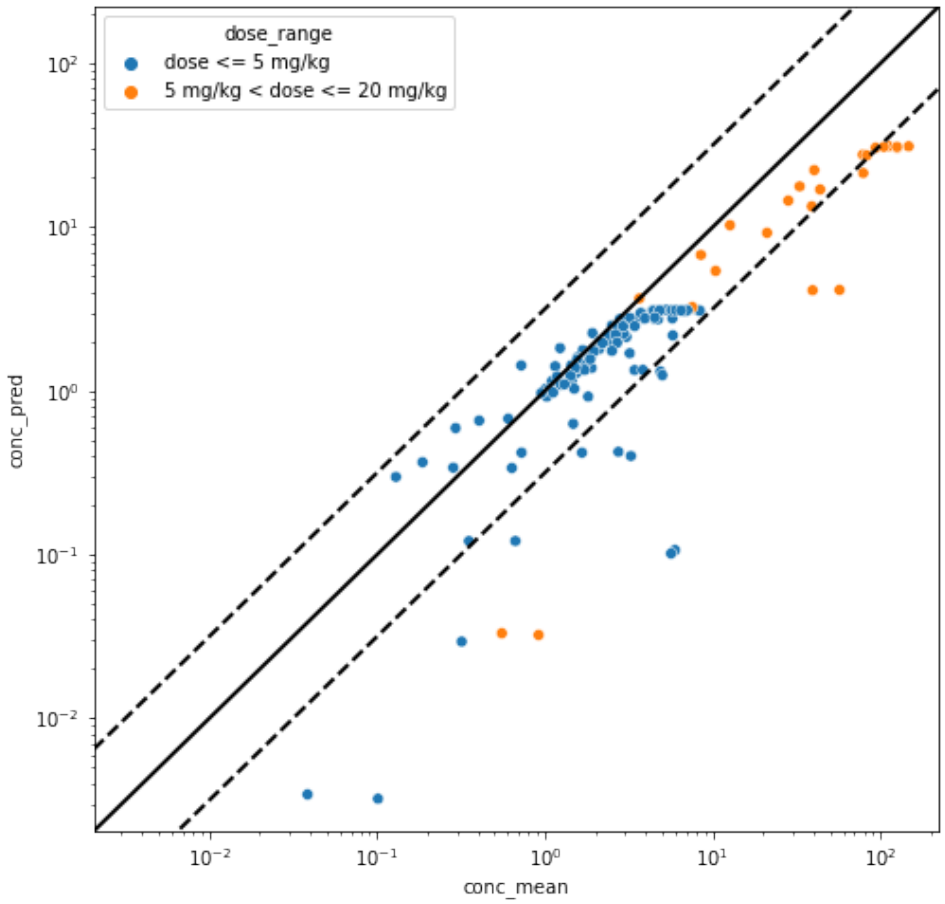


Figure F-10. Model Prediction Summary for PFOS Test Data

Model predictions on the adult, single-dose test data result in a mean squared log error (MSLE) of 0.384. Dashed lines represent +/- one-half $\log_{10}$. Developmental pharmacokinetic summary results not shown as only one study (presented in main text) is available for comparison.

F.3 Human Model Validation

As mentioned in the Toxicity Assessment (U.S. EPA, 2024), the human model was implemented in R/MCSim from the original AcslX model (Verner et al., 2016). Comparison with model output from the original model shows that, with the original parameters, the R model exactly replicates the original model (Figure F-11). The only difference remaining was that the start of pregnancy occurs at slightly different times in the two models, but this does not affect predictions outside of that very narrow time. Validation figures shown in this section include data for PFOA as well as PFOS. This is because model validation and decisions related to model structure were made for both chemicals together due to the preference for a similar model structure for the two chemicals.

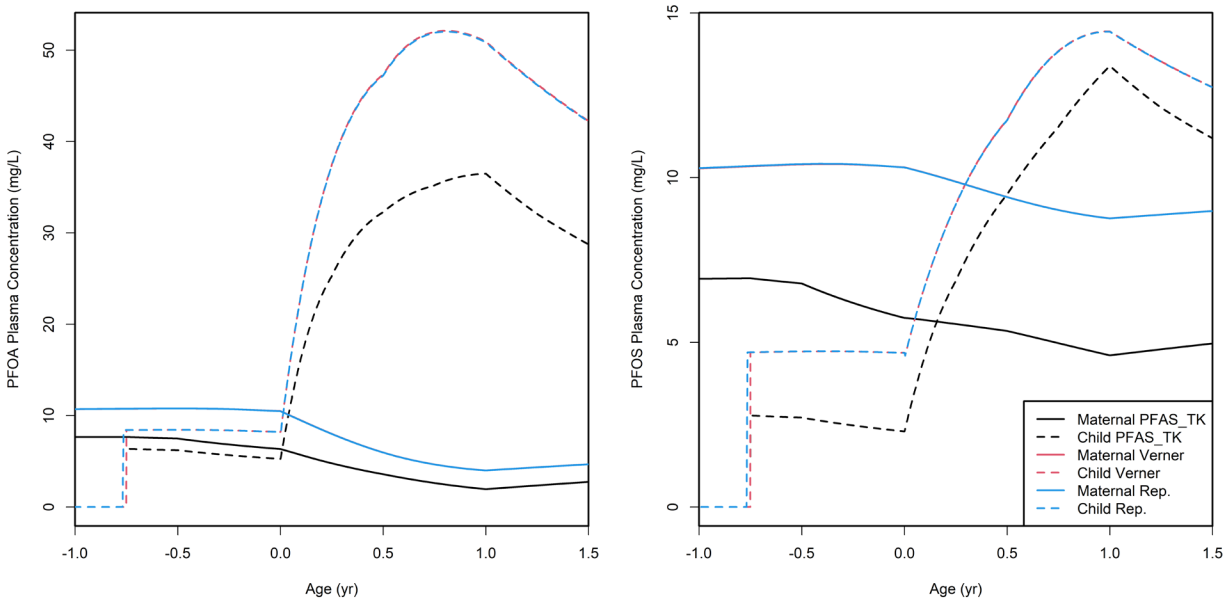


Figure F-11. Model Comparison

Comparison of the original AcslX model output (red, "Verner" label), the R model output with original model parameters (blue, "Rep." label), and the R model output with updated parameters (black, "PFAS_TK" label). Note that the red lines are almost entirely obscured by the blue lines.
The updated parameters result in lower serum concentrations for both the maternal and child. This is mainly due to lower half-lives selected during the parameter update.

Application of the updated parameters to predictions of serum levels in children showed good agreement between model predictions and reported values (Figure F-11;Figure F-12). This simulation was performed using mean breastmilk consumption estimates rather than the 95th percentile values from EPA's *Exposure Factors Handbook* (U.S. EPA, 2011b). Exposure in the validation scenario was assumed to be constant relative to body weight and was the same in the mother and child. This exposure was set such that predicted maternal serum level at delivery matched the reported value. Unlike the version of the model applied for human exposure prediction, validation was performed using the age-dependent mean breastmilk consumption estimates. The main application of the model used the 95th quantile of breastmilk consumption to provide a health-protective estimate of exposure. Each validation scenario was customized based on information about the length of breastfeeding typical in that cohort. As a reminder, the default modeling scenario consisted of 1 year of breastfeeding, with an instantaneous transition to non-breastfeeding exposure (i.e., with exposure to other PFAS sources at weaning). One year is more typical of total (exclusive and partial) breastfeeding, as opposed to exclusive breastfeeding which typically lasts up to around 6 months of age.

For the simulation of the Fromme et al. (2010) cohort, information on breastfeeding status was only available 6 months after birth. At this point 37 of 50 participants were exclusively breastfed, 6 predominantly breastfed, 6 partially breastfed, and 1 received no breast milk. As in the analysis by Verner et al. (2016), this scenario was modeled as exclusive breastfeeding to 6 months of age at which point the constant per bodyweight exposure starts equivalent to maternal exposure. For the cohort of the MOBA study (Granum et al., 2013), the average breast-feeding duration was 12.8 months. Because breastfeeding parameters were only developed in the model up to 1 year,

and the information used to inform the model only extended to 1 year, the simulation for this scenario used the default 1 year of breastfeeding. In the Mogensen et al. (2015b) study, the median length of exclusive breastfeeding was 4.5 months, and the median length of partial breastfeeding was 4.0 months so 8.5 months was chosen as the breastfeeding duration for simulation of this study.

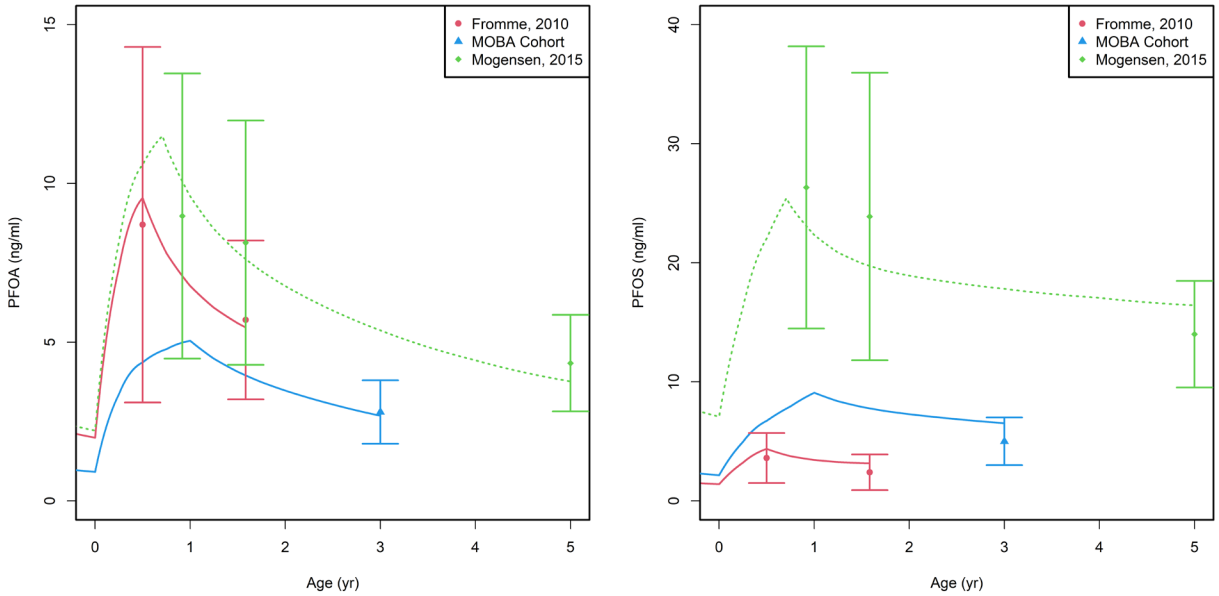


Figure F-12. Predicted Child Serum Levels Compared to Reported Values

These values were calculated using the updated parameters with constant Vd and exposure relative to body weight.

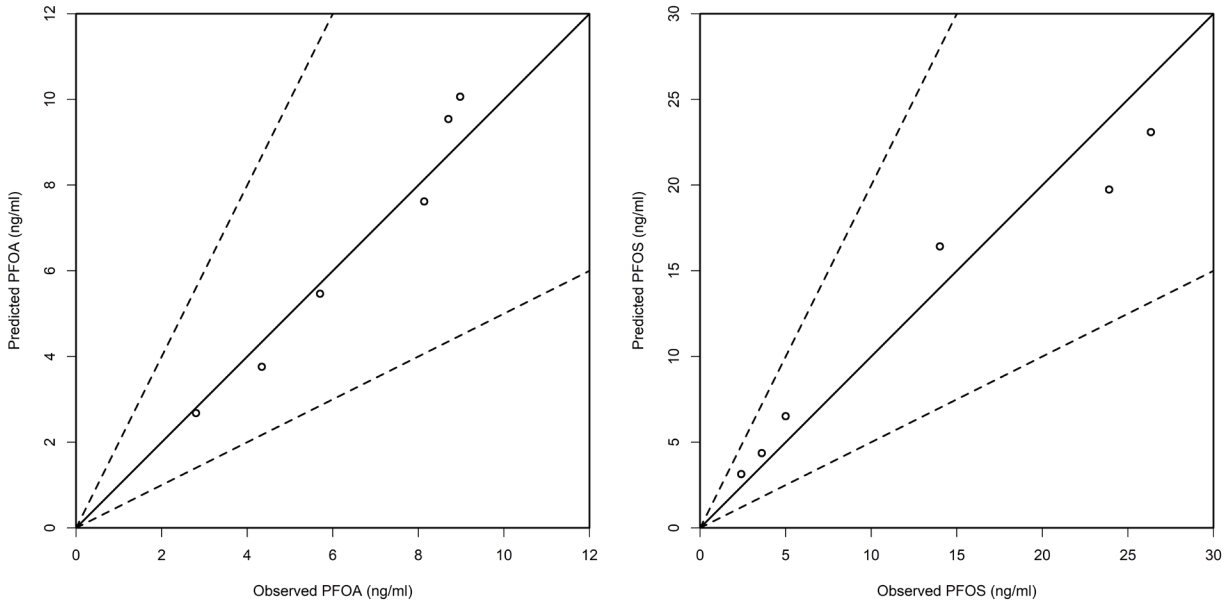


Figure F-13. Comparison of Predicted and Observed Child Serum Concentration

Dashed guidelines represent a 2-fold difference between observed and predicted concentration.

Local, one-at-a-time sensitivity analysis was performed to examine how parameter sensitivity varied across age and between maternal and child serum (Figure F-13). Sensitivity coefficients describe the change in a dose metric, in this case serum concentration, relative to the proportional change in a parameter value, in this case a 1% increase. A sensitivity coefficient of 1 describes the situation where a 1% increase in a parameter resulted in a 1% increase in serum concentration. Half-life and V_d were sensitive for every dose metric because they govern the distribution and excretion in all life stages and have a synergistic effect on child levels because they influence the serum levels in children directly as well as the indirect exposure to the child early in life through maternal exposure.

For maternal serum at delivery, only the half-life and the V_d influenced the serum concentration. This was expected as the other parameters evaluated govern distribution of PFOS to the child and are not in play at this point. For cord blood, a similar effect is observed from V_d and half-life as in the maternal serum, because cord blood levels are based on maternal levels in the model, but a high sensitivity is also seen on the cord blood:maternal serum ratio parameter. This was not unexpected but emphasizes the importance of this parameter for this endpoint. The 1-year timepoint occurs at the peak serum concentration associated with the end of breastfeeding. Consistent with this observation, the parameters that govern lactational transfer of PFOS (i.e., breastmilk intake and the milk:maternal serum ratio) have high sensitivity coefficients. Additionally, sensitivity to V_d is high because that governs the relationship between exposure and serum levels by accounting for the amount of PFOS distributed to tissues. At the 5-year timepoint the sensitivity to parameters associated with lactational exposure has decreased. The sensitivity to V_d is somewhat lower compared with the value at 1 year, and the sensitivity to half-life has slightly increased. This reflects the increased importance of excretion relative to the distribution of incoming PFOS during the time period following lactational exposure.

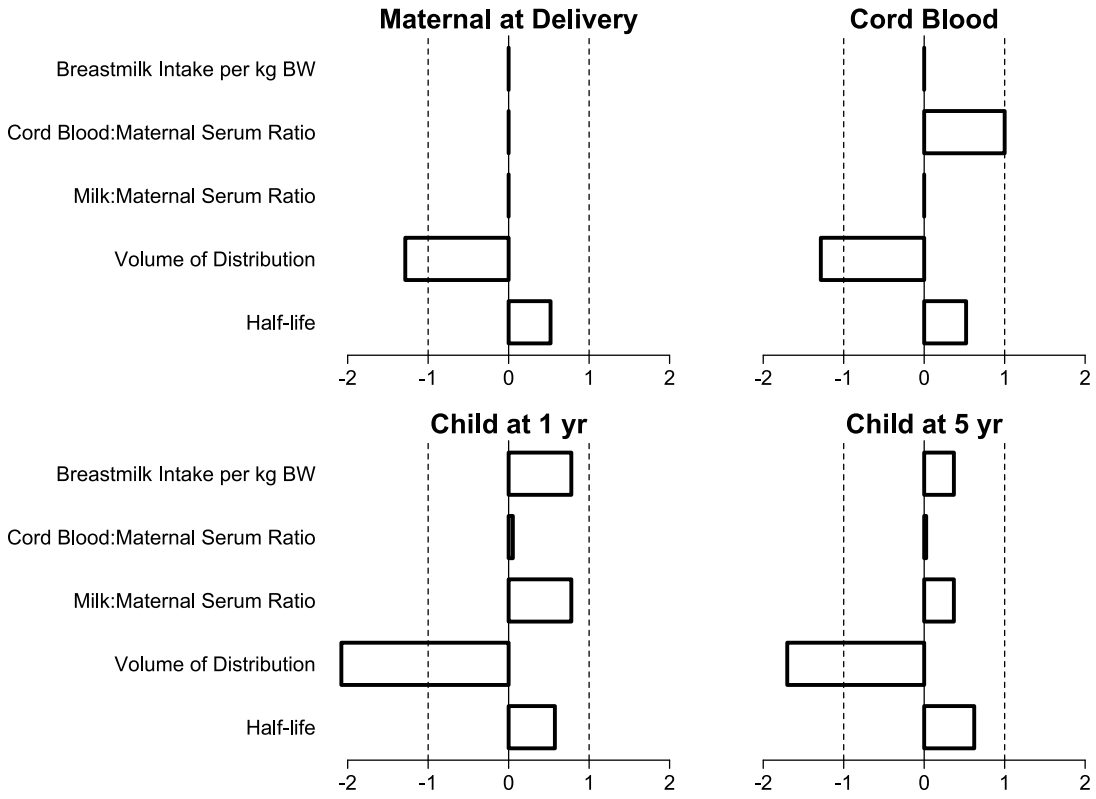


Figure F-14. Sensitivity Coefficients

Sensitivity coefficients from a local sensitivity analysis of maternal serum at delivery, cord blood at delivery, and child serum at 1 and 5 years old. The child was female. Results for a male child were similar (not shown).
BW = body weight; yr = year.

A model developed by the Minnesota Department of Health (MDH model) (Goeden et al., 2019) was also considered for application to this assessment. This model has a similar model structure to the chosen model, with single compartments to represent the mother and child and excretion handled by first-order clearance.

To evaluate the effect of V_d in children, the V_d scaling in the MDH model was integrated into model shown in Figure F-14. The main effect is to reduce the peak serum levels in children that occurs due to exposure through breastmilk. Based on root mean squared error, it was determined that the model with constant V_d had better performance (Table F-1).

Table F-1. Root mean squared error comparison between the baseline model (as applied in the main risk assessment) and alternative models with features inspired by the MDH model.

Chemical	Root Mean Squared Error		
	Baseline Model	Model with Variable V_d	Model with Drinking Water Exposure
PFOA	0.65	1.59	1.27
PFOS	2.48	5.06	4.82

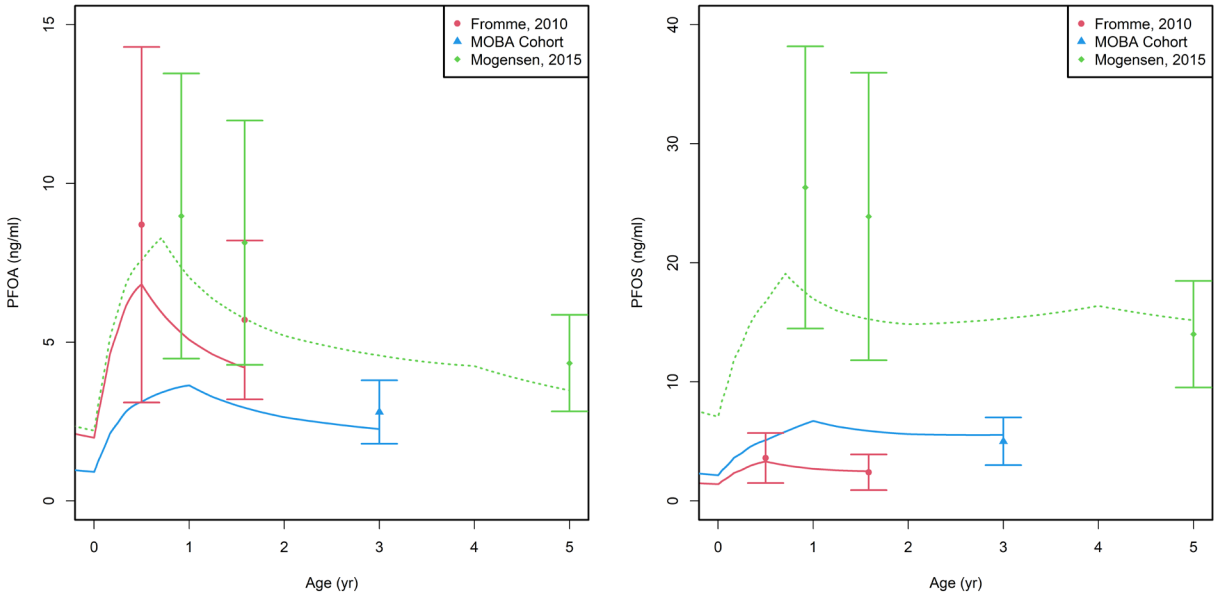


Figure F-15. Predicted Child Serum Levels Compared to Reported Values with Increased Volume of Distribution in Children as was Implemented in the Minnesota Department of Health Model

MOBA = Norwegian Mother, Father, and Child Cohort Study.

EPA also implemented exposure based on drinking water consumption in the modified Verner model to examine the effect on model predictions and especially on the results of the risk assessment (Figure F-15). Based on root mean squared error, it was determined that the model with constant exposure relative to bodyweight had better performance than a model that explicitly adjusts for drinking water consumption (Table F-1). An MCLG based on constant exposure does not greatly underestimate the risk to populations with greater water consumption per body weight (e.g., children and lactating women) because the method for calculating the MCLG from a RfD that assumes constant exposure accounts for the greater drinking water consumption in these populations.

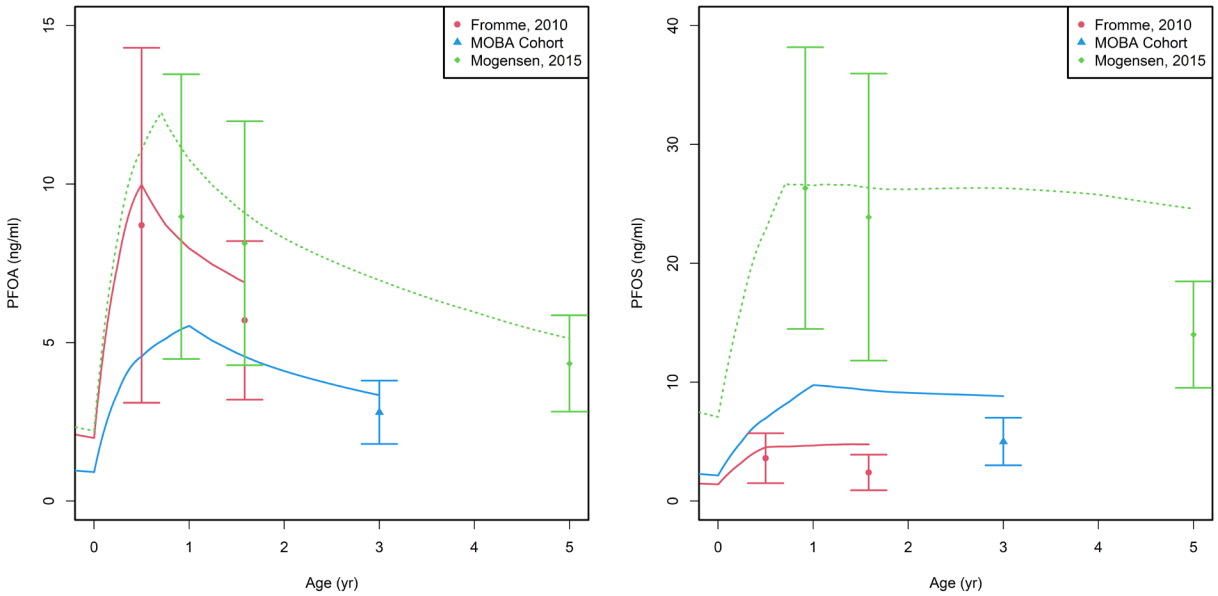


Figure F-16. Predicted Child Serum Levels Compared to Reported Values with Constant Volume of Distribution and Variable Exposure Based on Drinking Water Intake

MOBA = Norwegian Mother, Father, and Child Cohort Study.

Appendix G. Relative Source Contribution

G.1 Background

The EPA applies an RSC to the RfD when calculating an MCLG based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (U.S. EPA, 2000). The EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. The EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (U.S. EPA, 2000). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (USEPA, 2000). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (U.S. EPA, 2000). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively-derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (U.S. EPA, 2000). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies (U.S. EPA, 2000).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of 20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD

is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (U.S. EPA, 2000). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

G.2 Literature Review

In 2019, EPA's Office of Research and Development (ORD) conducted a literature search to evaluate evidence for pathways of human exposure to PFOA and PFOS. This search was not date limited and spanned the information collected across the Web of Science, PubMed, and ToxNet/ToxLine (now ProQuest) databases. An updated literature search was conducted and captured relevant literature published through March 2021. Literature captured by this search is housed in EPA's HERO database (https://hero.epa.gov/).

Results of this broad literature search were further distilled to address two questions. First, a systematic review was conducted to investigate evidence for important PFAS exposure pathways from indoor environment media including consumer products, household articles, cleaning products, personal care products, and indoor air and dust (Deluca et al., 2022a). Literature that reported exposure measures from household media paired with occupant PFAS concentrations in blood serum was identified. Second, systematic evidence mapping was conducted for literature reporting measured occurrence of PFAS in exposure media (Holder et al., 2023). This review focused on real-world occurrences (measured concentrations) primarily in media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil).

G.2.1 Systematic Review

Deluca et al. (2022b) investigated evidence for important PFAS exposure pathways from indoor environment media including consumer products, household articles, cleaning products, personal care products, and indoor air and dust. The authors adapted existing systematic review methodologies and study evaluation tools to identify and screen exposure studies that presented concordant data on PFAS occurrence in indoor media and PFAS concentrations in blood or serum. Studies included in the systematic review report exposure measures from household media paired with occupant PFAS concentrations in blood serum, focusing on PFOS and seven other frequently measured PFAS (PFOA, perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), PFDA, PFHxA, PFHxS, and PFNA). Machine learning approaches were used during the literature scoping and title/abstract screening to prioritize exposure pathways of interest by automated tagging and to select studies for inclusion using an iterative predictive screening model. Title/Abstract screening for the PECO criteria identified 486 studies for full text screening; only 6 studies fully addressed the protocol requirements (Balk et al., 2019; Kim et al., 2019; Poothong et al., 2019; Byrne et al., 2017; Makey et al., 2017; Wu et al., 2014). The extraction of exposure measurement data and study characteristics from each included study was performed in DistillerSR software. Exposure intake calculations were used to estimate a percentage of occupant serum concentrations that could be attributed to indoor exposure pathways other than drinking water and diet. The included studies were evaluated using an

approach modified from EPA's IRIS Handbook (U.S. EPA, 2022c). Along with providing evidence for an estimated range of indoor exposure media's contribution to serum PFAS concentrations, this systematic review highlights the limited availability of concordant measurement data from indoor exposure media and participant serum.

The Deluca and coworkers review (2022a) described above focused on indoor pathways and therefore excluded non-indoor pathways such as surface water or soil. Ninety-seven articles fell into this excluded group (i.e., PFOS was measured in non-indoor environmental medium). These 97 papers were reviewed for this effort, though are not fully described in this appendix.

G.2.2 Evidence Mapping

Holder et al. (2023) investigated evidence for important pathways of exposure to PFAS by reviewing literature reporting measured occurrence of PFAS in exposure media. The review focused on eight PFAS (PFOA, PFOS, PFBA, PFBS, PFDA, PFHxA, PFHxS, and PFNA) and their real-world occurrences primarily in human matrices and media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil). The initial review identified 3,622 peer-reviewed papers matching these criteria that were published between 2003–2020. ICF's *litstream*^{TM} software was used to conduct title-abstract (TiAb) and full-text screening, and to extract relevant primary data into a comprehensive evidence database. Parameters of interest included: sampling dates and locations (focused on locations in the United States, Canada, and Europe), numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics.

Detailed data on PFAS occurrence in high-priority household and environmental media from 210 studies were extracted, as well as limited data on human matrices from 422 additional papers. Published studies of PFAS occurrence became numerous after about 2005 and were most abundant for PFOA and PFOS. Co-measurements for PFAS occurrence in human matrices plus other media, while relatively infrequent, were typically for occurrence in food and drinking water. Most studies found detectable levels of PFAS, and half or more of the limited studies of indoor air and products detected PFAS in 50% or more of their samples. Levels of PFOS in these media ranged widely.

Literature search results were categorized into 7 types of exposure pathway categories, including environmental media, home products/articles/building materials, cleaning products, food packaging, personal care products, clothing, and specialty products. The environmental media pathway category included the sub-categories of food, water, air, dust, soil, wastewater, and landfill. The identified studies were reviewed for this effort, though are not fully described in this appendix.

G.3 Summary of Potential PFOS Sources

PFOS is a synthetic, fully fluorinated, organic compound that is used in many types of consumer products and is resistant to metabolic and environmental degradation (U.S. EPA, 2016c). It has been associated with releases from manufacturing sites, industrial sites, fire/crash training areas, and industrial and municipal waste sites. PFOS is one of a large group of perfluoroalkyl substances that are widely used in consumer and industrial products to improve their resistance

to stains, grease, and water. PFOS was a major component of AFFF which were used to extinguish petroleum-based fires. Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007 EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (U.S. EPA, 2016a). Exposure to PFOS can occur through food, including fish and shellfish, house dust, air, and contact with consumer products (U.S. EPA, 2016c).

G.3.1 Dietary Sources

Ingestion of food is a potentially significant source of exposure to PFOS and is often claimed to be the dominant source of exposure for the general population based on early studies that modeled the relative contributions of various sources among the general populations of North America and Europe (Fromme et al., 2009; Vestergren and Cousins, 2009; Trudel et al., 2008). The exposure among adults in western countries is typically estimated to be about 1 ng/kg/day, but studies on the dietary exposure among the U.S. population are limited (East et al., 2021; Domingo and Nadal, 2017). The dominance of the food ingestion pathway is attributed to bioaccumulation in food from environmental emissions, relatively large amounts of foods being consumed, and high GI uptake (Trudel et al., 2008). However, the estimates are highly uncertain due to limited data availability, relatively low detection frequencies, and relatively large differences in composition of diets across geographic locations (EFSA, 2020; Domingo and Nadal, 2017).

There is currently no comprehensive, nationwide Total Diet Study (TDS) for PFOS that can be used to draw conclusions about the occurrence and potential risk of PFOS in the U.S. food supply for the general population. In 2021, the U.S. Food and Drug Administration (FDA) released PFAS testing results from their first survey of nationally distributed processed foods, including several baby foods. Results of the survey showed that 164 of the 167 foods tested had no detectable levels of the PFAS measured. Three food samples had detectable levels of PFAS: fish sticks (PFOS (33 parts per trillion (ppt)) and PFNA), canned tuna (PFOS (76 ppt) and PFDA), and protein powder (PFOS (140 ppt)). In another recent FDA study, PFOS was detected in one sample (baked cod, 98 ppt) out of 94 food samples collected nationally (FDA, 2021). In a 2019 national survey of produce, meats, dairy and grain products, PFOS was detected in three of the 179 food samples tested (two samples of tilapia, one sample of turkey) (FDA, 2019a, b). PFOS was also detected in produce samples (collard greens and lettuce) in a 2018 focused study near a PFAS production plant in the Fayetteville, North Carolina area (FDA, 2018). The sample size in all of these studies is limited, and thus, the results cannot be used to draw definitive conclusions about the levels of PFAS in the U.S. food supply more generally (FDA, 2021). In a 2010 study of 31 types of food collected from 5 grocery stores in Texas, PFOS was not detected in any of the samples (Schecter et al., 2010).

As a component of a scientific evaluation on the risks to human health related to PFAS in food, the European Food Safety Authority (EFSA) conducted an exposure assessment using consumption data from the EFSA Comprehensive Food Consumption Database and 69,433 analytical results for 26 PFAS in 1,528 samples of food and beverages obtained from 16 European countries (EFSA, 2020). Samples were collected between the years 2000 and 2016 (74% after 2008), mainly from Norway, Germany, and France. With 92% of the analytical

results below the LOD or LOQ, lower bound dietary exposure estimates were obtained by assigning zero to values below LOD/LOQ. Median chronic dietary exposures of PFOS for children and adults were estimated as 1.02 and 0.58 ng/kg-body weight/day, respectively. The most important contributors for PFOS were "Fish and other seafood," "Eggs and egg products," and "Meat and meat products." It is unclear whether the contribution from food contact material is reflected in the data. The authors determined diet to be the major source of PFAS exposure for most of the population but noted that dust ingestion and indoor air inhalation may provide substantial contributions for some individuals.

The 2020 EFSA report highlighted a recent study of aggregate exposure to PFAS from diet, house dust, indoor air, and dermal contact among Norwegian adults (Poothong et al., 2020). Dietary exposures were estimated for 61 study participants using food diaries and data on concentrations from an extensive Norwegian database of concentrations in sixty-eight different food and drinks (including drinking water). For PFOS, the authors concluded that dietary intake was by far the greatest contributor to aggregate exposure (contributing 95% of total estimated PFOS intake), but intake from ingestion of house dust represented the dominant pathway for some of the top 20% most highly exposed individuals. While the authors did not separately quantify intake from food and drinking water, an earlier article from the same research group (Papadopoulou et al., 2017) reported measured concentrations in duplicate diets with median estimated intake of PFOS approximately 150 times higher from solid food than from liquids.

De Felip et al. (2015) investigated correlations of blood concentrations of PFOS with dietary intake among Italian women. They estimated daily intake of PFOS based on the reported food consumption frequencies of specific food items and found strongly significant correlations of blood levels with consumption of beef, pork, and vegetables ($p < 0.01$), and moderate correlation with consumption of fish ($p < 0.05$).

EPA's *Emerging Issues in Food Waste Management Persistent Chemical Contaminants* (U.S. EPA, 2021b) further describes global PFOS and other PFAS occurrence in food items, waste, and compost, as well as food contact materials, described below (Section G.3.1.2).

G.3.1.1 Fish and Shellfish

PFOS has been shown to bioaccumulate and biomagnify with increasing trophic level in a variety of freshwater ecosystems (Penland et al., 2020; Xu et al., 2014; Kannan et al., 2005; Martin et al., 2004) and saltwater ecosystems (Loi et al., 2011; de Vos et al., 2008; Powley et al., 2008; Houde et al., 2006; Tomy et al., 2004) in North America, Europe, and Asia. PFOS is often the most abundant PFAS in aquatic organisms, and this high relative abundance is at least partially explained by the biotransformation of PFOS precursor chemicals into PFOS (Kelly et al., 2009; Haukås et al., 2007; Kannan et al., 2005; Martin et al., 2004; Tomy et al., 2004). Higher trophic level organisms have a greater capacity to metabolize PFOS precursor chemicals, which have been found in lower concentrations in increasing trophic level (Fang et al., 2014; Kannan et al., 2005; Martin et al., 2004).

Global distribution of PFAS chemicals in tissues of aquatic species has been demonstrated in studies conducted in freshwater and marine environments across every continent, including remote regions far from direct sources, such as the high arctic, Antarctica, and oceanic islands (Houde et al., 2006; Giesy and Kannan, 2001).

EPA collaborates with federal agencies, states, tribes, and other partners to conduct freshwater fish contamination studies as part of a series of statistically based surveys to produce information on the condition of U.S. lakes, streams, rivers, and coastal waters. PFOS was detected in nearly all freshwater fish fillet samples collected during several national studies in rivers and the Great Lakes (Table G-1).

Table G-1. Summary of EPA national fish tissue monitoring results for PFOS

Reference	Most Commonly Sampled Species	Site Description	Results
U.S. EPA (2010)	Smallmouth bass Largemouth bass Channel catfish	162 urban river sites across the United States	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 77 percent of samples. Maximum detected concentration 127 ng/g.
U.S. EPA (2015)	Largemouth bass Smallmouth bass Black crappie White crappie Walleye/sauger Yellow perch White bass Northern pike Lake trout Brown trout Rainbow trout Brook trout	349 urban and nonurban river sites across the United States.	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 99 percent of samples. Maximum detected concentration 283 ng/g.
U.S. EPA (2011a)	Lake trout Smallmouth bass Walleye	157 nearshore sites along the U.S. shoreline of the Great Lakes	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 100 percent of samples. Maximum detected concentration 80 ng/g; median 15 ng/g.
U.S. EPA (2016e)	Freshwater Drum Longnose Sucker White Sucker Lake Whitefish Northern Pike Channel Catfish Burbot Smallmouth Bass White Perch White Bass Coho Salmon Rainbow Trout Chinook Salmon Yellow Perch Brown Trout Lake Trout Walleye	152 nearshore sites along the U.S. shoreline of the Great Lakes	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 100 percent of samples. Maximum detected concentration 64 ng/g; median 11 parts per billion (ppb).

Guo et al. (2012) measured PFOS in lake trout muscle tissues in Canadian waters of Lake Superior, Huron, Erie, and Ontario. Average PFOS concentrations correlated with watershed urbanization, and were 0.85 ng/g, 8.3 ng/g, 27 ng/g, and 46 ng/g wet weight (ww), respectively. Delinsky et al. (2010) measured PFOS in bluegill, black crappie, and pumpkinseed muscle tissue in 59 lakes in Minnesota, including four lakes in the Minneapolis–St. Paul metropolitan area. PFOS was detected in muscle tissues of fish collected in 13 of the 59 lakes, and concentrations ranged from 1.08 ng/g ww to 52.4 ng/g ww in lakes where it was detected. In the four lakes in the Minneapolis–St. Paul metropolitan area, PFOS concentrations in fish muscle tissues ranged from 4.39 ng/g ww to 47.3 ng/g ww.

Penland et al. (2020) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin – Pee Dee River, in North Carolina and South Carolina in 2015. PFOS was measured in whole body tissues of snails (6.47 ng/g ww) but was not detected whole body tissues of in Asian clam, unionid mussels, or crayfish. The highest concentrations in invertebrates were measured in aquatic insect whole body samples (132.8 ng/g ww) and was hypothesized to result from dietary uptake of aquatic biofilms. PFOS was measured in muscle tissue of all 11 sampled fish species and ranged from 11.42 ng/g ww in channel catfish to 37.36 ng/g ww in whitefin shiner. The highest PFOS concentration that Penland et al. (2020) measured was 482.9 ng/g ww, from the eggs of a redhorse fish sample.

Houde et al. (2006) measured whole body PFOS in six fish species in Charleston Harbor, South Carolina, and whole body PFOS in zooplankton and five fish species in Sarasota Bay, Florida. Charleston Harbor was the more developed of the two sites and had higher overall PFOS concentrations. Average PFOS concentrations in Charleston Harbor ranged from 19 ng/g in pinfish to 92 ng/g in spot. In Sarasota Bay, PFOS concentrations averaged 0.2 ng/g in zooplankton, and ranged from 3.1 ng/g in pigfish to 8.8 ng/g in spotted seatrout, suggesting evidence of trophic biomagnification.

Zafeiraki et al. (2019) analyzed about 250 samples of marine fish, farmed fish, crustaceans, bivalves and European eel, caught in Dutch waters or purchased at Dutch markets between 2012 and 2018. Of the 16 PFAS that were analyzed, PFOS was generally detected at a higher frequency and concentration across the tested species. Shrimps and seabass had the highest average concentrations of PFOS (each over 4 ng/g ww). PFOS was also detected in mussels, brown crab, eel (100% detection, ranging from 3.3 to 67 ng/g ww) and several farmed and marine fish species.

Ruffle et al. (2020) analyzed marine and freshwater finfish and shellfish from four regions of the United States and seven countries with significant imports to the United States. A total of 70 samples were analyzed for 26 PFAS. PFOS represented 80% to 100% of total PFAS measured in all but one sample. The highest PFOS concentrations (1.2 ng/g ww to 19.1 ng/g ww) were found in whitefish, walleye, and yellow perch from the Great Lakes region.

In seafood samples collected for the FDA 2021–22 seafood survey, Young et al. (2022), analyzed concentrations of 20 PFAS, including PFOS, in 8 of the most highly consumed seafood products in the U.S. PFOS was detected most frequently (100% of samples; n=10) and at the highest average concentrations (422.9 ppt) in clams. The study also reported detections in crab

(45.5% of samples; n=11; 151.6 ppt average concentration in samples with detections), tuna (50% of samples; n=10; 86.8 ppt average concentration in samples with detections), tilapia (20% of samples; n=10; 57.5 ppt average concentration in samples with detections), and cod (60% of samples; n=10; 62.5 ppt average concentration in samples with detections). PFOS was not detected above the method detection limits (39 or 45 ppt) in salmon, shrimp, or pollock.

Based on National Oceanic and Atmospheric Administration (NOAA) National Centers for Ocean and Coastal Science, National Status and Trends Data, PFOS concentrations (in ww) were not detected in mussels, oysters, and fish liver samples. However, PFOS was detected in marine fish fillet samples, up to 75.1 ppb (NOAA, 2017).

PFOS concentrations in aquatic biota tend to be higher in areas with known PFAS manufacturing, industrial use, and/or application of AFFF, which also tend to be more populated areas and where recreational and subsistence fishing is more common. Several states have developed fish consumption advisories for PFOS (e.g., Alabama, Wisconsin, Minnesota, Michigan).

G.3.1.2 Food Contact Materials

The FDA has authorized the use of PFAS in food contact substances due to their non-stick and grease, oil, and water-resistant properties since the 1960s. There are four categories of products that may contain PFAS:

- "Non-stick cookware: PFAS may be used as a coating to make cookware non-stick.
- Gaskets, O-Rings, and other parts used in food processing equipment: PFAS may be used as a resin in forming certain parts used in food processing equipment that require chemical and physical durability.
- Processing aids: PFAS may be used as processing aids for manufacturing other food contact polymers to reduce build-up on manufacturing equipment.
- Paper/paperboard food packaging: PFAS may be used as grease-proofing agents in fast-food wrappers, microwave popcorn bags, take-out paperboard containers, and pet food bags to prevent oil and grease from foods from leaking through the packaging." (FDA, 2020)

Paper products used for food packaging are often treated with PFAS for water and grease resistance. In previous testing, sandwich wrappers, french-fry boxes, and bakery bags were all been found to contain PFAS (Schreder and Dickman, 2018). Older generation PFAS (e.g., PFOA, PFOS) were manufactured and used in products for decades, and the bulk of the information available on PFAS toxicity relates to the older compounds. However, because newer-generation PFAS are more mobile than their predecessors, they migrate more readily into food. In 2016, the FDA deauthorized the remaining uses of long-chain "C8" PFAS in food packaging, which are therefore, no longer used in food contact applications sold in the United States (FDA, 2020).

Under FDA rules, there are dozens of PFAS chemicals still approved for food contact materials. In 2020, Safer Chemicals Healthy Families and Toxic-Free Future co-published a report where 78 samples of food packaging including take-out containers and deli or bakery paper, among others, were collected from 20 stores in 12 states (Schreder and Dickman, 2018). An independent laboratory tested the samples for fluorine. The utility of measuring fluorine content is limited

because it does not allow for identification and quantification of individual PFAS; however, this method can be used to determine if a food-packaging material has been treated with PFAS. Over 10% of 78 samples tested contained PFAS. The sample size was not large enough to indicate how widespread the use of PFAS in food packaging is at this time. However, the study demonstrated that PFAS in food packaging is still a concern, especially for fiber bowls and trays.

Several other relatively recent studies found PFAS in fast-food packaging collected in the United States, China, or Europe. The data from the references described below and other publications likely contributed to the recent regulatory actions of the FDA and a number of states to ban or restrict the presence of PFAS in food contact materials (Keller & Heckman LLP, 2021). Schaider at al. (2017) collected 407 samples of food contact papers, beverage containers, and paperboard boxes from locations throughout the United States. As was the case with Schreder & Dickman (2018), inorganic fluoride was the analyte for the initial analysis. 56% of the dessert and bread wrappers were positive for fluoride, 38% of the sandwich and burger wrappers, and 20% of the paper-board containers. None of the 30 (hot/cold) paper beverage cups tested positive in contrast to 16% of beverage containers (milk/juice) made from other materials. Generally, food contact papers had higher fluoride detection frequencies than food contact paperboard.

An analysis of popcorn bags, snack bags, and sandwich bags purchased in 2018 from international vendors and grocery stores in the United States found no evidence of PFOS at concentrations above the LOD (0.63 ng/g paper) (Monge Brenes et al., 2019). The authors presented these results as evidence of a reduction in PFOS concentrations in microwave packaging between 2005 and 2018. In an analysis of microwave popcorn bags from around the world, Zabaleta et al. (2017) reported no measurable concentrations of any PFSA, including PFOS, in any of the samples. In a second study, Zabaleta et al. (2020) looked at PFAS in 25 paper- and paperboard packaging materials primarily collected in Spain. Again, no PFSAs, including PFOS, were found above the level of detection. The packaging materials with the largest number of detectable analytes was a popcorn bag from China and the inside paper lining from three individual pet food products, which contained a spectrum of C-3 to C10 perfluorinated carboxylates.

G.3.2 Consumer Product Uses

An early investigation of consumer exposure to PFOS by Trudel et al. (2008) used mechanistic modeling together with information on product-use habits to estimate exposures from mill-treated carpets and impregnated clothing. The authors concluded that contact with consumer products represents less than 1% of total exposure to PFOS, but also pointed out that because carpets have a relatively long lifetime, the exposure is expected to continue long after cessation of use of PFOS in carpet treatments. Liu et al. (2014) also investigated trends in PFAS content of household goods between 2007 and 2011. They reported a decrease in the availability of consumer products that contain PFOS is declining but were still able to find products that contained PFOS. In an analysis of 52 European products collected between 2014–2016, Borg and Ivarrson (2017) reported that PFSAs were rarely detected in the samples; PFOS was the only PFSA detected and was only present in one sample, a microwave popcorn bag. Notably, the authors specifically targeted products that were known or suspected to contain PFAS in their analyses.

In contrast, Kotthoff et al. (2015) reported broad detection of PFOS in a 2010 sampling effort that collected 115 European consumer products, including carpets, leather, outdoor materials, cooking materials, and others. PFOS was detected in all but two sample types, often at the highest median concentration compared to other PFSAs. However, PFSAs were detected at concentrations often several orders of magnitude lower than perfluorinated carboxylic acids (PFCAs) and fluorotelomers. The products with the highest concentrations of total PFAS included ski wax (median concentration of 1.6 µg/kg), leather products (maximum concentration of 5.6 µg/m^2), and outdoor materials (median concentration of 9.5 µg/m^2). PFOS was the most frequently and abundantly detected PFAS in paper-based cooking materials. PFOS has also been detected in textile samples of outdoor apparel from Europe and Asia (van der Veen et al., 2020; Gremmel et al., 2016). PFOS was detected in one-third of the jackets tested by Gremmel et al. (2016) at relatively low concentrations ranging from 0.01 µg/m^2–0.59 µg/m^2. Interestingly, while the concentrations of almost all individual PFAS and total PFAS concentrations increased when the textiles were subjected to weathering (i.e., increased ultraviolet light radiation, temperature, and humidity for 300 hours to mimic the average lifespan of outdoor apparel), PFOS concentrations declined after weathering in the one sample that exceeded European Commission restrictions on PFOS content of coated materials (1 µg/m^2) (van der Veen et al., 2020).

G.3.3 Indoor Dust

Several studies suggest that PFOS and its precursors in indoor dust may be an important exposure source for some individuals (Poothong et al., 2020; NJDWQI, 2018; Gebbink et al., 2015; Shoeib et al., 2011). PFOS is generally a dominant ionic PFAS constituent in household dust, frequently occurring above detection limits and at relatively high concentrations in all or most samples (Poothong et al., 2020; Kim et al., 2019; Byrne et al., 2017; Makey et al., 2017; Wu et al., 2014; Fraser et al., 2013; Shoeib et al., 2011).

PFOS was measured at the second highest concentrations (geometric mean concentrations ranging from 29.0 ng/g–34.6 ng/g) and frequencies (ranging from 85%–87% detected) in dust sampled from Californian households. Similarly, PFOS was found at the highest levels (mean concentration of 3.06 ng/g) of 15 PFAS measured in dust samples taken from households in Seoul, Republic of Korea (Kim et al., 2019). One study of Alaska Natives noted that PFOS was the predominant compound in dust samples (Byrne et al., 2017).

G.3.4 Ambient Air

Air concentrations of PFOS in the atmosphere vary widely across the globe. Areas near wastewater treatment facilities, waste incinerators, and landfills can be point sources of PFOS to air (Ahrens et al., 2011). In an urban area in Albany, NY, perfluorinated acids were measured in air samples in both the gas and particulate phase in May and July 2006 (Kim and Kannan, 2007). PFOS in the gas phase had a mean concentration of 1.70 pg/m^3 (range: 0.94–3.0 pg/m^3) and in the particulate phase had a mean concentration of 0.64 pg/m^3 (range: 0.35–1.16 pg/m^3). However, at Lake Ontario, concentrations of PFOS in the particulate phase measured in air samples over the lake were higher (Boulanger et al., 2005). The mean concentration of PFOS at Lake Ontario was 6.4 ± 3.3 pg/m^3; with a range of concentrations from detected to 8.1 pg/m^3. In an urban area in Minneapolis, Minnesota, PFOS was measured in both the particulate and gas

phase (MPCA, 2008). PFOS in the particulate phase ranged from 2.1 pg/m^3 –7.9 pg/m^3 and the gas phase ranged from 1.8 pg/m^3–5.0 pg/m^3 across the five samples.

In Canada, PFOS air concentrations measured in 2009 showed widespread distribution with remote sites having similar concentrations to urban sites (ECCC, 2018). Using passive samplers, PFOS concentrations were detected in Toronto, Ontario (8 pg/m^3), an agricultural site in Saskatchewan (5 pg/m^3), Whistler, British Columbia (4 pg/m^3), and Alert, N Nunavut (2 pg/m^3) (ECCC, 2018).

Other reported concentrations of PFOS in air samples from Sydney, Florida (3.4 pg/m^3), Tudor Hill, Bermuda (6.1 pg/m^3), Malin Head, Ireland (3.3 pg/m^3), and Hilo, Hawaii (6.6 pg/m^3) are similar to the concentrations reported in Canada (ECCC, 2018) and Japan (Sasaki et al., 2003). The annual geometric mean concentration of PFOS in air samples collected monthly from 2001–2002 in the town of Oyamazaki and Fukuchiyama City were 5.3 and 0.6 pg/m^3, respectively (Sasaki et al., 2003).

Across Europe, PFOS air concentrations were reported to be variable. In the particulate phase PFOS concentrations ranged from < 1.8 pg/m^3–46 pg/m^3 (Martin et al., 2004). Most locations had low (~1 pg/m^3–2 pg/m^3) to less than the reported Minimum Detection Limit (MDL) and included Hazelrigg, United Kingdom, Kjeller Norway, and Mace Head, Ireland (Barber et al., 2007). The highest concentrations were reported in Manchester, United Kingdom. Similarly, high concentrations, 150 pg/m^3 for were reported Paris, France (ECCC, 2018).

Even in the Arctic, PFOS, its precursors, and degradation products, have been detected in air samples in Resolute Bay, Nunavut, Canada, during the summer of 2004 (Stock et al., 2007). PFOS in the filter samples were 1–2 orders of magnitude greater than other compounds, with a mean concentration of 5.9 pg/m^3. These concentrations are greater than PFOS concentrations measured in the particle phase of air samples measured in Zeppelinstasjonen, Svalbard, Norway (Butt et al., 2010). PFOS was measured in September and December, 2006 and August and December, 2007, with mean concentrations of 0.11 pg/m^3 (range: 0.03 pg/m^3–0.50 pg/m^3) and 0.18 pg/m^3 (range: 0.02 pg/m^3–0.97 pg/m^3), respectively.

G.3.5 Other Possible Exposure Sources

PFOS has also been detected in soils and dust from carpets and upholstered furniture in homes, offices, and vehicles. Incidental exposure from soils and dust is an important exposure route, particularly for small children because of their increased level of hand-to-mouth behaviors compared to adults. Also, the levels in soils and surface waters can affect the concentrations in local produce, meat/poultry, dairy products, fish, and particulates in the air.

G.4 Recommended RSC

EPA followed the Exposure Decision Tree approach to determine the RSC for PFOS, as outlined in Figure G-1 (U.S. EPA, 2000). EPA first identified several potential populations of concern (Box 1): pregnant women and their developing fetuses, infants, children, lactating women, and women of childbearing age. However, limited information was available regarding specific exposure of these populations to PFOS in different environmental media. EPA considered exposures in the general U.S. population as likely being applicable to the majority of these

populations. Second, EPA identified several relevant PFOS exposures and pathways (Box 2), including dietary consumption, incidental oral, inhalation, or dermal exposure via dust, consumer products, and soil, and inhalation exposure via ambient air. Several of these may be potentially significant exposure sources. Third, EPA determined that there was inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, studies from the U.S. indicate that dust may be a significant source of exposure to PFOS. Although several studies report PFOS detections in consumer products, most examined samples from specific locations that may not be nationally representative. Therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, based on the studies summarized in the sections above, there are significant known or potential uses/sources of PFOS other than drinking water (Box 6), though there is not information available on each source to make a characterization of exposure (Box 8A). For example, there are several studies from the U.S., Canada, and Europe indicating that PFOS may occur in multiple food products, most notably, seafood. The physico-chemical properties of PFOS indicate that it is likely to bioaccumulate. However, the available evidence about the occurrence of PFOS in other food types (e.g., eggs, meats, vegetables, fruit) is less substantive; the majority of studies examined very few samples (i.e., n=1-5) of various food products and a nationally representative total diet study does not exist. Therefore, it is not possible to determine whether food or other types of media can be considered a major or minor contributor to total PFOS exposure. Given these

considerations, following recommendations of the Exposure Decision Tree (U.S. EPA, 2000), EPA recommends an RSC of 20% (0.20) for PFOS.

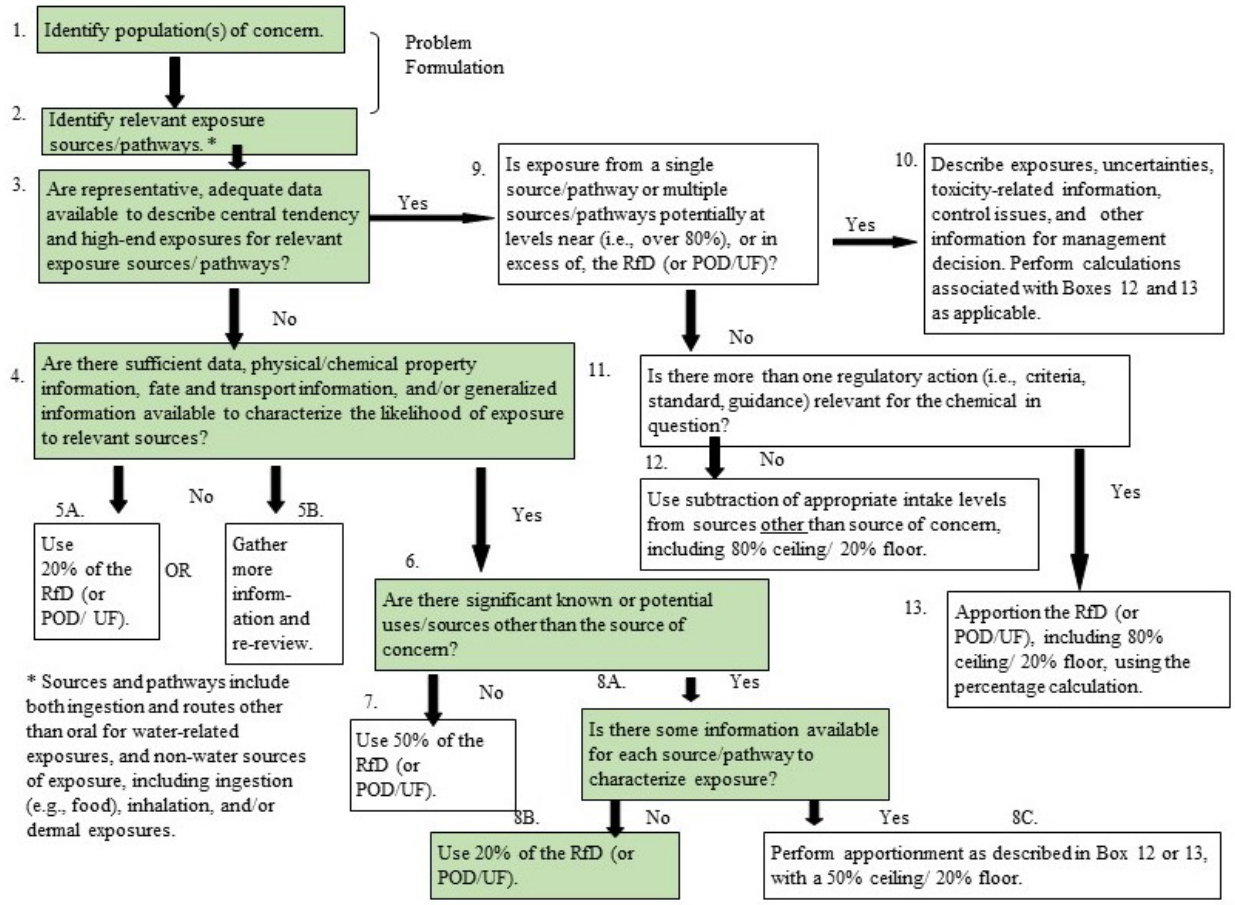


Figure G-1. Application of the Exposure Decision Tree (U.S. EPA, 2000) for PFOS

Green highlighted boxes indicate selections made at each branch of the Decision Tree.
POD = point of departure; RfD = reference dose; UF = uncertainty factor.

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