March 2023
EPA Document No.
822P23008

PUBLIC COMMENT DRAFT
APPENDIX: Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water

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EPA Document Number: EPA 822P23008

March 2023

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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, 5099062}. The number of PFAS used globally in commercial products at the time of the drafting of this document is approximately 250 substances {Buck, 2021, 9640864}.

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States since the 1950s. PFAS have strong, stable, carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism {Ahrens, 2011, 2657780; Beach, 2006, 1290843; Buck, 2011, 4771046}. 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, 2011, 4771046}. 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, 2007, 1290899; Calafat, 2019, 5381304}. Due to 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, 2016, 3603365} (hereafter referred to as the 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, 2021, 10428576}, 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 (*Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal (MCLG) for PFOS*) 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, 2020, 7006986; U.S. EPA, 2022, 10367891} (hereafter referred to as the Integrated Risk Information System (IRIS) Handbook) and a companion publication {Thayer, 2022, 10259560}. 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" {NAS, 2021, 9959764}. Furthermore, EPA's IRIS program has used the IRIS Handbook to develop toxicological reviews for numerous chemicals, including some PFAS. Though the IRIS Handbook was finalized concurrently with this assessment, the alterations in the final IRIS Handbook compared to the draft version did not conflict with the methods used in this assessment. In fact, many of the NAS recommendations incorporated into the final IRIS handbook (e.g., updated methods for evidence synthesis and integration) were similarly incorporated into this assessment protocol {NAS, 2021, 9959764}. However, some of the study evaluation refinements recommended by NAS {2021, 9959764}, including clarifications to the procedure for evaluating studies for sensitivity and standardizing the procedure for evaluating reporting quality between human and animal studies, were not included in this assessment protocol, consistent with a 2011 NASEM recommendation not to delay releasing assessments until systematic review methods are finalized {NRC, 2011, 710724}. The assessment team concluded that implementing these minor changes in study quality evaluation 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.

The Safe Drinking Water Act (SDWA) regulatory process enables EPA to receive comments and feedback on the systematic review protocol, including the 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 *Proposed Maximum Contaminant Level Goal (MCLG) for PFOS* (hereafter referred to as the PFOS MCLG main document) and is provided for context. Please refer to the PFOS MCLG main document 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, 10369707}. 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 PFOS MCLG main document.

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 {Ahrens, 2011, 2657780; Benskin, 2012, 1274133; Dinglasan-Panlilio, 2014, 2545254; Nakayama, 2007, 2901973; Remucal, 2019, 5413103; Zareitalabad, 2013, 5080561}.

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, 2017, 9419085; U.S. EPA, 2021, 7487276; U.S. EPA, 2023, 10692764}. 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 Chapter 1 of the PFOS MCLG main document, EPA conducted this updated assessment of PFOS to support development of an MCLG and national primary drinking water regulation (NPDWR). 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 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*) as well ongoing 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).

[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 nation-wide 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, 2021, 9640861}.

The 2016 PFOS HESD identified several adverse health outcomes associated with PFOS exposure based on results from animal toxicological and epidemiological studies, including: developmental effects (e.g., low birth weight, accelerated puberty, skeletal variations), cancer (e.g., testicular, kidney), 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 is "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, 2021, 10428576} evaluated PFOS in relation to all health outcomes. The SAB recommended that the scope be narrowed to focus on the five main 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 HESD for PFOS. 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 primary ones, 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, 2020, 8642427}, 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 toxicokinetic (see PFOS MCLG main document). The human relevance of effects in animals that involve PPARα was investigated in the mechanistic syntheses of the five main health outcomes (see PFOS MCLG main document). 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, 2718645) 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 draft for public comment is to derive an MCLG for PFOS to support the NPDWR for PFAS. To derive an MCLG, a cancer classification, toxicity values (i.e., a reference dose (RfD) and cancer slope factor (CSF)), and relative source contribution (RSC) for PFOS are potentially needed. 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, 2021, 10428576} and in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and Health Advisory {U.S. EPA, 2016, 3982043}.

A.1.3.2 Specific Aims

The specific aims of the PFOS MCLG document, which support the overall objective of deriving an MCLG for PFOS, are to:

- Provide a description of the literature searches conducted and systematic review methods used to identify health effects information (epidemiological, animal toxicological studies, and physiologically-based pharmacokinetic (PBPK) models) published since the 2016 PFOS HESD.
- Describe literature screening methods, including use of the Populations, Exposures, Comparator, and Outcomes (PECO) criteria and procedures for tracking studies throughout the literature screening process.
- Identify epidemiological and animal toxicological literature reporting effects of exposure to PFOS (and its associated salts and isomers) as outlined in the PECO criteria.
- 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 for the five main health outcomes (developmental, hepatic, immune and cardiovascular effects, and cancer).
- Describe and document study quality evaluations conducted for epidemiological and animal toxicological studies considered potentially useful for point-of-departure (POD) derivation.
- Describe and document data from *high* and *medium* confidence epidemiological and animal toxicological studies (as determined by study quality evaluations) that could be used for POD derivation. For dose-response assessment, only *high* and *medium* confidence studies were used to quantify health effects.
- Synthesize and document the adverse health effects evidence reported across studies, assessing similar health outcomes using a narrative approach. (The assessment focuses on synthesizing the available evidence for five main health outcomes—developmental, hepatic, immune and cardiovascular effects, and cancer—and also provides secondary syntheses of evidence for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects; reproductive effects in males or females; and general systemic toxicity.
- Develop and document strength-of-evidence judgments across studies (or subsets of studies) separately for epidemiological and animal toxicological lines of evidence for the five main health outcomes and integrate mechanistic analyses into the judgments.

- Develop and document integrated expert judgments across lines of evidence (i.e., epidemiological and animal toxicological lines of evidence) as to whether and to what extent the evidence supports that exposure to PFOS has the potential to be hazardous to humans. The judgments will be directly informed by the evidence syntheses and based on structured review of an adapted set of considerations for causality first introduced by Austin Bradford Hill {Hill, 1965, 71664}.
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation.
- Derive candidate RfDs and/or CSFs and select the RfD and/or CSF for PFOS and describe the rationale.
- Determine PFOS's cancer classification using a weight-of-evidence approach.
- Characterize the effects associated with PFOS exposure, including uncertainties and data gaps.

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, 2022, 10476098}, 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 literature search 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 life stage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any life stage (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*).

[2] Note: Although this appendix and its accompanying main document 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
	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 days 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 life stage (occupational or general population, including children and other sensitive populations): whole organism, tissues, individual cells, or biomolecules. **Animal:** Select non-human mammalian animal species: only non-human primates, rats, and mice (whole organism, tissues, individual cells, or biomolecules) of any life stage (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).

PECO Element	Inclusion Criteria
	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. 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 life stage (occupational or general population, including children and other sensitive populations). **Animal:** Select mammals (i.e., non-human primates and rodents (i.e., rats, mice, rabbits, guinea pigs, other rodent models) and fish (i.e., zebrafish) of any life stage (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, 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 literature inventories of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this updated toxicity assessment based on three data streams: 1) literature published from 2014 through 2019 and then updated in the course of this review (i.e., through February 3, 2022) 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 and studies shared with EPA by the SAB), and 3) literature identified in EPA's 2016 HESDs for PFOA and PFOS, which captured literature through 2013 {U.S. EPA, 2016, 3603279; U.S. EPA, 2016, 3603365}.

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 into the literature inventory. 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, animal, *in vitro*, *in silico*) or health outcomes. These searches comprised all literature related to health effects in animals and humans 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) and another covering September 2020 through February 3, 2022 (2022 literature search). 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 focused on the five main 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	4/10/2019

Database	Search String	Date Run
	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=(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]	4/10/2019

Database	Search String	Date Run
	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]))	
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+335-67-1+@TERM+@rn+1763-23-1+@TERM+@rn+45298-90-6)+@NOT+@org+pubmed+@AND+@RANGE+yr+2013+2019	4/11/2019
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 and February 2022 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	9/3/2020, 2/2/2022

Database	Search String	Date Run
	"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])	
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-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)	9/3/2020, 2/3/2022
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, and February 2 and 3, 2022 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, 2016, 4149688}. 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).

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 literature search, the *in vitro* evidence stream filter was not used). 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, 9642134} (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, 9416932} (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 potentially 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).

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

The 2016 HESD for PFOS contained a comprehensive summary of relevant literature based on searches conducted through 2013, as described in that document and in the related 2016 Drinking Water Health Advisory for PFOS. The 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 main health outcomes into this updated PFOS assessment, as described below.

Over 140 epidemiological studies were captured in the 2016 PFOS HESD. The 2016 HESD did not use the epidemiological data quantitatively. For this updated assessment, EPA reviewed the epidemiological studies that were included in the HESD summary tables and identified those that were relevant to one or more of the five main health outcomes (i.e., developmental, immune, hepatic, cardiovascular, and cancer). A total of 51 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

HERO ID	Reference	Title
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
2850925	Geiger et al., 2014	The association between PFOA, PFOS and serum lipid levels in adolescents
2851286	Geiger et al., 2014	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., 2003	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., 2014	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., 2007	Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth
1290900	Apelberg et al., 2007	Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland
1332466	Chen et al., 2012	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., 2008	Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy
2349574	Fei et al., 2008	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

HERO ID	Reference	Title
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
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., 2003	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 years 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., 2014	The association between PFOA, PFOS and serum lipid levels in adolescents

HERO ID	Reference	Title
1290820	Lin et al., 2009	Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults
3981585	Maisonet et al., 2015	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
1290020	Olsen et al., 2003	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., 2014	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 HESD summary tables that were identified as relevant for all health outcomes. A total of 9 animal toxicological studies 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., 2005	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	Sub-chronic 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., 2005	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

HERO ID	Reference	Title
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., 2005	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al., 2005	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	Sub-chronic 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., 2005	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	Sub-chronic 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	Sub-chronic 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., 2005	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	Sub-chronic 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., 2005	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
		Renal

HERO ID	Reference	Title
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)
1290852	Seacat et al., 2003	Sub-chronic 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., 2005	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al., 2005	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., 2005	Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters
1276160	Luebker et al., 2005	Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats
1290852	Seacat et al., 2003	Sub-chronic 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 life stage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any life stage (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." **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 days 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 published since development of the 2016 PFOS HESD and which could influence the derivation of an oral RfD and/or CSF. 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 (e.g., mechanistic, ADME). Studies that met the PECO criteria were tagged as having relevant

human data, relevant animal data (in a mammalian model), or a PBPK model. 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 independent reviewers using structured forms in DistillerSR, with a process for conflict resolution. Literature inventories for PECO-relevant studies and studies tagged as containing potentially relevant supplemental material during full-text screening were created to facilitate review of studies by topic-specific experts by identifying evidence types and health effect systems. These procedures are consistent with those outlined in the IRIS Handbook {U.S. EPA, 2022, 10476098}.

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. Details on the screening and data extraction methods for ADME 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, 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 • No[a] • 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] Erratums and corrections were considered not relevant.
[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 • No, exclude the reference[a]
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] Erratums and corrections were considered not relevant.
[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, non-cancer, or unclear/unknown.
PK or PBPK Models	Studies reporting the application of existing PK or PBPK models.
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 days (unless the study is a developmental/reproductive study)

Category	Evidence
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
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, life stage, 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-11). 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 **Error! Reference source not found.**.

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. Health Effect Categories Considered for Epidemiological StudiesTable 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 General immune assays (e.g., white blood cell counts)	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
	Immune responses in the respiratory system Stress-related factors in blood (e.g., glucocorticoids or other adrenal markers)	
Metabolic	Obesity BMI Adiposity Diabetes (including gestational diabetes) Insulin resistance Blood glucose	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 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.
- Sub-chronic: 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) and 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 PFOS Main Document) 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 breast feeding 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 • Non-human 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, life stage 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 life stage when the ADME data was collected?** Use the free text field to provide additional life stage notes. *[Select one; Free-text]*	• Prenatal: conception to birth • Infancy: 0–12 months • Childhood: 13 months to 11 years • Adolescence: 12 to 20 years • Adult: 21 to 65 years • Elderly: > 65 years	• If there is more than one life stage 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). ○ 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). ○ 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, life stage 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 life stage when the animal was dosed?** *[Select all that apply]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Prenatal o Non-human primates: conception to birth o Rodents: GD0 to birth • Weaning o Non-human primates: 1–130 days (0.35 years) o Rodents: PND 1–21 • Adolescent o Non-human primates: 130–1,825 days (0.35–5 years) o Rodents: 21–50 days (3–7 weeks) • Adult o Non-human primates: 5–35 years o Rodents: > 50 days (> 7 weeks) • Elderly o Non-human primates: > 35 years
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 life stage when the ADME data was collected?** *[Select all that apply; Free-text]*	• Prenatal • Weaning • Adolescent	• Prenatal o Non-human primates: conception to birth o Rodents: GD 0 to birth

Question/Prompt	Response Options	Suggested Considerations
	• Adult • Elderly	• Weaning o Non-human primates: 1–130 days (0.35 years) o Rodents: PND 1–21 • Adolescent o Non-human primates: 130–1,825 days (0.35–5 years) o Rodents: 21–50 days (3–7 weeks) • Adult o Non-human primates: 5–35 years o Rodents: >50 days (> 7 weeks) • Elderly o Non-human primates: > 35 years; use the free text field to provide additional life stage notes. • If there is more than one life stage 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, mon, y). • For reproductive and developmental studies, where possible instead include abbreviated age descriptions such as "GD1-10" or "GD2-PND10" o Examples: 14 d, 13 w (6 h/d x 5 d/wk); GD 2–PND 10 • Use "Not Reported" if appropriate.

	Question/Prompt	Response Options	Suggested Considerations
3.16	**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) ○ Examples: 14 or 28 d; 13 wk; 2 y • 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, life stage 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**	–	• Examples: A549; liver tissue from adult Sprague Dawley female rats

	Question/Prompt	Response Options	Suggested Considerations
	Use the free text field to list the cell line name or tissue source the cells were derived from. *[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) ○ 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.	–	• Use abbreviations (h, d, wk, mon, y) ○ Examples: 28 d; 13 wk; 2 y

	Question/Prompt	Response Options	Suggested Considerations
	[Free-text]		• Use "Not Reported" if appropriate.
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 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 life stage when the mechanistic data was collected?** *[Select one; Free-text]*	• Prenatal • Infancy • Childhood • Adolescence • Adult • Elderly	• Free text for life stage 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]*	• Non-human primate • Zebrafish • Rat • Mouse	• Free text field to list species for "other rodent model" option.

	Question	Options	Suggested Considerations
		• Rabbit • Guinea pig • 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 life stage when the animal was dosed?** *[Select one; Free-text]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Free text field for life stage notes.
3.7	**What is the life stage when the mechanistic data was collected?** *[Select one; Free-text]*	• Prenatal • Weaning	• Free text field for life stage notes.

	Question	Options	Suggested Considerations
		• Adolescent • Adult • 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	• Free text field for "other" option.

	Question	Options	Suggested Considerations
		• 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	
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 • Non-human primate • Rat • Mouse	• Free text field to list "other rodent model" option.

	Question	Options	Suggested Considerations
		• Rabbit • Guinea pig • 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	• Free text field for "other" option.

	Question	Options	Suggested Considerations
		• 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	
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, 2022, 10476098}, 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, 2022, 10476098} (previously Figure 6-1 in the draft IRIS Handbook {U.S. EPA, 2020, 7006986}). Study quality evaluations were performed using the structured platform for study evaluation housed within EPA's Health Assessment Workplace Collaborative (HAWC).

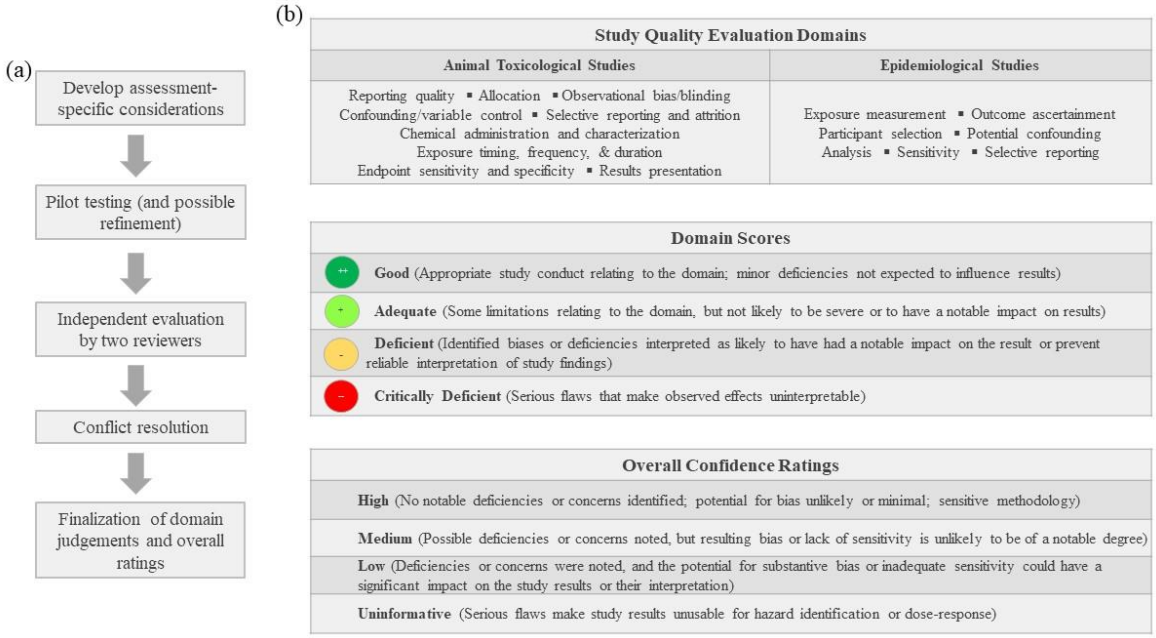


Figure A-1. Overview of Study Quality Evaluation Approach

(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 Figure A-2). 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 Figure A-3) 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 (including the direction and/or magnitude of influence) 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 mechanistic or ADME data did not undergo study quality evaluation as study quality domains for these types of studies are not currently available in HAWC.

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.

Figure A-2. Possible Domain Scores for Study Quality Evaluation

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.

Figure A-3. Overall Study Confidence Classifications

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: exposure measurement, outcome ascertainment, participant selection, 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-17).

Table A-17. 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-18 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-18. 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,

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?			
			tasks performed, or materials used) – may be Critically Deficient, depending on severity of this limitation.
		Critically Deficient	• JEM with data indicating it cannot differentiate between exposure levels over time, area, or between individuals.

A.1.7.1.3 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-19).

Table A-19. 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 non-differential 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).

Rating	Criteria
	• Direct evidence that bias was likely because the exposure was assessed using methods with poor validity. • 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.1.7.1.4 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, different sets of criteria may be applied for different outcomes in the same study. Table A-20 presents criteria that apply across outcomes.

Table A-20. 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 health care, 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.1.7.1.5 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, what the direction and magnitude of the effect of the confounder might be and whether it was considered in the design and/or analysis of the study (Table A-21).

Table A-21. 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: ○ 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); ○ Consideration that potential confounders were rare among the study population, or were expected to be poorly correlated with exposure of interest; ○ Consideration of the most relevant functional forms of potential confounders; ○ Examination of the potential impact of measurement error or missing data on confounder adjustment; ○ 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 Deficient	• 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.

Core Question: Is confounding of the effect of the exposure likely?		
		• If confounders not considered by design or in the analysis (e.g., only simple correlations presented).

Notes: ADHD = attention deficit hyperactivity disorder.

A.1.7.1.6 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-22).

Table A-22. 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.1.7.1.7 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-23).

Table A-23. 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.1.7.1.8 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-24). 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-24. 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, life stage, 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.1.7.1.9 Overall Confidence

Table A-25. 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-26).

Table A-26. 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/life stage the animals were at outcome evaluation.

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?			
	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).	• **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.

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-27. 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

Core Question: Were animals assigned to experimental groups using a method that minimizes selection bias?			
			randomly assigned to the control and 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-28. 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, 2004, 51763}. 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, 2004, 51763}, 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

Core Question: Did the study implement measures to reduce observational bias?			
			non-targeted 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, 2004, 51763}.
	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, 2004, 51763}. 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

Core Question: Did the study implement measures to reduce observational bias?			
			behavioral testing in treated mice. There 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, 2004, 51763}.

A.1.7.2.4 Confounding/Variable Control

Table A-29. 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. Based on 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.

ppm = parts per million; DMSO = dimethyl sulfoxide.

A.1.7.2.5 Reporting and Attrition Bias

Table A-30. 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-31. 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	• **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.		concentrations are 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. Based on the doses tested (and inferences regarding the administered doses of the impurity), this is likely to be a significant driver of any observed effects. • **Example 2 (inhalation):** Critically Deficient. Dams were exposed in static

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
			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-32. 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. The experimental design and exposure period were appropriate for evaluation of potential male reproductive and developmental effects. The experiment was designed to evaluate reproductive and developmental outcomes and followed recommendations in {OECD, 2001, 3421602} and {U.S. EPA, 1998, 2229410} 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?			
		• 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 week and fertility was assessed on week 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 weeks" {U.S. EPA, 1996, 30019}.
	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 weeks 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-33. 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 is consistent with NTP pathology

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			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.
		–	• **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. • **Example 3:** Deficient. Motor activity: Concerns were raised regarding the small sample sizes used to evaluate these

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			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. [Endpoint name]: [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-34. 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): Performance on the rotarod was presented as incidence of falling off the rod within an arbitrary time, rather than as time

Core Question: Are the results presented in a way that makes the data usable and transparent?			
			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-35).

Table A-35. 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. • **Example 2:** *Medium* Confidence. Histopathology: The study authors did not

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?			
			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 non-specific 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 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

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?			
			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, 2022, 10476098}, 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 published prior to 2016 (i.e., from the 2016 HESD and the 2021 ATSDR *Toxicological Profile for Perfluoroalkyls*) or identified in the updated literature searches were extracted if they received a *medium* or *high* confidence study quality evaluation rating. In cases where data was limited (e.g., thyroid cancer) or when there was a notable effect, results from *low* confidence studies were extracted. Studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction. Extraction was targeted towards the five main 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 on a case-by-case basis (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 evaluation team.

Table A-36 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-36. 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 years • 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 years later" but do not provide dates), extract

	Question/Prompt	Response Options	Suggested Considerations
			"recruitment 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: • Weight (ln-grams) • 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: • High cholesterol (> 5.0 mg/dL)
11.3	**If developmental, when was the outcome measured?** *[Select all that apply]*	• ≤ 2 years of age • > 2–5 years of age • > 5 years 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 months of age • Participants were > 6 months of age	–
11.6	**Sub-population** *[Free-text]*	–	• If relevant, specify sub-group 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 sub-group (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.

	Question/Prompt	Response Options	Suggested Considerations
			The following format is preferred: 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. • Example: • 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 tertile 2 (0.83–1.4 ng/mL) vs. tertile 1 (< 0.83 ng/mL)] <u>Bad Examples/Formatting:</u> • beta coefficient • linear regression coefficient (standard error) with one unit increase in log-PFC in adults

	Question/Prompt	Response Options	Suggested Considerations
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., extract OR (for Q2 vs Q1), but don't extract the OR of 1 for the reference group Q1).

	Question/Prompt	Response Options	Suggested Considerations
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
11.19	**Results Comments** *[Free-text]*	–	• Enter the location of the extracted data (e.g., "Table 3" or "in-text p. 650").

	Question/Prompt	Response Options	Suggested Considerations
			• 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 <u>not</u> 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 <u>not</u> need to specify how values below the LOD were handled. • If data is presented by **sub-group/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; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; 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-37 were used throughout full-text screening and data extraction for epidemiological studies.

Table A-37. 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; cancer survivors). All cohort studies (prospective or retrospective) consider exposure data from before the occurrence of the health outcome.

Study Design	Description
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, 2022, 10476098}, 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-38). 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-38. 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.

	Questions/Prompts and Options	Suggested Considerations
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.
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 4h single day study can be represented as 0.17 days. 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.

	Questions/Prompts and Options	Suggested Considerations
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. • 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.

	Questions/Prompts and Options	Suggested Considerations
		• 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; 90min • For developmental and reproductive studies, specify observation time in terms of development (e.g., GD 16, PND_0).
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 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.

Questions/Prompts and Options	Suggested Considerations
• 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 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.	• '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. • 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 to *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. Available 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-39 and Table A-40.

Following evidence synthesis, the evidence for human and animals were integrated for each health outcome. 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 and other supplemental information. 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. 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, 71664} 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-41) (“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-39. 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 exposure levels, or uncertainties or methodological limitations that result in an inability to discern effects from exposure.

Strength-of-Evidence Judgment	Description
	• 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-40. 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

Strength-of-Evidence Judgment	Description
	a 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-41. 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:
[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 HESDs

For all non-priority health outcomes, epidemiological studies identified and reviewed in the 2016 HESD were included 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 HESD studies. Inferences drawn from evidence in the current literature search were compared to the results described from 2016 studies.

For the 5 main health outcomes (i.e., developmental, immune, hepatic, cardiovascular and cancer), epidemiological studies identified and reviewed in the 2016 HESD and other pre-2016 assessments were included in the evidence synthesis, including discussion of study quality considerations, according to the recommendations from the SAB. Inferences drawn from studies included from the 2016 HESD were considered in drawing health effects conclusions.

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, 2020, 8642427}. Briefly, the evidence integration involved evidence stream evaluation, including evaluation of the qualitative summaries on the strength of evidence from studies in animals and humans, and inference across evidence streams. Across evidence streams, human relevance of animal models and mechanistic evidence were considered. 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.

A.1.10.2 Epidemiological Studies Excluded from Synthesis

Some epidemiological studies were not included in the evidence synthesis narrative if they included factors that could lead to 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 largely 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 judgments about 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, 1239433}, *Review of the Reference Dose and Reference Concentration Processes* {U.S. EPA, 2002, 88824}, *Guidelines*

for Carcinogen Risk Assessment {U.S. EPA, 2005, 6324329}, and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* {U.S. EPA, 2005, 88823}.

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, 88824}. 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

Selection of specific endpoints for toxicity value derivation is primarily a result of the evidence integration and hazard characterization. Specific issues that may be considered for their potential to affect the feasibility of dose-response modeling for individual data sets are described in more detail in the *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}. In general, studies and endpoints that are most useful for dose-response analysis will generally have at least one exposure level in the region of the dose-response curve near the benchmark response (BMR; the response level to be used for deriving toxicity values) to minimize low-dose extrapolation. Such studies will also have more exposure levels and larger sample sizes overall {U.S. EPA, 2012, 1239433}. 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, and
- studies with a design or analysis that addresses relevant confounding for a given outcome are preferred.

The number of studies considered for toxicity value derivation will be reduced based on these considerations and others described in EPA {2012, 1239433; 2022, 10476098}.

A.1.11.2 Conducting Dose-Response Assessments

Several EPA guidance and support documents provide background for the derivation of toxicity values {U.S. EPA, 2002, 88824; U.S. EPA, 2005, 6324329; U.S. EPA, 2022, 10476098}. Steps of the dose-response process include: 1) selecting BMR values; 2) dose characterization and dose-response modeling, including conversion of administered doses to internal doses (animal studies only) and conversion of PODs to human equivalence doses; 3) candidate toxicity value development; 4) characterizing uncertainty; and 5) selection of final toxicity values.

The recommended EPA human health risk assessment (HHRA) approach described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* describes a multistep approach to dose-response assessment, including analysis in the range of observation followed by extrapolation to lower levels {U.S. EPA, 2002, 88824}. In this effort, EPA conducted a dose-response assessment to define a POD and extrapolated from the POD to an RfD. For PFOS, EPA performed benchmark dose (BMD) modeling of animal and human studies to refine the critical effect POD in deriving the RfD. 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 lower limit of the BMD (BMDLs) to serve as potential PODs for deriving quantitative estimates below the range of observation {U.S. EPA, 2012, 1239433}. EPA used several approaches for dose-response modelling. EPA generally 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 pre-determined level of response (i.e., BMR).

Considerations for BMR selection are discussed in detail in EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}. 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, 2012, 1239433; U.S. EPA, 2022, 10476098}.
- 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, 2012, 1239433; U.S. EPA, 2022, 10476098}.

- For outcomes for which there is no accepted percent change that is considered adverse, EPA used the hybrid approach to derive the BMR.

Deviations of these recommendations, if any, will be described in the assessment.

The preferred approach for dose estimation for dose-response modeling is PBPK modeling because it can incorporate a wide range of relevant chemical-specific information, describe the active agent more accurately, and provide a better basis for extrapolation to human equivalent exposures. For animal studies, EPA used a pharmacokinetic model to make predictions of the internal dose in laboratory animals used in toxicity studies or in humans based on the administered dose used in the study (see PFOS MCLG main document for additional detail). Concentrations of PFOS in blood are considered for all the internal dose-metrics. For animal studies, this conversion would occur prior to BMD modeling.

If multiple studies are suitable for exposure-response modeling and if no single study is judged to be appreciably better than the others for the purposes of deriving toxicity values, data or results from multiple studies may be derived from different studies for comparison. For each modeled response, a POD from the observed data will be estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses. The POD will be used as the starting point for subsequent extrapolations and analyses. For noncancer dose-response data not amenable to BMD modeling, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD.

Subsequent to POD derivation, EPA used a pharmacokinetic model for human dosimetry to estimate human equivalent doses (HEDs) from both animal and epidemiological studies. For the human and animal endpoints of interests, serum concentration was identified, based on the available data, as a suitable internal dosimetry target. The selected pharmacokinetic models are discussed in Section 4 of the PFOS Main Document.

A.1.12 Candidate Toxicity Value Derivation and Selection

For each noncancer data set analyzed for dose-response, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HED}s) to account for five possible areas of uncertainty and variability: human variation, extrapolation from animals to humans, extrapolation to chronic exposure duration, the type of POD being used for reference value derivation, and extrapolation to a minimal level of risk (if not observed in the data set). The particular value for these adjustments is usually 10, 3, or 1, but different values based on chemical-specific information may be applied 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, 88824}.

- 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, 88824}. 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, 88824}. 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, 88824; U.S. EPA, 1991, 732120}. Otherwise, a factor of 10 is generally used to account for this variation.
- LOAEL to NOAEL: If a POD is based on an 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, 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, 88824; U.S. EPA, 1991, 732120}. 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.

For each cancer data set analyzed for dose-response, the approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). If the chemical causes cancer through a mutagenic change to deoxyribonucleic acid (DNA), or if the MOA for causing cancer is not known, this extrapolation is conducted by drawing a line from the POD to the origin (zero dose, zero tumors). The slope of the line (Δresponse/Δdose) gives the CSF which can be interpreted as the risk per mg/kg/day. In addition, under the supplemental guidance {U.S. EPA, 2005, 88823}, affirmative determination of a mutagenic MOA (as opposed to defaulting to a mutagenic MOA based on insufficient data or limited data indicating potential mutagenicity) determines if age-dependent adjustment factors are applied in the quantification of risk to account for additional sensitivity of children. A CSF is derived by dividing the BMR by the POD_{HED}.

If the chemical is shown to cause cancer via a 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. The 2005 guidelines 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, 2005, 88823}.

The next step is to select an organ/system-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, 2022, 10476098} and include: 1) the weight of evidence for the specific effect or health outcome; 2) study confidence; 3) 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 data sets. 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, 13 meta-analysis studies were identified and summarized below (Table A-41).

Table A-42. Epidemiologic Meta-Analysis Studies Identified from Literature Review

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Verner et al. (2015, 3150627)	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, 3981320[b])	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, 7643488)	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, 9959525)	5	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): OR = 1.44 (1.15, 1.72)
Deji et al. (2021, 7564388)	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, 9959601)	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. (2022, 10176603)	22	Belgium, Canada, China, Denmark, Netherlands, Norway, Slovakia, Spain, Sweden, United States	Developmental	**PTB:** • 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, 10285082[b])	25	Asia (NOS), Europe (NOS), United States	Hepatic	**ALT:** • In adults and adolescent, Cross-sectional (6 studies) weighted z-score = 3.55, p < 0.001 ○ One longitudinal study reported positive associations • ALT in children <12 years of age, GGT, AST, liver enzymes: associations not statistically significant
Abdullah Soheimi et al. (2021, 9959584)	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 triglyceride levels
			Metabolic (3 studies)	**GDM:** • Inconsistent associations between serum PFOS and increased GDM in pregnant mothers compared to non-pregnant mothers
Kim et al. (2018, 5079795)	12	Canada, China, Korea, Japan, Norway, Taiwan, United States	Endocrine – Thyroid	**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 = −0.04 (−0.06, −0.01),
Zare Jeddi et al. (2021, 8347183)	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, 10176437)	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, 9959569)	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%

Notes: LBW = low birth weight; OR = odds ratio; 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; T4 = thyroxine; T3 = triiodothyronine; TSH = thyroid stimulating hormone; BMI = body mass index; ADHD = attention deficit-hyperactivity disorder.

[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 After Assessment Literature Cut-Off Date

Studies identified after the updated literature review (February 2022) did not undergo the systematic review protocol. Studies were reviewed for major findings and how those findings may affect the assessment. For PFOS, 8 studies were identified after the updated literature review and are summarized below (Table A-43).

Table A-43. Studies Identified After Updated Literature Review (Published or Identified After February 2022)

Reference	Major Findings	Assessment Implications
Ding et al. (2022, 10328874)	Cohort study of 1,058 midlife women initially free of hypertension from the multiethnic and multiracial SWAN. Compared with the lowest tertile, women in the highest tertile of baseline serum PFOS concentrations had adjusted HRs of 1.42 (95% CI: 1.19, 1.68) (p-trend = 0.01). In the mixture analysis, women in the highest tertile of overall PFAS concentrations had a hazard ratio of 1.71 (95% CI: 1.15, 2.54; p-trend=0.008), compared with those in the lowest tertile.	PFOS might be associated with increased risk of hypertension in women. Possible mixture effects with hypertension in women. No change.
Feng et al. (2022, 10328872)	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. Quantile g-computation analysis observed a 19% increased incident risk of breast cancer along with each simultaneous quartile increase in all ln-transformed PFCA concentrations (HR = 1.19, 95% CI: 1.01, 1.41), with PFOA accounting for 56% of the positive effect.	No change.
Goodrich et al., 2022 (10369722)	Nested case-control study within the Multiethnic Cohort (MEC) Study, including incident, non-viral hepatocellular carcinoma (HCC) cases (n=50) and healthy controls (n=50). Significant increase in risk in in those with high exposure (>85th percentile; >54.9 ug/L) *vs.* low exposure (<85th percentile; < 54.9 ug/L) (OR = 4.50, 95% CI: 1.20, 16.00).	PFOS may be associated with incident, non-viral HCC. Contrasts findings in Eriksen et al., 2009 (2919344), however, Eriksen et al., 2009 (2919344) did not specify cancer type or etiology in their analysis.
Gui et al., 2022 (10365824)	Meta-analysis of 23 studies, pooled change in birthweight per 1-ln ng/mL increase in PFOS (unadjusted for gestational age/unstandardized birth weight): –34.88 g (95% CI: −52.53, −17.24), I^2= 66.1%. Significant effects observed for birth length and ponderal index. No associations observed for preterm birth, low birth weight or small for gestational age. Subgroup analyses were included, by fetal gender, time of blood sample collection, blood sample type and whether adjusted for GA/parity, study design, and geographic region. Included assessment of risk of bias for studies included in the meta-analyses.	Supports an association between PFOS and birth weight, birth length and ponderal index. Similar conclusions as previous meta-analyses.

Reference	Major Findings	Assessment Implications
Jiang et al. (2022, 10328207)	Meta-analysis of 8 studies across 8 countries. No association between PFOS and breast cancer risk (OR = 1.01; 95% CI: 0.87, 1.17), I^2 = 99.8%.	No change. Serious methodological limitations warrant cautious interpretation of results from this publication.
Luo et al., 2022 (10273290)	Prospective study in the Danish National Birth Cohort, 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.	No change.
Velarde et al. (2022, 9956482)	Case-control study of 150 Filipino women (75 breast cancer cases and 75 controls). Serum PFOS levels were significantly higher in cases than on controls. PFOS was positively but not statistically significant 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.	No change.
Wen et al. (2022, 10328873)	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 all-cause (OR = 1.57; 95% CI: 1.22, 2.07), heart disease (OR = 1.65; 95% CI: 1.09, 2.57) or cancer mortality (OR = 1.75; 95% CI: 1.10, 2.83), but only in the highest tertile (≥ 17.1 ng/mL) compared to the lowest tertile (< 7.9 ng/mL).	No change.
Zhang et al., 2022 (9944433)	Prospective cohort study (the Shanghai Birth Cohort) of 2,395 mother-infant pairs. Prenatal PFOS exposure measured in early pregnancy (median, 15 gestational weeks) was not associated with infant length, weight, and head circumference at birth, 42 days, 6 months, and 12 months.	No change.

Notes: SWAN = Study of Women's Health Across the Nation; HR = hazard ratio; OR = odds ratio; NHANES = National Health and Nutrition Examination Survey.

Appendix B. Detailed Toxicokinetics

B.1 Absorption

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, 4234856) 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 Phospholipidicity as Reported by Sanchez-Garcia et al. (2018, 4234856)

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, 4234856) 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, 6505873) 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, 2008, 9641966}. 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 (log P_{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, 1289832) administered a single oral dose of 4.2 mg/kg of PFOS-^{14}C in solution to three male Sprague-Dawley rats. At 48 hours after dosing, only 9.08 ± 0.51% of the total PFOS-^{14}C 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, 7561179}. 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, 2016, 3749289}. 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 hr and 11.5 hr, respectively). In a similar study {Huang, 2019, 5387170}, 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 hr and 12.2 hr 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. (2016, 3749289)	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. (2019, 5387170)	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. (2019, 5387170).
[b] Converted published T_{max} (days) to T_{max} (hours) for Kim et al. (2016, 3749289).

B.2 Distribution

B.2.1 Protein Binding

Kerstner-Wood et al. (2003, 4771364) 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, 2919350) 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, 1280480) 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, 2919252) 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, 5084740) 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, 2919350) 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, 2850292) 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, 3856708) 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, 6565171) 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, 5387135) 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 to 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 vs. cord blood, lower cord blood albumin levels compared to maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017, 3981900) 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, 1291067), Zhang et al. (2013, 5081488), and Yang et al. (2020, 6356370) 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, 2004, 5212239} and constitutes 2%–5% of the cytosolic protein in hepatocytes. Luebker et al. (2002, 1291067) 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. (2013, 5081488) 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. (2020, 6356370) 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, 2018, 5024207}. The authors found that predicted free energies correlated well with binding affinities measured in 3 previous studies {Woodcroft, 2010, 2919284; Zhang, 2013, 5081488; Sheng, 2018, 4199441}. 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, 2020, 6833715}.

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, 6311640) 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, 3859825) 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, 3859825) study was lower (1.5 ± 0.42) compared to the mean plasma:whole blood (2.2–2.3) {Ehresman, 2007, 1429928} and serum:whole blood (1.2–2.3) {Kärrmen, 2006, 2159543; Hanssen, 2013, 3859848} 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 to 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., 2017, 4239163}. 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. (2003, 3005572) 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, 2732071) 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, 2325349) 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.

PFOS also accumulates in follicular fluid. Kang et al. (2020, 6356899) measured 6.82 ng/mL 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 ($r^2 = 0.93$, $p < 0.001$). Exposure of oocytes to PFOS raise the possibility of reproductive toxicity in humans.

Stein et al. (2012, 1332468) 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 µg/mL–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 {Harada, 2007, 2919450; Wang, 2018, 5080654}. 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 {Apelberg, 2007, 1290900; Von Ehrenstein, 2009, 194805; Völkel, 2008, 3103448}. Kärrman et al. (2010, 2732071) 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 to levels in the liver.

B.2.2.2 Animal Studies

Studies of tissue distribution are available for several species of animals including non-human primates, rats, and, to a lesser extent, mice. Studies of non-human 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 accumulate far more PFOS in liver than serum.

B.2.2.2.1 Non-Human Primates

Two long-term studies in monkeys examined PFOS accumulation in the serum and liver. Seacat et al. (2002, 757853) 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 2 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 non-linear 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 to 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, 2017, 3981378}, 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, 757853). 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 3 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 µg/mL –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, 757853) study reported a ratio closer to 2:1. Chang et al. (2017, 3981378) 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, 758419) 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.

In another acute study performed by Yu et al. (2011, 1294541), 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, 5400978}. 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, 757868) 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, 757868)

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: PFOS = perfluorooctane sulfonate; 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, 757871), 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, 757871)

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, 3859701) 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 to 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, 3859701)

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 {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}. 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, 3859701) 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, 5029075)

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

Timepoint (weeks)	0 ppm		0.5 ppm		2 ppm		5 ppm		20 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
106[c]	–	–	–	–	–	–	–	–	2.42	9.51
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.

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, 5080641) 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).

Bogdanska et al. (2011, 2919253) 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, 2019, 5387049}. 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, 1937260), 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, 3981396) 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, 2019, 757868}, PFOS exposure is found to cross the blood-brain barrier. In Yu et al. (2019, 5918598), 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 finding 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, 2850956) 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, 1294541) 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, 3789332) 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. (2020, 6505874) 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 life stages.

B.2.3.1 Human Studies

Zhang et al. (2013, 3859792) 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 to 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. (2017, 3859806; 2017, 3981340) 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. (2017, 3859806), 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. (2017, 3981340) followed a similar pattern, however, the PFOS accumulation in the placenta was approximately 14.5% less in Chen et al. (2017, 3981340) than in Chen et al. (2017, 3859806).

Zhang et al. (2013, 3859792) (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. Based on RPM, 59% of maternal PFOS is accumulated in the placenta. This study and the Chen et al. (2017, 3859806; 2017, 3981340) 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. (2013, 3859792) is parity. About 82% of the mothers in Zhang et al. (2013, 3859792) were primiparous whereas only 46.8% were primiparous in Chen et al. (2017, 3859806; 2017, 3981340), which may explain the higher PFOS concentrations in maternal serum and placenta found in the Zhang et al. (2013, 3859792) study. Primiparous mothers also tend to have higher levels of PFAS in breast milk than women who have had multiple children {Lee, 2013, 3983576}, adding to the evidence that pregnancy and lactation durations are critical for PFAS distribution.

Mamsen et al. (2019, 5080595) 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. (2015, 2851103) 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, 2017, 3859806}. 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, 2851230) 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).

Porpora et al. (2013, 2150057) 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, 1290877) 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. (2019, 5083694) 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 ng/mL–29.85 ng/mL) in maternal serum and 1.33 ng/mL (range 0.12 ng/mL–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, 2020, 6318671; Li, 2020, 6505874}. 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, 2020, 6318671}. Similarly, Li et al. (2020, 6505874) 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, 6318671) found an 8% increase in branched PFOS accumulation compared to linear PFOS isomers. Similarly, Li et al. (2020, 6505874) showed a 6% increase in branched PFOS accumulation compared to linear PFOS isomers. Zhao et al. (2017, 3856461) observed higher TTEs for 1m, 4m, 3+5m, and m2 compared to 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 to linear isomers.

In summary, these studies suggest that maternal serum levels of PFOS is 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, 5412430), 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 (R_{CM})[d]
Manzano-Salgado et al. (2015, 3448674)	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. (2017, 3981340) and Chen et al. (2017, 3859806)	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 (2017, 3981340) and total PFOS was reported in Chen (2017, 3859806).						
Cariou et al. (2015, 3859840)	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, 5412430)	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, 6318671)	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 (R_{CM})[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. (2020, 6505874)	Maoming Birth Cohort, China (pre-term 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. (2020, 6506038)	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. (2019, 5083694)	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, 3981900)	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. (2017, 3856461)	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 (R_{CM})[d]
	Note: Authors reported that samples < LOD were not included in RCM analysis. Mean ratios reported for matched pairs.						
Beeson et al. (2011, 2050293)	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], 1005775)	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, 2919297)	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, 2994839)	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, 1424975)	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 (R_{CM})[d]
	Note: Median serum concentrations reported. Values in parentheses are 25–75% IQRs						
Fromme et al. (2010, 1290877)	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, 1312781)	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, 2919240)	Jinhu, China	50 (all)	NR	total PFOS	1.686	3.184	0.57
		26 (males 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, 1290901)	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. (2015, 3299692)	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, 2920555).						

Notes: 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 (2013, 3859792) 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, 2013, 2150057} to 183 ng/mL in Hubei, China {Zhao, 2017, 5085130}. Cord serum values ranged from < LOD in Wuhan, China {Chen, 2017, 3859806} and Toulouse, France {Cariou, 2015, 3859840} to 13.89 ng/mL in Faroe Islands, Denmark {Eryasa, 2019, 5412430}. 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, 2017, 3981340} to 21.4 ng/g in Tianjin, China {Zhang, 2013, 3859792}. 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, 2014, 2850251} to 0.121 ng/mL {Zhang, 2013, 3859792}. 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, 5381552) 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 brain, but not in 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, 2150057) (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, 2850251) (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. (2016, 3858535) (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, 3448674) (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. (2017, 3859806) (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. (2017, 3859806) (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: 0.06–0.1.38 ng/g	NR
Pan et al. (2017, 3981900) (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, 4728855) (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. (2019, 5083694) (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. (2017, 3856461) (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, 5381552) (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, 5387135) (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, 5412430) (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, 6318671) (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. (2020, 6505874) (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. (2020, 6506038) (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. (2013, 2639569) (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, 3859840) (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, 3859848) (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, 3859848) (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	NR	NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
	range: < 0.08– 0.89 ng/mL				
Mamsen et al. (2017, 3858487) (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, 5080595) (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, 2851230) (Ohio, USA)[f]	Maternal Serum at 16 weeks Median: 12.70 µg/L Maternal serum at delivery Median: 8.50 µg/L	Cord serum at delivery Median: 3.50 µg/L			

Notes: CI = confidence interval; INMA = INfancia y Medio Ambiente; 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.

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

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

[c] Eryasa et al. (2019, 5412430) 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 birth from 2008 to 2005). Both cohorts had the same source of exposure and are similar in maternal characteristics

[d] Li et al. (2020, 6505874) measured PFOS in matched maternal-cord serum pairs with pre-term deliveries and full-term deliveries.

[e] Hanssen et al. (2013, 3859848) collected whole blood and plasma from women in 2 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, 2851230) 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, 3858487) 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, 5080595) quantified PFAS levels in embryos and fetuses at gestational weeks 7–42 and serum from their matched maternal pairs. Like Mamsen et al. (2017, 3858487), 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 to 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 to the third trimester, and lowest in the lung in the second trimester compared to the first and third trimesters. Interestingly, PFOA concentration in the liver was also highest in the second trimester compared to 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, 9642023). 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, 5080595) 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 (BBB). 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, 2014, 2850916} compared to subjects analyzed in France, Denmark (Faroe Islands), or Sweden {Cariou, 2015, 3859840; Mogensen, 2015, 3859839; Gyllenhammar, 2018, 4778766}. In the Mondal study, geometric mean (GM) maternal serum PFOS concentrations were lower in breastfeeding mothers (11.63 ng/mL) vs. 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, 3859840) 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 to 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. (2015, 3859839) 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. (2018, 4778766) 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, 2850916)	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 years 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 & 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 & 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. (2015, 3859839)[a]	80 singleton children in Faroese birth cohort born between 1997–2000. 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, 3859840)	Female volunteers hospitalized between June 2010 and January 2013 for planned caesarean 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. (2018, 4778766)	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

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, 1312781).

Mondal et al. (2014, 2850916) 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. (2015, 3859839) 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. (2018, 4778766) study included only five exclusively bottle-fed infants. In this group, they observed a higher percentage of branched PFOS compared to exclusively breast-fed infants, which may be the result of the higher efficiency of placental transfer of branched PFOS isomers vs. linear isomers. 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, 2850916)

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. (2015, 3859839)

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, 2019, 5919402}. 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, 5 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, 2005, 1276160}. 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 three 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, 2005, 1276160}

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 main document (See PFOS Main Document) as reported in Butenhoff et al. (2009, 757873). 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, 757876) (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, 757876)

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, 2009, 757876}. The concentrations in the brains of fetuses was about 10 times higher than in their dams for all doses. Based on 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, 3981472) 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, 1326732), 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 to 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, 1326732)

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 to the lung in offspring on both PND 0 and PND 21 {Chen, 2012, 1276152} (Table B-15).

Table B-15. Serum and Lung PFOS Concentration of Sprague-Dawley Rat Pups {Chen, 2012, 1276152}

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, 2919287) 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, 2017, 3981375}. 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 to 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, 2017, 3981375}

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, 2009, 757877}. 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, 2009, 757877}

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: PFOS = perfluorooctance sulfonic acid; 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 {Thompson, 2010, 2919278; Egeghy, 2011, 723765} 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 {Thompson, 2010, 2919278; Egeghy, 2011, 723765}. The models developed were designed to estimate intakes of PFOS by young children and adults {Egeghy, 2011, 723765} and the general population of urban areas on the east coast of Australia {Thompson, 2010, 2919278}. 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, 2919278) 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, 818501). The original Andersen et al. (2006, 818501) 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, 2919278) used a V_d of 230 mL/kg for humans in their model.

Egeghy and Lorber (2011, 723765) 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 modelling 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, 3981280) used a V_d of 235 mL/kg by averaging of 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 non-pregnant 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. (2015, 2851103)	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, 3859800)	> 12 years	Males and Females	Blood (2016)	230 mL/kg bodyweight	Mean: 23.4 (18.5, 28.4)	–
	> 12 years	Males and Females	Blood (2010)	230 mL/kg bodyweight	Mean: 39.8 (30.9, 48.9)	–
Fu et al. (2016, 3859819)	Adult, occupational	Males and females	Serum	230 mL/kg	Mean: 5624; median: 1725	–
Zhang et al. (2013, 3859849)	Adults	Males and Females	Serum and whole blood	230 mL/kg	Mean: 31	–
Gomis et al. (2017, 3981280)	Humans and Animals	Males and Females	Serum	235 mLe/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.

B.2.4.2 Animal Studies

The Chang et al. (2012, 1289832) 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. Based on these studies, the authors concluded that the V_ds for monkeys, rats, and mice are likely in the range of 200 mL/kg–300 mL/kg.

Two recent studies in rats {Kim, 2016, 3749289; Huang, 2019, 5387170} measured toxicokinetic parameters including V_d (Table B-19). In the Kim et al. (2016, 3749289) 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. (2019, 5387170) calculated the apparent volume of central (V_1) and peripheral (V_2) distribution in rats using standard equations {Gabrielsson, 2000, 9642135}. 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. (2016, 3749289). 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. (2016, 3749289). 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 mL/kg–280 mL/kg) for both compartments, they were notably higher in the central compartment (222 ± 84 mL/kg) compared to the peripheral compartment (93.4 ± 93 mL/kg) in females.

In a third study {Iwabuchi, 2017, 3859701}, 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. (2016, 3749289)	Dose × AUMC/(AUC0-∞)[2]	IV	2 mg/kg	Sprague-Dawley	8–12 weeks	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 weeks	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. (2019, 5387170)	Standard equations {Gabrielsson, 2000, 9642135}	IV	2 mg/kg	Sprague-Dawley	8 weeks	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 weeks	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
							93.4 ± 93 ml/kg	Peripheral	AUC: 17.74 ± 1.02 µM-hr	0.02 ± 0.01 mM	NR
			2 mg/kg (x 5 d)	Sprague-Dawley	8 weeks	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 weeks	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, 3859701)	Dose / elimination rate constant (ke) × plasma concentration (AUC).	Oral	100 µg/kg	Wistar	7–9 weeks 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
							0.043 kg tissue volume/kg BW	Liver	240000 µg/kg tissue volume - day	2730 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
							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	2300 µg/kg tissue volume - day	197 µg/kg tissue volume	NR
							2.5 kg tissue volume/kg BW	Whole blood	1800 µg/kg tissue volume - day	52.6 µg/kg tissue volume	NR
							0.96 kg tissue volume/kg BW	Serum	2200 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.

Unlike the sex differences observed in rats, V_d calculations were similar in male and female monkeys as shown in **Error! Not a valid bookmark self-reference.** {Chang, 2017, 3981378}. 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 3 separate PFOS doses (11 mg/kg–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, 2017, 3981378}

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

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, 2016, 3603279}, and it is likely that PFOS is similarly resistant to metabolism in humans, primates, and rodents.

B.4 Excretion

B.4.1 Urinary and Fecal Excretion

B.4.1.1 Human Studies

Three major studies highlight the urinary excretion of PFOS in humans. Zhang et al. (2015, 2851103) 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 to nonpregnant females (0.0004 and 0.0013, respectively), suggesting the placenta and cord blood as possible elimination pathways.

Zhang et al. (2013, 3859849) 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, 3859819) 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 5624 and 1725 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:

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

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 to 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, 1289832), three Sprague-Dawley rats/sex/timepoint were administered 14C-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, 2016, 3749289}. 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 to 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 ten 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, 2010, 2919335}. 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, 2015, 2851191} 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 to 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. (2005, 1276160), 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 to 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. (2013, 3859849) 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, 5085553), 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, 2919450), in which serum and bile samples from patients (2 male and 2 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, 2583643) self-study of the potential for CSM to lower the levels of PFAS in blood. This was a case report and 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, three 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 (2015, 3856550; 2017, 3856461} 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, 2015, 3856550}. The opposite trend was seen for OATP-mediated uptake {Zhao, 2017, 3856461}. For these 5 OATPs, PFOS was transported with the highest affinity compared to 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 vs. 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 as unknown whether and to what extent these transporters function *in vivo*.

Table B-21. Enterohepatic Transporters of PFOS

	Human Transporters		Rat Transporters	
Organ	Liver	Intestine	Liver	Intestine
Cell type	Hepatocyte	Enterocyte	Hepatocyte	Enterocyte
Sodium-dependent {Zhao, 2015, 3856550}	NTCP	ASBT	NTCP	
Sodium- independent {Zhao, 2017, 3856461}	OATP1B1[a]	OATP2B1[a]	OATP1A1[a]	OATP1A5
	OATP1B3[a]		OATP1B2	OATP2B1
	OATP2B1[a]		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, 2015, 3856550}{Zhao, 2017, 3856461}.

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, 2014, 2850251}. The ratio of branched:total PFOS isomers in cord blood was 0.27 and was statistically greater in cord blood compared to maternal blood and

placenta. These finding suggests 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. (2013, 3859792) 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, 5080595) 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, 3858487) 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, 2017, 3858487}. 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 to 1.3 ng/g in placenta and 8.2 ng/g in maternal plasma. Increasing fetal PFOS levels with fetal age suggest that the rate of elimination of PFOS from mother to fetus may increase through the gestational period.

The same group {Mamsen, 2019, 5080595} measured PFOS accumulation in fetal tissues across the 3 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 to 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 life-stage when excretion is altered, Zhang et al. (2015, 2857764) 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 non-pregnant women (0.0013) and may be affected by the increase in blood volume during pregnancy {Pritchard, 1965, 9641812}.

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, 2008, 1290895}. 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, 2017, 3983576}. 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 ng/L–63.8 ng/L) and the median concentration for all PFAS chemicals measured was 151 ng/L (range of 105 ng/L–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, 759807) 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 1 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, 2016, 3981718}. 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, 2008, 2349575}. 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 caesarean delivery in Tolouse, France {Cariou, 2015, 3859840}. Mean PFOS concentrations were 3.67, 1.38 and 0.040 in maternal serum, cord serum and breast milk respectively (compared to 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, 2851239) 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) data sets (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, 2851239) 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, 2850226) suggested a need to consider the non-blood portion of the menstrual fluid and its albumin content in the Wong et al. (2014, 2851239) estimate for the menstrual fluid volume. A yearly estimate for serum loss of 868 mL/year by Verner and Longnecker (2015, 2850226) compared to the 432 mL/year estimate of Wong et al. (2014, 2851239) 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 {Knox, 2011, 1402395; Taylor, 2014, 2850915}. However, a re-analysis of this data {Ruark, 2017, 3981395} 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, 5079732) failed to find evidence of associations between menstrual cycle length and PFAS concentrations.

Furthermore, Lorber et al. (2015, 2851157) 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, 2015, 2851191}. 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 2086 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 ng/g–444 ng/g) and PFNA (14.2 ng/g–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, 2851191) 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, 2851191)

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
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; PFNA = perfluorononanoic acid.
[a] Data is presented in % total perfluoroalkyl acids administered. Animals exposed to 0.05 mg/L (in Gao, 2015, 2851191)

A single case report study {Genuis, 2010, 2583643} 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 vs. other excreta matrices.

B.4.5 Half-life Data

B.4.5.1 Overview

We recognize that 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). Exposures 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 to other measures of lipophilicity. Also, phospholipid binding affinity could distinguish between high and low accumulating compounds as well as half-life measures {Sanchez Garcia, 2018, 4234856}.

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 to 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, 2012, 1289830; Loccisano, 2012, 1289833}.

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, 2000, 8568548; 3M, 2002, 6574114}. Both of these studies exhibited some deficiencies in sample collection and methods.

More recently, Olsen et al. (2007, 1429952) 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 µg/mL–1.740 µg/mL). Semi-log graphs of concentration vs. 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, 2018, 4238434}. 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 8000 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, 3859819) 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 to PFOA may also reflect its stronger affinity for serum albumin as reported previously {Salvalaglio, 2010, 2919252}. 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 to 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, 3859800) 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 3.5–4.1, and 3.0–3.6 years). The authors suggest these parameters have a significant impact on half-life estimates.

Xu et al. (2020, 6781357) estimated the half-life of PFAS by sampling urine (4 times) and blood (5 times) from 26 airport employees between 2 weeks to 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, 2020, 6781357; Worley, 2017, 3859800; Fu, 2016, 3859819; Zhang, 2013, 2639569} (Table B-23). PFOS half-life values among these 4 studies varied from

1.04 years in Xu et al. (2020, 6781357) to 60.9 years in Fu et al. (2016, 3859819). These comparisons support principles suggested by the broader literature. First, sex-related differences with males exhibiting somewhat longer half-lives compared to (especially females of reproductive age) may relate, at least in part, to menstruation as routes of elimination {Zhang, 2013, 3859849}. 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, 2020, 6781357; Worley, 2017, 3859800}. Altogether, there is insufficient data to correlate PFOS intake measurements to serum/plasma and urine concentrations. These factors, as well as age and health status of subjects, likely contribute to the 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. (2020, 6781357)	26 19 Males 7 Females	22– 62 years	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, 3859800)	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

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half Life (y)	Considerations
								230 ml/kg body weight. • Clearance rate was not reported
Fu et al. (2016, 3859819)	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. (2013, 3859849)	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

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half Life (y)	Considerations
								PFOS: mean 0.050 mg/kg/day (young females), 0.037 mg/kg/day (males and older females)

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

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, 2014, 2851045} to up to 60.9 years for males occupationally exposed in a plant in China {Fu, 2016, 3859819}. Second, with one exception {Genuis, 2014, 2851045}, 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, 2014, 2851045}{Zhang, 2013, 2639569}. Fourth, linear isomers exhibit longer half-lives than branched isomers {Zhang, 2013, 3859849}.

Gomis et al. (2017, 3981280) 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. (2012, 1578499) 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, 2010, 379025}. Other studies of subjects exposed to contaminated drinking water in Sweden {Li, 2017, 4238434} 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, 2008, 2919368}. 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 vs 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, 6574114)	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, 379025)	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, 3859819)	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, 2851045)	53 Father 47 Mother 22 1st Male Child 19 2nd Female child 17 3rd Male child 16 4th Male child 3	16–53	Father: 1.14 Mother: 1.93 First Male child: 0.65 2nd Female child: 1.03 3rd Male child: 0.78 4th 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-year period.
Glynn et al. (2012, 1578498)	413 women	19–41	8.2	Primiparous women 3 weeks after delivery in Uppsala County, Sweden 1996–2010 (the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas)
Gomis et al. (2017, 3981280)	Australia: A total of 24–84 pools per survey containing between 30–100 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. (2017, 4238434)	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, 1429952)	26 24 Males 2 Females	55–75	5.4	Retirees from the 3M plant in Decatur, Alabama where PFOS was produced.
Olsen et al. (2012, 1578499)	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-year 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, 2919368)	240	Newborn infant (1–2 days)	4.1	New York State newborn screening program blood spot specimens from newborn infants
Wong et al. (2014, 2851239)	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 data sets 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, 3859800)	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. (2020, 6315709)	26 19 Males 7 Females	22–62 years	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, 2850973)	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. (2013, 3859849)	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; GM = geometric mean; NHANES = National Health and Nutrition Examination Survey; PROS = perfluorooctane sulfonic acid; PFAA = Per- and polyfluoroalkyl acids.

B.4.5.3 Animal Studies

B.4.5.3.1 Non-Human Primates

In the study by Chang et al. (2012, 1289832), 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 vs. 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, 757853) administered 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS orally in a capsule by intragastric intubation to 6 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, 1289832) 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, 1289832) measured slightly higher half-lives in males (36–43 days) compared to 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, 2019, 5387170}{Kim, 2016, 3749289}. 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, 2019, 5387170}, 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, 1289832)	Cynomolgus Monkey	IV	NR	Male	2 mg/kg and followed for 161 days	132 ± 7
				Female	2 mg/kg and followed for 161 days	110 ± 15
		Oral	4–6 years	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, 757853)	Cynomolgus Monkey	Oral	Young-adult to adult	Male	0.15 mg/kg	~200
				Female	0.75 mg/kg	~200
Chang et al. (2012, 1289832)	Mice, CD-1	Oral	8–10 weeks	Male	1 mg/kg, followed for 20 weeks	42.81
					20 mg/kg, followed for 20 weeks	36.42
				Female	1 mg/kg, followed for 20 weeks	37.80
					20 mg/kg, followed for 20 weeks	30.45
Benskin et al. (2009, 1274133)	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, 1289832)	Rat, Sprague-Dawley	IV	8–10 weeks	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 weeks	Male	4.2 mg/kg, followed for 144 hr	8.23 ± 1.53
					2 mg/kg, followed for 10 weeks	38.31 ± 2.32

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex/Half-Life Approach	Dose	Estimated Half Life[a]
					15 mg/kg, followed for 10 weeks	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 weeks	62.30 ± 2.09
					15 mg/kg, followed for 10 weeks	71.13 ± 11.25
Huang et al. (2019, 5387170)	Rat, Sprague-Dawley	IV	8 weeks	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 weeks	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

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex/Half-Life Approach	Dose	Estimated Half Life[a]
				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. (2016, 3749289)	Rat, Sprague-Dawley	IV	8–12 weeks	Male	2 mg/kg	28.70 ± 1.85
				Female	2 mg/kg	24.80 ± 1.52
		Oral	8–12 weeks	Male	2 mg/kg	26.44 ± 2.77
				Female	2 mg/kg	23.50 ± 1.75

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

Appendix C. Non-priority Health Systems Evidence Synthesis and Integration

C.1 Reproductive

EPA identified 60 epidemiological and 22 animal studies that investigated the association between 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 multiple judgments 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 Main PFOS Document).

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, 2016, 3982042} and HESD {U.S. EPA, 2016, 3603365} 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, 2011, 1424973} 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 (2013, 2851244) 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, 2015, 2851189; Joensen, 2009, 1405085; Toft, 2012, 1332467} out of nine observed associations with morphologically abnormal sperm. In a cross-sectional sample of military recruits (n = 105), Joensen (2009, 1405085) observed significantly lower sperm counts in men with higher combined PFOS/PFOA exposure. A Texas- and Michigan-based cohort (n = 462), the Longitudinal Investigation of Fertility and the Environment (LIFE) study (Buck Louis, 2015, 2851189), 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[3] (24 publications) report on the association between PFOS and endocrine effects since the 2016 document. Eleven of the studies were in children and

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

adolescents {Di Nisio, 2019, 5080655; Ernst, 2019, 5080529; Goudarzi, 2017, 3981462; Itoh, 2016, 3981465; Jensen, 2020, 6311643; Lind, 2017, 3858512; Liu, 2020, 6569227; Lopez-Espinosa, 2016, 3859832; Wang, 2019, 5080598; Zhou, 2016, 3856472; Zhou, 2017, 3858488}, one study was in pregnant women {Anand-Ivell, 2018, 4728675} and the remainder of the publications were in the general population. Different study designs were utilized, including four cohort studies {Ernst, 2019, 5080529; Goudarzi, 2017, 3981462; Itoh, 2016, 3981465; Jensen, 2020, 6311643}, one case-control study {Anand-Ivell, 2018, 4728675} 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, 2020, 6833614; Di Nisio, 2019, 5080655; Pan, 2019, 6315783; Song, 2018, 4220306} and amniotic fluid in one study {Anand-Ivell, 2018, 4728675}. 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 {Kvist, 2012, 2919170; Leter, 2014, 2967406), the Odense Child Cohort (OCC) (Lind, 2017, 3858512; Jensen, 2020, 6311643}, the Genetic and Biomarkers study for Childhood Asthma (GBCA) {Zhou, 2016, 3856472; Zhou, 2017, 3858488}, and a cross-sectional sample of men from an infertility clinic in Nanjing, China {Pan, 2019, 6315783; Cui, 2020, 6833614}. Two studies assessed populations from related cohorts belonging to the Hokkaido Study on the Environment and Children's Health {Itoh, 2016, 3981465; Goudarzi, 2017, 3981462}.

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, 2016, 3603365} 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, 2018, 4220306} 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, 2016, 3856472; Zhou, 2017, 3858488} 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, 2019, 5080655} 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, 2015, 3749030} 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*.

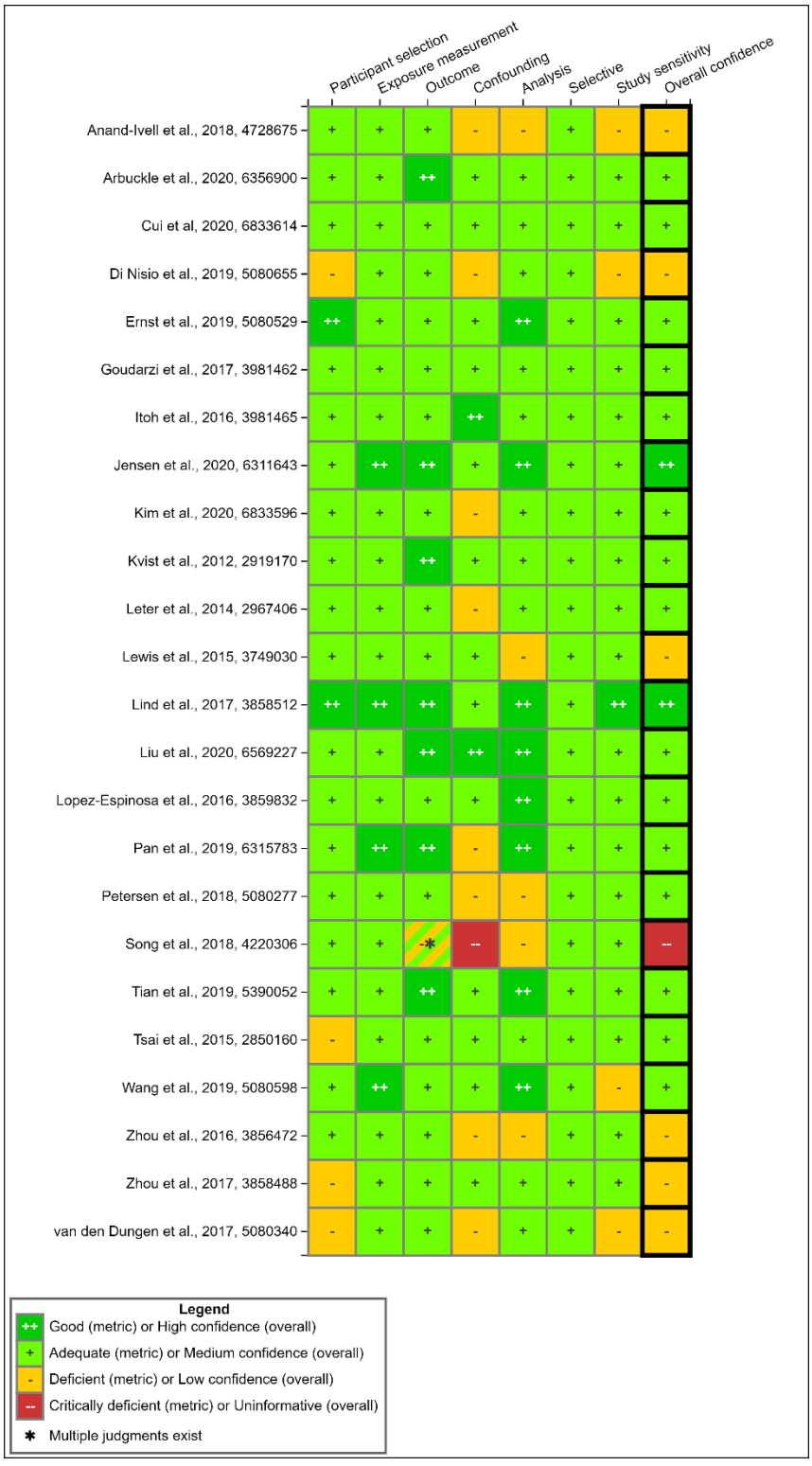


Figure C-1. Summary of Study Evaluation for Epidemiology Studies of PFOS and Male Reproductive Effects

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 {Di Nisio, 2019, 5080655; Goudarzi, 2017, 3981462; Itoh, 2016, 3981465; Jensen, 2020, 6311643; Liu, 2020, 6569227; Lopez-Espinosa, 2016, 3859832; Wang, 2019, 5080598; Zhou, 2016, 3856472; Zhou, 2017, 3858488} and three observed significant effects (Appendix D). A *high* confidence prospective study on the Odense cohort {Jensen, 2020, 6311643; Lind, 2017, 3858512} 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, 2017, 3981462} 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, 2016, 3981465} 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, 2016, 3859832) 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, 2019, 5080598} 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, 2016, 3856472; Zhou, 2017, 3858488} 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, 2017, 3858488} 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, 2019, 5080655} 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 to unexposed controls.

Pubertal development and semen parameters were examined in two studies {Di Nisio, 2019, 5080655; Ernst, 2019, 5080529} and effects were seen in one (Appendix D). One *medium* confidence study {Ernst, 2019, 5080529} 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, 2019, 5080655} 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, 2020, 6356900; Di Nisio, 2019, 5080655; Lind, 2017, 3858512; Tian, 2019, 5390052} and three observed effects (Appendix D). A *high* confidence Danish study {Lind,

2017, 3858512} 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, 2019, 5390052} 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, 2020, 6356900} 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, 5080655) reported smaller AGD in exposed compared to 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, 2020, 6833614; Lewis, 2015, 3749030; Petersen, 2018, 5080277; Tsai, 2015, 2850160} and two observed effects (Appendix D). A *medium* confidence study {Cui, 2020, 6833614} 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, 2018, 5080277} 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 {Kvist, 2012, 2919170; Leter, 2014, 2967406; Pan, 2019, 6315783; Petersen, 2018, 5080277; Song, 2018, 4220306} and three observed effects (Appendix D). One *medium* confidence study {Kvist, 2012, 2919170} 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, 2014, 2967406} 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, 2019, 6315783} on a sample of men from Nanjing, China, described above {Cui, 2020, 6833614}, 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, 2016, 3603365} concluded that there was suggestive evidence of an association with risk of gestational hypertension or preeclampsia {Darrow, 2013, 2850966; Zhang, 2015, 2857764; Stein, 2009, 1290816}. There was generally consistent evidence of associations between serum PFOS and reduced female fertility and fecundity {Bach, 2015, 3981738; Fei, 2009, 1291107; Jørgensen, 2014, 2851025; Vélez, 2015, 2851037}. 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, 2012, 1332476}.

There are 48 studies (50 publications) that have investigated relationships between PFOS exposure and female reproductive outcomes since the 2016 document {U.S. EPA, 2016, 3603365}. 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, 2016, 3603365} 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, 2018, 5079713; Zhang, 2018, 5079665}. Several *low* confidence studies lacked an appropriate strategy for identifying potential confounders {McCoy, 2017, 3858475; Zhou, 2017, 3859799} or failed to adjust for key confounders, such as age and SES {Heffernan, 2018, 5079713; Zhou, 2016, 3856472}. *Low* confidence studies had deficiencies in participant selection {Zhang, 2018, 5079665; Bach, 2018, 5080557; Heffernan, 2018, 5079713}, exposure measurement methods {Campbell, 2016, 3860110}, reliance on self-reporting for exposure, outcome, or covariate information {Campbell, 2016, 3860110}, and small sample size {Heffernan, 2018, 5079713; McCoy, 2017, 3858475}. 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.

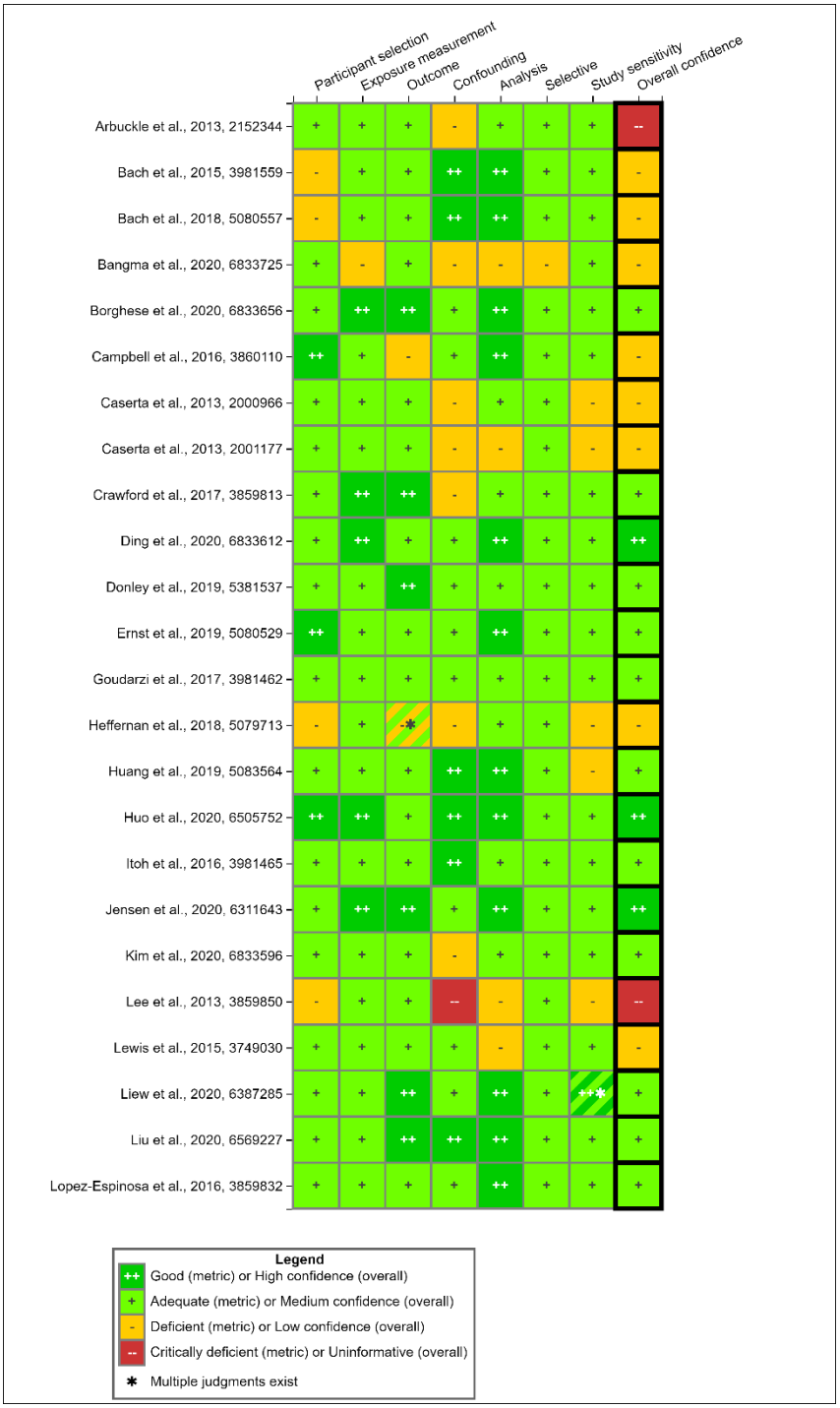


Figure C-2. Summary of Study Evaluation for Epidemiology Studies of PFOS and Female Reproductive Effects

Interactive figure and additional study details available on HAWC.

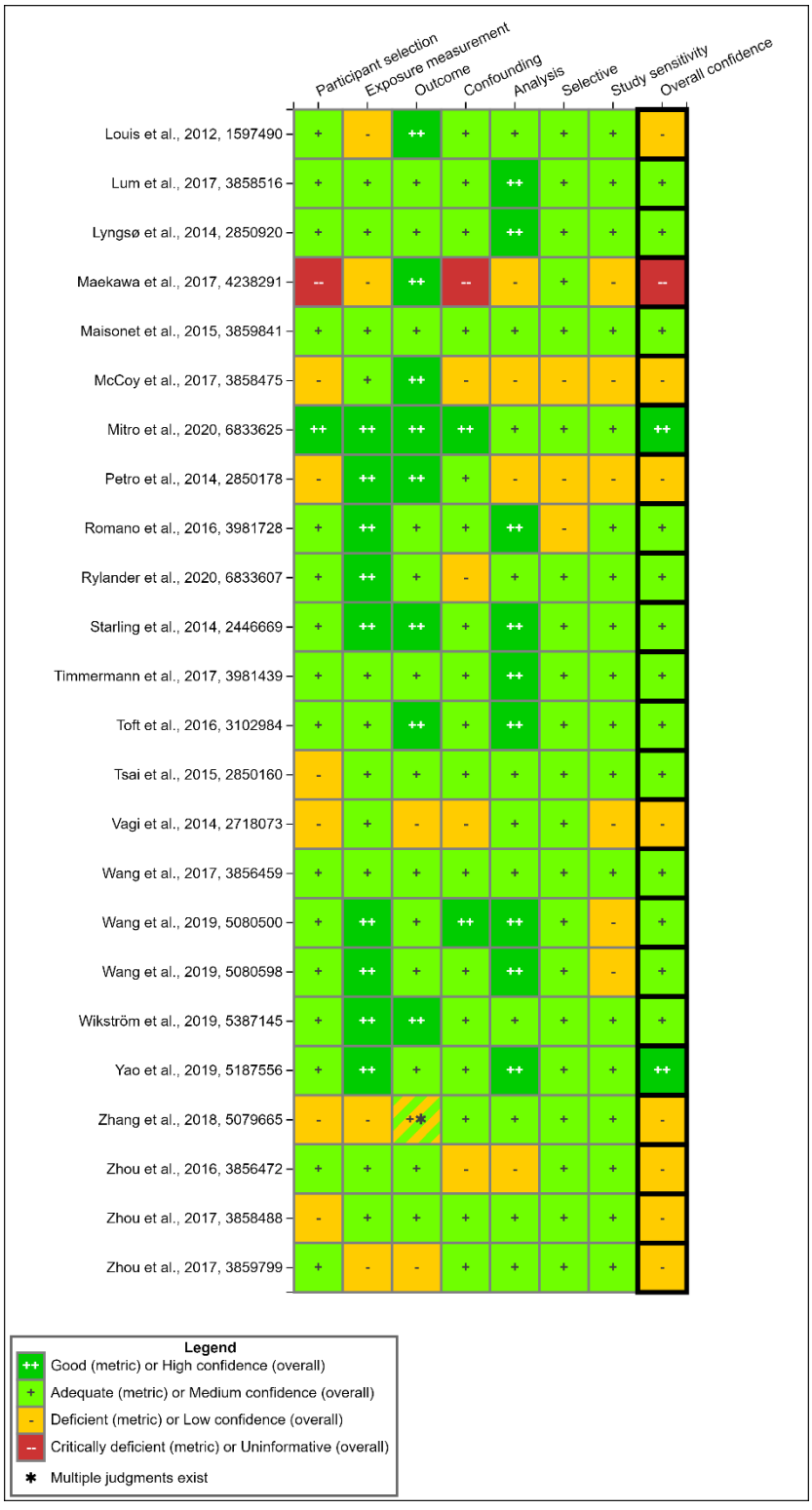


Figure C-3. Summary of Study Evaluation for Epidemiology Studies of PFOS and Female Reproductive Effects (Continued)

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 {Yao, 2019, 5187556; Jensen, 2020, 6311643} and four *medium* confidence studies {Itoh, 2016, 3981465; Liu, 2020, 6569227; Goudarzi, 2017, 3981462; Wang, 2019, 5080598} 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 (log_{10}-ng/mL) = −0.6; 95% CI: −0.9, −0.2) as well as prolactin in cord blood (regression coefficient per unit change in PFOS (log_{10}-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 (log_{10}-ng/mL) = 0.5; 95% CI: 0.3, 0.7) in another *medium* confidence study {Wang, 2019, 5080598}. 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, 2019, 5187556}; E2, testosterone, SHBG, the testosterone-to-SHBG ratio {Itoh, 2016, 3981465}; 17-OHP, androstenedione, FSH, LH, DHEA, dehydroepiandrosterone sulfate (DHEAS) {Jensen, 2020, 6311643}; 17-OHP, progesterone {Liu, 2020, 6569227}; androstenedione, DHEA {Goudarzi, 2017, 3981462}; β-E2, and estrone {Wang, 2019, 5080598}.

Three *medium* confidence {Lopez-Espinosa, 2016, 3859832; Maisonet, 2015, 3859841; Tsai, 2015, 2850160} and three *low* confidence {Lewis, 2015, 3749030; Zhou, 2016, 3856472; Zhou, 2017, 3858488} 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 {Lopez-Espinosa, 2016, 3859832; Zhou, 2016, 3856472; Zhou, 2017, 3858488}, testosterone {Lewis, 2015, 3749030; Zhou, 2016, 3856472}, SHBG (Maisonet, 2015, 3859841; Tsai, 2015, 2850160}, or FSH {Tsai, 2015, 2850160}.

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, 2019, 5080529}. 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 {Wikstrom, 2019, 5387145} 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, 2020, 6833607; Huang, 2019, 5083564; Rylander, 2020, 6833607; Huo, 2020, 6505752; Starling, 2014, 2446669}. 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 to 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, 2020, 6833656; Starling, 2014, 2446669}. Non-significant negative associations were observed in *medium* confidence case-control {Rylander, 2020, 6833607} and cross-sectional {Huang, 2019, 5083564} studies. A *low* confidence study found no association between median PFOS levels and hypertensive disorders of pregnancy {Bangma, 2020, 6833725}.

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 {Louis, 2016, 3858527; Liew, 2016, 6387285} and one *low* confidence study {Jensen, 2015, 2850253} 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, 2017, 3981439; Romano, 2016, 3981728}. 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, 2016, 3981728}. 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 to 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 to 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 to 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 to 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 three years postpartum.

One *medium* confidence study {Lyngsø, 2014, 2850920} 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 {Crawford, 2017, 3859813; Donley, 2019, 5381537; Kim, 2020, 6833596; Lum, 2017, 3858516; Wang, 2017, 3856459}, three *low* confidence studies { Bach, 2018, 5080557; McCoy, 2017, 3858475; Zhang, 2018, 5079665} and one *uninformative* study {Arbuckle, 2013, 2152344} 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, 2018, 5079665} and for the fecundity ratio for parous women in plasma PFOS quartiles {Bach, 2018, 5080557}. Non-significant positive associations were observed for day-specific probability of pregnancy (Lum 2017, 3858516) and cycle and day-specific time to pregnancy {Crawford, 2017, 3859813}. Associations with indicators of ovarian function were largely non-significant, including no association observed between serum PFOS and anti-Müllerian hormone (AMH) (Crawford, 2017, 3859813). Associations between maternal serum PFOS during pregnancy and female adolescent AMH levels were also not observed {Donley, 2019, 5381537}. No significant associations were reported for infertility measures including endometriosis-related infertility {Wang, 2017, 3856459}, and fertilization rate {Kim, 2020, 6833596}. 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, 2017, 3858516}, endometriosis, polycystic ovary syndrome (PCOS), genital tract infections, and idiopathic infertility {Kim, 2020, 6833596}.

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, 2020, 6833612}. Significant, positive associations were reported between serum Sm-PFOS and risk of natural menopause for women in Sm-PFOS tertile 3 vs. 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 vs. 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 vs. tertile 1.

One *medium* confidence {Tsai, 2015, 2850160} and five *low* confidence studies {Heffernan, 2018, 5079713; Lewis, 2015, 3749030; McCoy, 2017, 3858475; Petro, 2014, 2850178; Zhang, 2018, 5079665} 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, 2015, 3749030}; mean FSH and SHBG in young women (ages 12–30 years) {Tsai, 2015, 2850160};

testosterone, E2, and SHBG {Heffernan, 2018, 5079713}; E2 {Petro, 2014, 2850178}; or for LH and testosterone {Zhang, 2018, 5079665}.

C.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} 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.

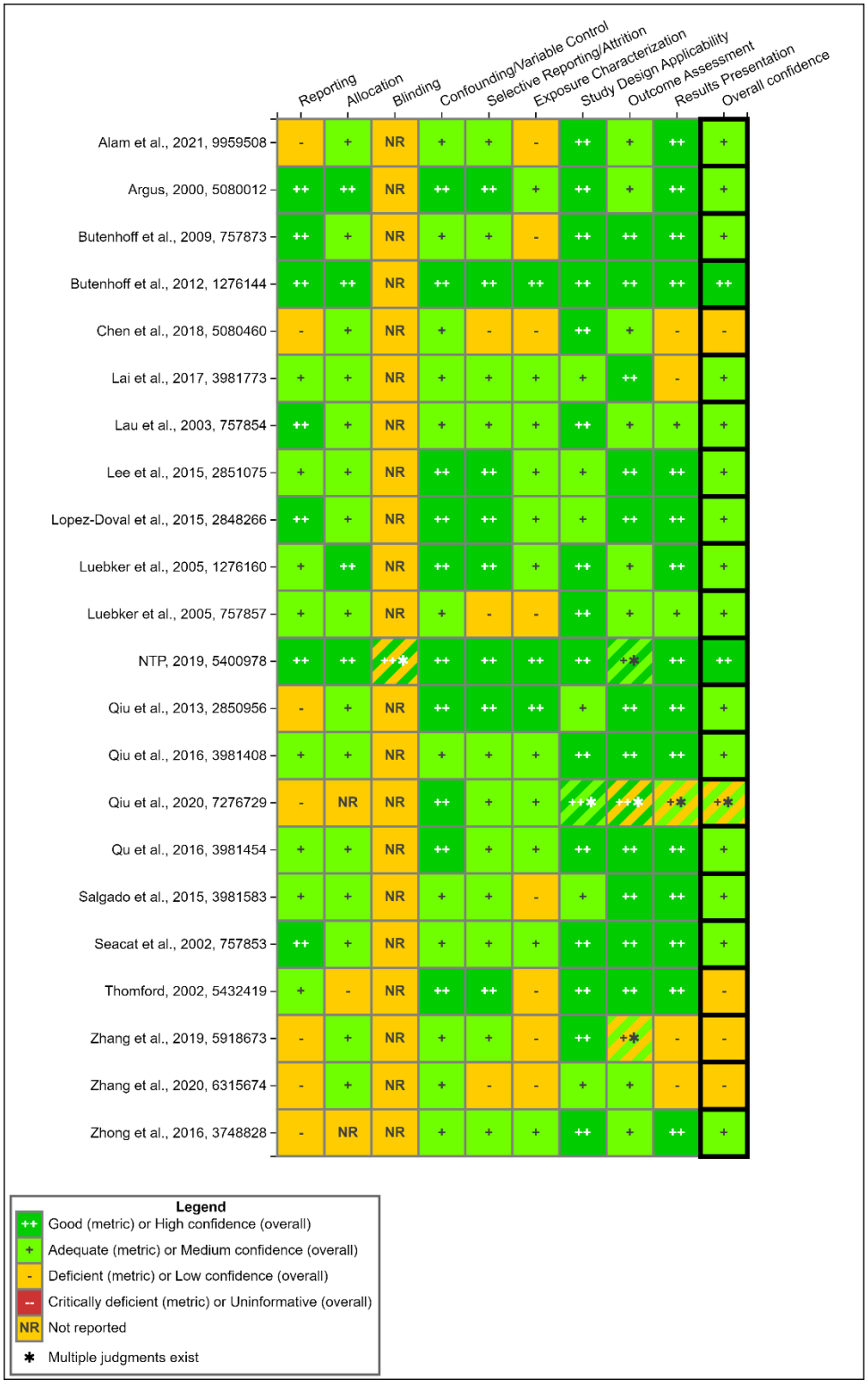


Figure C-4. Summary of Study Evaluation for Toxicology Studies of PFOS and Reproductive Effects

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, 2002, 757853}.

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{Luebker, 2005, 1276160; Butenhoff, 2009, 757873}. Gestation and fertility indices were unaffected in one- and two-generation rat reproduction studies {Luebker, 2005, 757857; Luebker, 2005, 1276160}; 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, 2005, 757857} and in P_0 dams exposed to 3.2 mg/kg/day in the two-generation study{Luebker, 2005, 1276160} (Figure C-5). Decreases in maternal bodyweight change were noted in both studies {Luebker, 2005, 757857; Luebker, 2005, 1276160}(see PFOS Main Document). In contrast, Butenhoff et al. (2009, 757873) 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, 2009, 757873}.

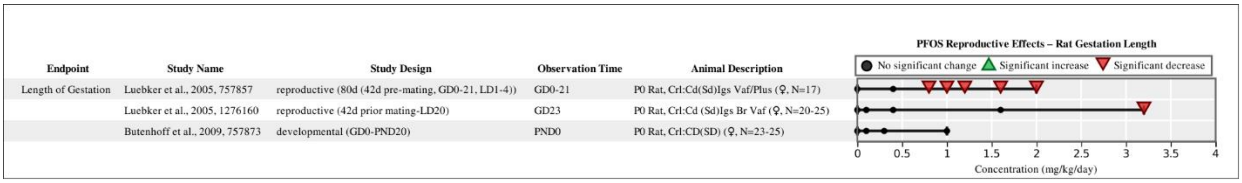


Figure C-5. Gestation Length in Rats Following Exposure to PFOS

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, 2006, 757859}. 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, 5080012}. 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 PFOS Main Document). 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 to 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, 5080012}.

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, 5400978}. 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, 2013, 2850956; Qiu, 2016, 3981408; Qu, 2016, 3981454; Lai, 2017, 3981773; Qiu, 2020, 7276729}. Qiu et al. (2016, 3981408) did not observe alterations in epididymis weight that may have influenced epididymal sperm counts.

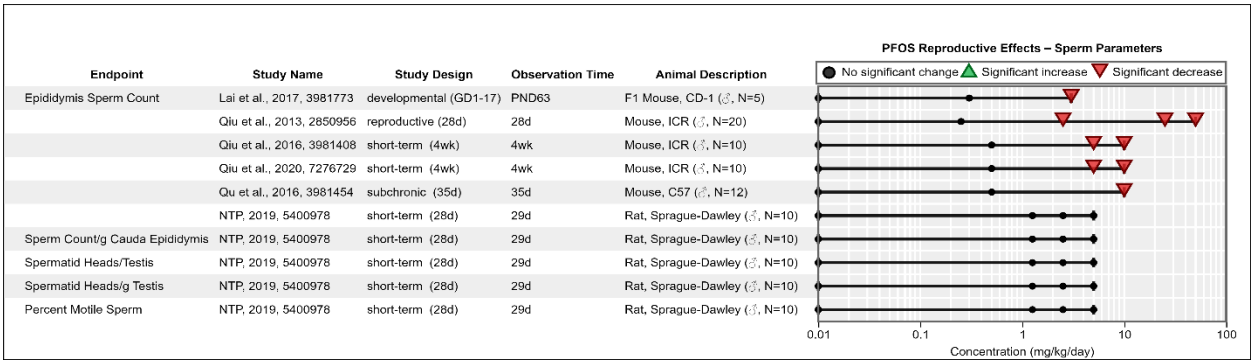


Figure C-6. Sperm Parameters in Male Rodents Following Exposure to PFOS

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, 2848266; 2014, 2850091) 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, 9959508) 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, 5400978), 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, 2016, 3981454}. 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, 2017, 3981773}. 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, 2016, 3748828}. 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, 7276729) 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, 2002, 757853}.

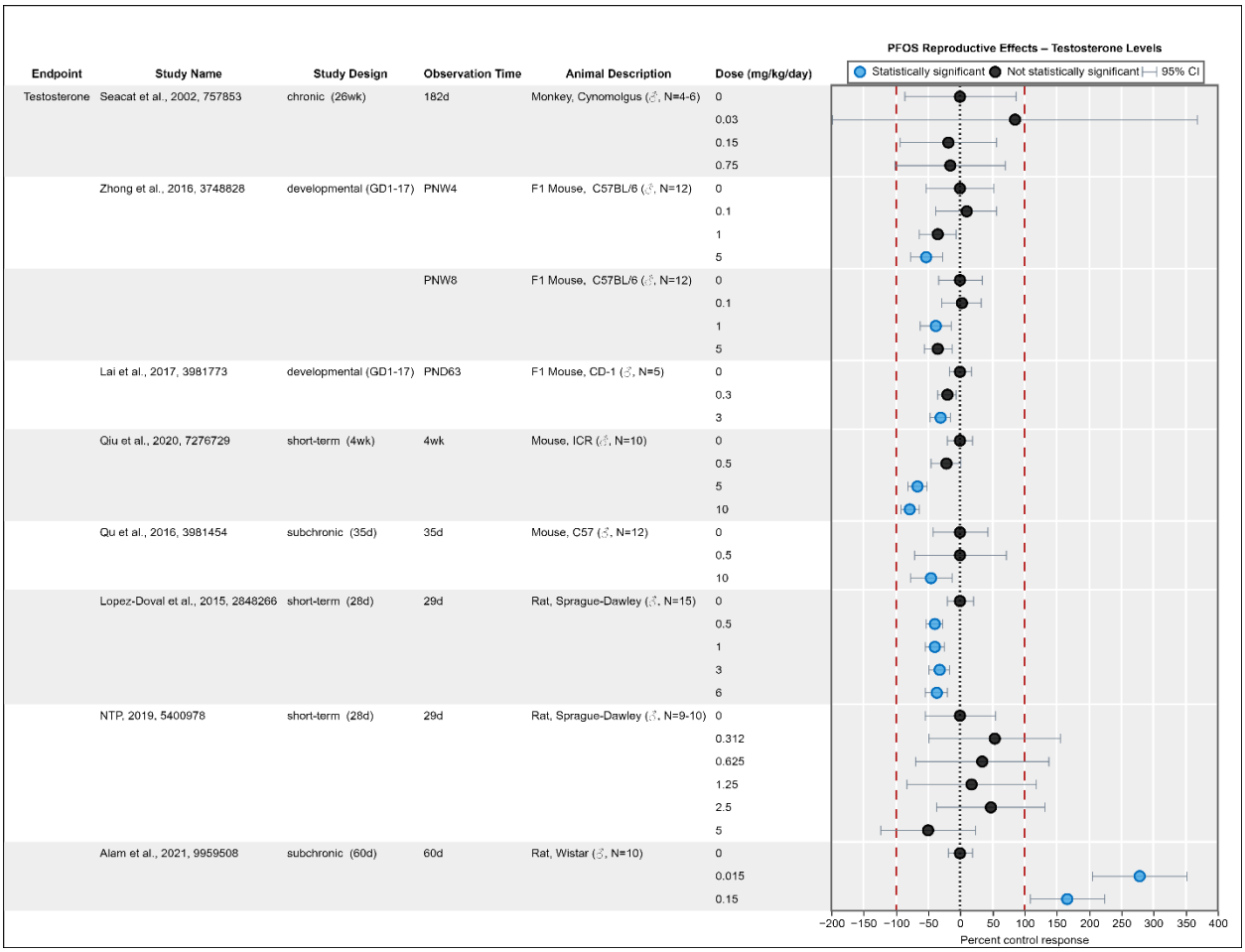


Figure C-7 Percent Change in Testosterone Levels Relative to Controls in Male Rodents and Non-Human Primates Following Exposure to PFOS

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 = post-natal 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, 3981583) and Lopez-Doval et al. (2015, 2848266) noted decreases in E2 ranging from 13–19% 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, 2016, 3981454}. 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, 2016, 3748828}. By PNW 8 the increase was no longer statistically significant but remained 28% higher than the control group {Zhong, 2016, 3748828}. 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 {Qui, 2020, 7276729}. Seacat et al. (2002, 757853) observed a 97% decrease in serum E2 in male cynomolgus monkeys treated at 0.75 mg/kg/day for 182 days {Seacat, 2002, 757853}.

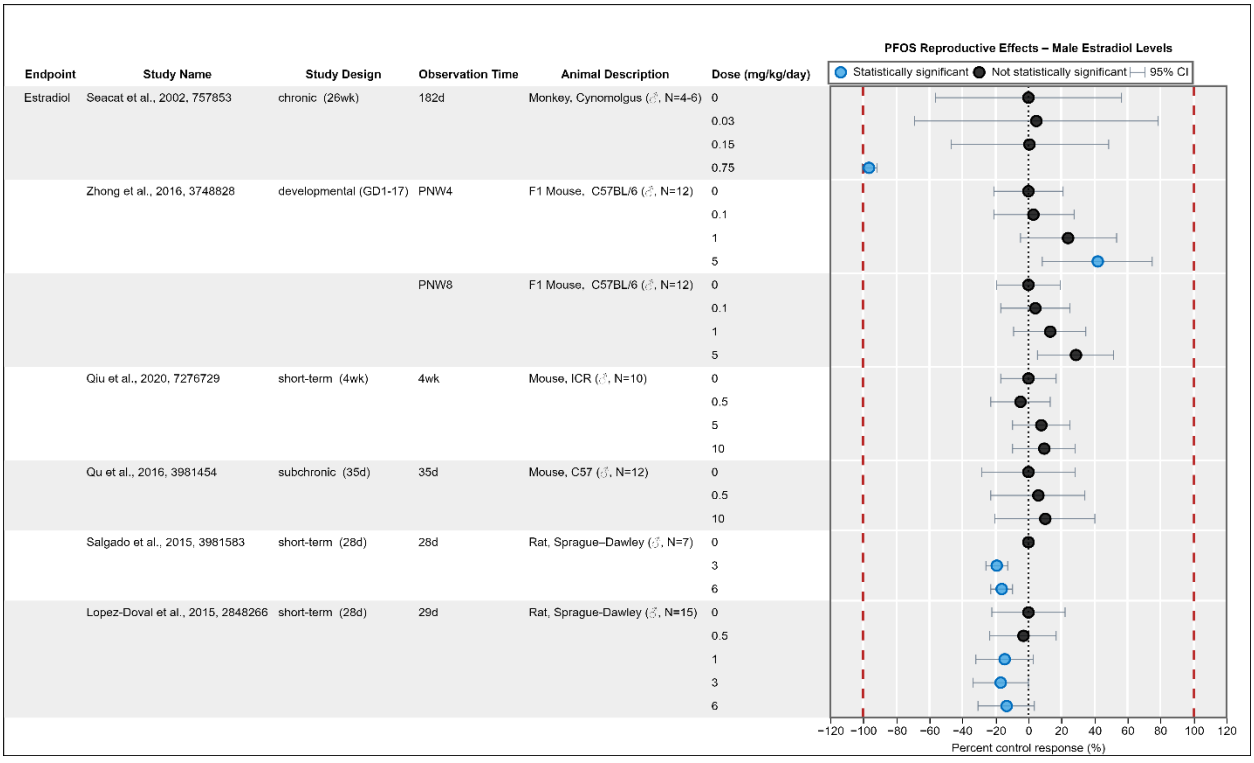


Figure C-8. Percent Change in Estradiol Levels Relative to Controls in Male Rodent and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; PNW = post-natal 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, 2014, 2850091; Lopez-Doval, 2015, 2848266; Salgado, 2015, 3981583}. Additionally, increases ranging from 88–133% in serum FSH levels were observed in all treated groups (0.5 mg/kg/day–6 mg/kg/day) when compared to controls {Lopez-Doval, 2014, 2850091}. However, in a study by Qiu et al. (2020, 7276729), PFOS exposure did not significantly alter serum FSH and LH levels.

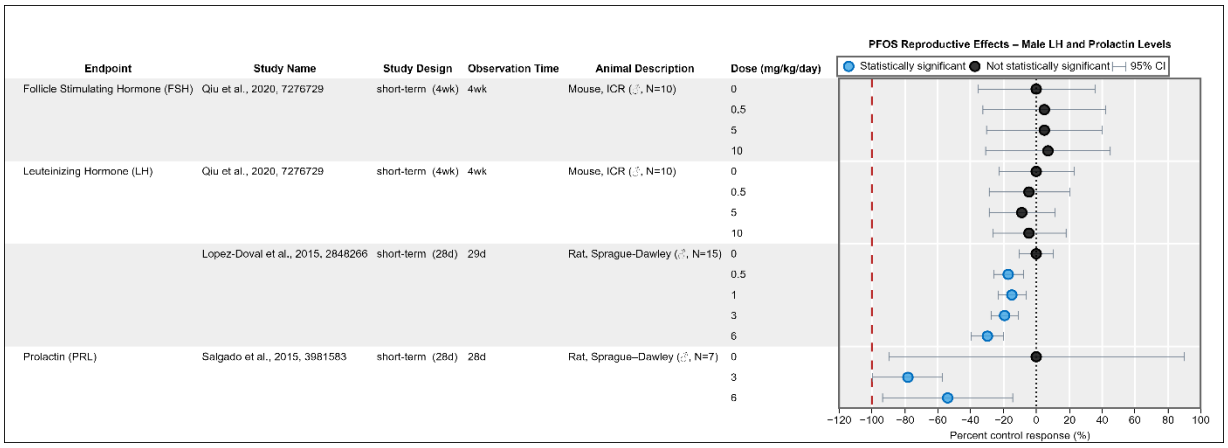


Figure C-9. Percent Change in LH and Prolactin Levels Relative to Controls in Male Rats Following Exposure to PFOS

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, 2851075) (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, 2015, 2851075}.

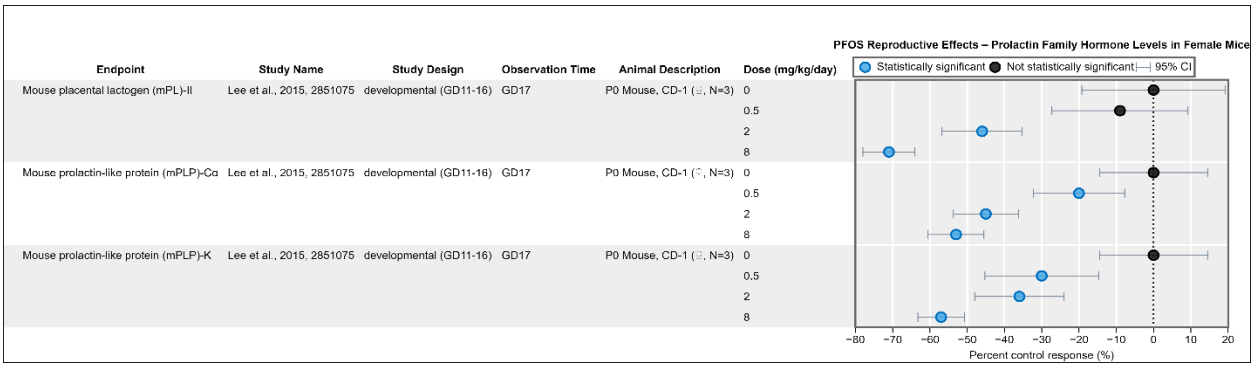


Figure C-10. Percent Change in Prolactin-Family Hormone Levels Relative to Controls in Female Mice Following Exposure to PFOS

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, 2002, 757853} (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, 2002, 757853}. 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, 2016, 3748828}. A dose-dependent decrease in testosterone levels when compared to 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, 2016, 3748828}. 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, 5400978}.

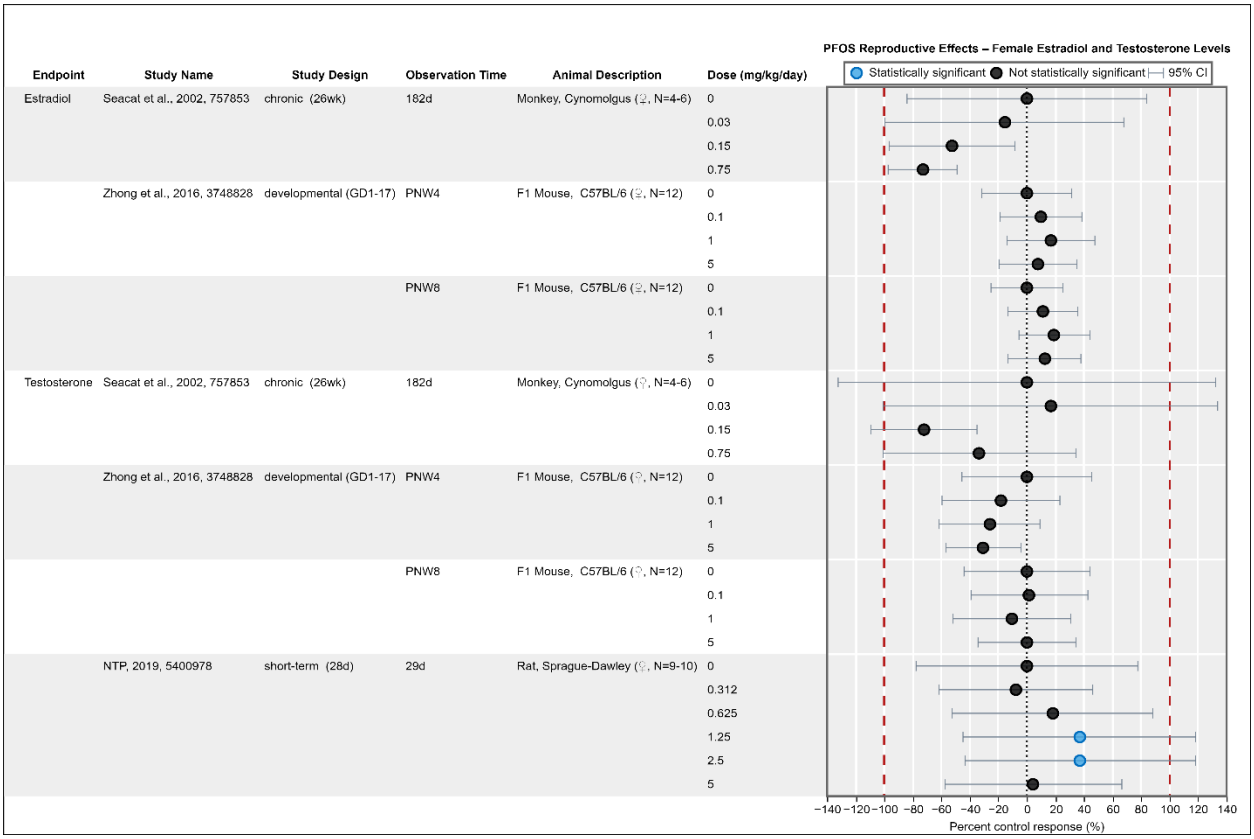


Figure C-11. Percent Change in Estradiol and Testosterone Levels Relative to Controls in Female Rodents and Non-Human Primates Following Exposure to PFOS

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 to controls); however, this finding was not statistically significant {NTP, 2019, 5400978}. 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 to controls. In the same study, the number of cycles was considered unaffected by treatment {NTP, 2019, 5400978}. 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, 2005, 1276160}.

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, 2005, 757857; Luebker, 2005, 1276160}. Likewise, no changes in the number of corpora lutea were seen in P_0 female rabbits exposed during gestation {Argus Research Laboratories, 2000, 5080012}. Reproductive and developmental studies additionally reported no impact of gestational PFOS exposure on the timing of preputial separation or vaginal opening in rats {Luebker, 2005, 1276160; Lau, 2003, 757854; Butenhoff, 2009, 757873}.

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, 5400978} 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, 2012, 1276144}. In a sub-chronic 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, 2021, 9959508}. 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, 2016, 3981454}. No effects were seen in relative epididymis or testis weights of mice treated up to 10 mg/kg/day for four weeks {Qiu, 2016, 3981408}, nor were any effects noted in the relative testes weight of mouse pups treated from GD 1 to GD 17 {Lai, 2017, 3981773}. 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, 2020, 7276729}. 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, 2002, 757853}.

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, 5400978; Butenhoff, 2012, 1276144}. However, Lopez-Doval et al. (2014, 2850091) noted edema around seminiferous tubules and malformed spermatids in male rats treated at $\geq$ 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, 2016, 3981454}.

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, 2013, 2850956}; 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, 2013, 2850956; Qiu, 2016, 3981408}. 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 to controls {Seacat, 2002, 757853}.

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, 5400978}. 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, 5400978}. A chronic study in rats {Butenhoff, 2012, 1276144} 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, 757853) 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 PFOS Main Document).

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, 2016, 3603365}. There are 56 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
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	4	1	1	5
Hormone Function	10	1	13	23
Oxidative Stress	2	0	5	7
Xenobiotic Metabolism	2	0	4	6
Other	2	0	1	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	20	3	38	56

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

Interactive figure and additional study details available on Tableau

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, 2019, 5080655; Zhou, 2016, 3856472; Zhou, 2017, 3858488} 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, 2019, 5080655}. Evidence was also inconsistent for AGD in infants. In adults, there was evidence in one study {Cui, 2020, 6833614} 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, 2013, 2850956; Qiu, 2016, 3981408; Qu, 2016, 3981454; Lopez-Doval, 2014, 2850091; Lopez-Doval, 2015, 2848266}. 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%–70% at the highest doses tested {Qiu, 2013, 2850956; Qiu, 2016, 3981408; Qu, 2016, 3981454; Lai, 2017, 3981773} and are consistent with effects seen in humans, fertility may be normal in male rodents even with sperm reductions as great as 90% {Gray, 1988,1332904}. 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 observed in a *medium* confidence study. In adults, one study (1/3)	• *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 adversity of the observed effects, a lack of

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 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.				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 two studies (2/3), non-significant positive associations were observed for sperm	• *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**	
	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 school students. Adverse effects were observed in adolescents with higher exposure levels, including smaller testicular volume, shorter penis length, and smaller penis circumference.	• *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**	
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 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	

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**	
				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 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	

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**	
				on testosterone levels, make it difficult to assess the relevance of these changes.	
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 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).	• *High and medium* confidence studies	• *Inconsistent direction* of effects across species • Changes in body weight may limit ability to interpret these responses		
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	• *High and medium* confidence studies • *Consistent direction* of effects within species •	• *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**	
	epididymal sperm motility were reported (1/1).				
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 •		
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		
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 weeks of exposure (3/6). These changes included vacuolations in	• *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**	
	spermatogonia, spermatocytes, Leydig cells, and Sertoli cells, and disturbed germ cell layers; however, results 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.

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 {Crawford, 2017, 3859813; McCoy, 2017, 3858475; Zhang, 2018, 5079665}, others found PFOS to be positively associated with female fertility indicators {Lum, 2017, 3858516; Kim, 2020, 6833596; Bach, 2018, 5080557}, and some did not observe any clear trends {Wang, 2017, 3856459}. Kim et al. (2020, 6833596) 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. (2017, 3981439) observed negative associations between PFOS exposure and exclusive and total breastfeeding duration, while Romano et al. (2016, 3981728) 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, 1290803) 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, 5080529) 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, 1402395) 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, 6833612) 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, 3981465) observed significant negative associations for maternal serum PFOS and cord blood prolactin and progesterone levels and Wang et al. (2019, 5080598) observed significant positive associations for cord blood PFOS and cord blood estrone and E3. In pregnant women, Yao et al. (2019, 5187556) observed significant, positive associations for cord blood PFOS and

testosterone and testosterone to E2 ratio and Toft et al. (2016, 3102984) 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, 5400978) 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, 2005, 1276160}. 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 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 associations with E2 (2/4), one *medium* study	• *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 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, and inconsistencies in 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**	
	observed increased progesterone, testosterone, and 17-OHP (1/4) and one observed an inverse association with free androgen index (1/4).				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*		
Female reproductive milestones	Three studies examined reproductive milestones	• *High* and *medium* confidence studies	• *Low* confidence study		

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**	
1 *High* confidence study 2 *Medium* confidence studies 1 *Low* confidence study	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.	• *Consistent direction* of effects	• 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 anti-Mullerian hormone levels or endometriosis. Twelve studies evaluated fertility indicators in non-pregnant women with mixed results. One *medium* confidence study reported significant inverse associations with endometriosis-driven	• *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**	
	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 anogenital distance, 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 anofourchette distances were inverse but not	• *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**	
	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 in testosterone concentrations in females, but the response in the highest dose was affected	
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 female monkeys exposed for 26 weeks or in female mice exposed *in utero* from GD1-17. One mouse	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Limited number* of studies examining specific outcomes • 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**	
	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).			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 to 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		
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		

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**	
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; 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 multiple judgments 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 Main PFOS Document).

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, 2016, 3982043} and HESD {U.S. EPA, 2016, 3603365} 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 {Melzer, 2010, 1290811;Wen, 2013, 2850943} reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease. One study {Melzer, 2010, 1290811} reported associations with thyroid disease in men, and another study {Wen, 2013, 2850943} 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, 2015, 2851002;Wang, 2013, 4241230;Webster, 2014, 2850208}. Increasing PFOS was associated with increased T4 in children aged 1 to 17 years from the C8 cohort {Lopez-Espinosa, 2012, 1291122}; 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, 2011, 1424975} 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, 2015, 2851002;Wang, 2013, 4241230}. Pregnant women testing positive for the anti-thyroid peroxidase (TPO) biomarker for autoimmune thyroid disease showed a positive association with PFOS and TSH {Webster, 2014, 2850208}. A case-control study of hypothyroxinemia (normal TSH and low free T4) in pregnant women {Chan, 2011, 1402500}, did not show associations of hypothyroxinemia with PFOS exposure.

For this updated review, 34 studies (35 publications)[4] report on the association between PFOS exposure and endocrine effects. Seven of the publications were studies in pregnant women {Aimuzi, 2020, 6512125;Berg, 2017, 3350759;Dreyer, 2020, 6833676;Inoue, 2019, 5918599;Itoh, 2019, 5915990;Reardon, 2019, 5412435;Shah-Kulkarni, 2016, 3859821}, and the remainder of the publications were on the general population. Different study designs were utilized, including seven cohort studies {Berg, 2017, 3350759, Blake`, 2018`, 5080657;Crawford, 2017, 3859813;Kim, 2020, 6833758;Lebeaux, 2020, 6356361;Liu, 2018, 4238396;Reardon, 2019, 5412435}, seven cohort and cross-sectional studies {Dreyer, 2020, 6833676;Itoh, 2019, 5915990;Kato, 2016, 3981723;Preston, 2018, 4241056;Wang, 2014, 2850394;Xiao, 2019, 5918609}, one case-control study {Predieri, 2015, 3889874}, one case-control and cross-sectional study {Zhang, 2018, 5079665}, and 19 cross-sectional studies {Abraham, 2020, 6506041;Aimuzi, 2019, 5387078;Aimuzi, 2020, 6512125;Audet-Delage, 2013, 2149477;Byrne, 2018, 5079678;Caron-Beaudoin, 2019, 5097914;Dufour, 2018, 4354164;Heffernan, 2018, 5079713;Inoue, 2019, 5918599;Jain, 2013, 2168068;Jain, 2019, 6315816;Kang, 2018, 4937567;Khalil, 2018, 4238547;Lewis, 2015, 3749030;Li, 2017, 3856460;Seo, 2018, 4238334;Shah-Kulkarni, 2016, 3859821;Tsai, 2017, 3860107;van den Dungen, 2017, 5080340;Yang, 2016, 3858535}. All observational studies measured PFOS in blood components (i.e., blood, plasma, or serum). Six studies measured PFOS in cord blood {Aimuzi, 2019, 5387078;Dufour, 2018, 4354164;Liu, 2020, 6569227;Shah-Kulkarni, 2016, 3859821;Tsai, 2017, 3860107;Yang, 2016, 3858535} and eight studies measured PFOS in maternal blood or serum during pregnancy {Dreyer, 2020, 6833676;Kato, 2016, 3981723;Lebeaux, 2020, 6356361;Preston, 2018, 4241056;Reardon, 2019, 5412435;Wang, 2014, 2850394;Xiao, 2019, 5918609;Yang, 2016, 3858535}. The studies were conducted in different study populations including populations from Belgium {Dufour, 2018, 4354164}, Canada {Caron-Beaudoin, 2019, 5097914;Reardon, 2019, 5412435}, China {Aimuzi, 2019, 5387078;Aimuzi, 2020, 6512125;Li, 2017, 3856460, Liu`, 2020`, 6569227;Yang, 2016, 3858535;Zhang, 2018, 5079665}, Denmark {Dreyer, 2020, 6833676;Inoue, 2019, 5918599;Xiao, 2019, 5918609}, Germany {Abraham, 2020, 6506041}, Italy {Predieri, 2015, 3889874}, Japan {Itoh, 2019, 5915990;Kato, 2016, 3981723}, Republic of Korea {Kang, 2018, 4937567;Kim, 2020, 6833758;Shah-Kulkarni, 2016, 3859821}, Taiwan {Tsai, 2017, 3860107;Wang, 2014, 2850394}, the United Kingdom {Heffernan, 2018, 5079713}, and the United States {Blake, 2018, 5080657;Byrne, 2018, 5079678;Crawford, 2017, 3859813;Jain, 2013, 2168068;Jain, 2019, 6315816;Khalil, 2018, 4238547;Lebeaux, 2020, 6356361;Lewis, 2015, 3749030;Liu, 2018, 4238396;Preston, 2018, 4241056}. Two studies {Itoh, 2019, 5915990;Kato, 2016, 3981723} 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

[4] Itoh et al. (2019, 5915990) reports thyroid-related hormone levels in a population overlapping with Kato et al. (2016, 3981723).

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 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, 2018, 4238434}, 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, 2016, 3603365} 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, 2020, 6506041;Predieri, 2015, 3889874} as *uninformative*.

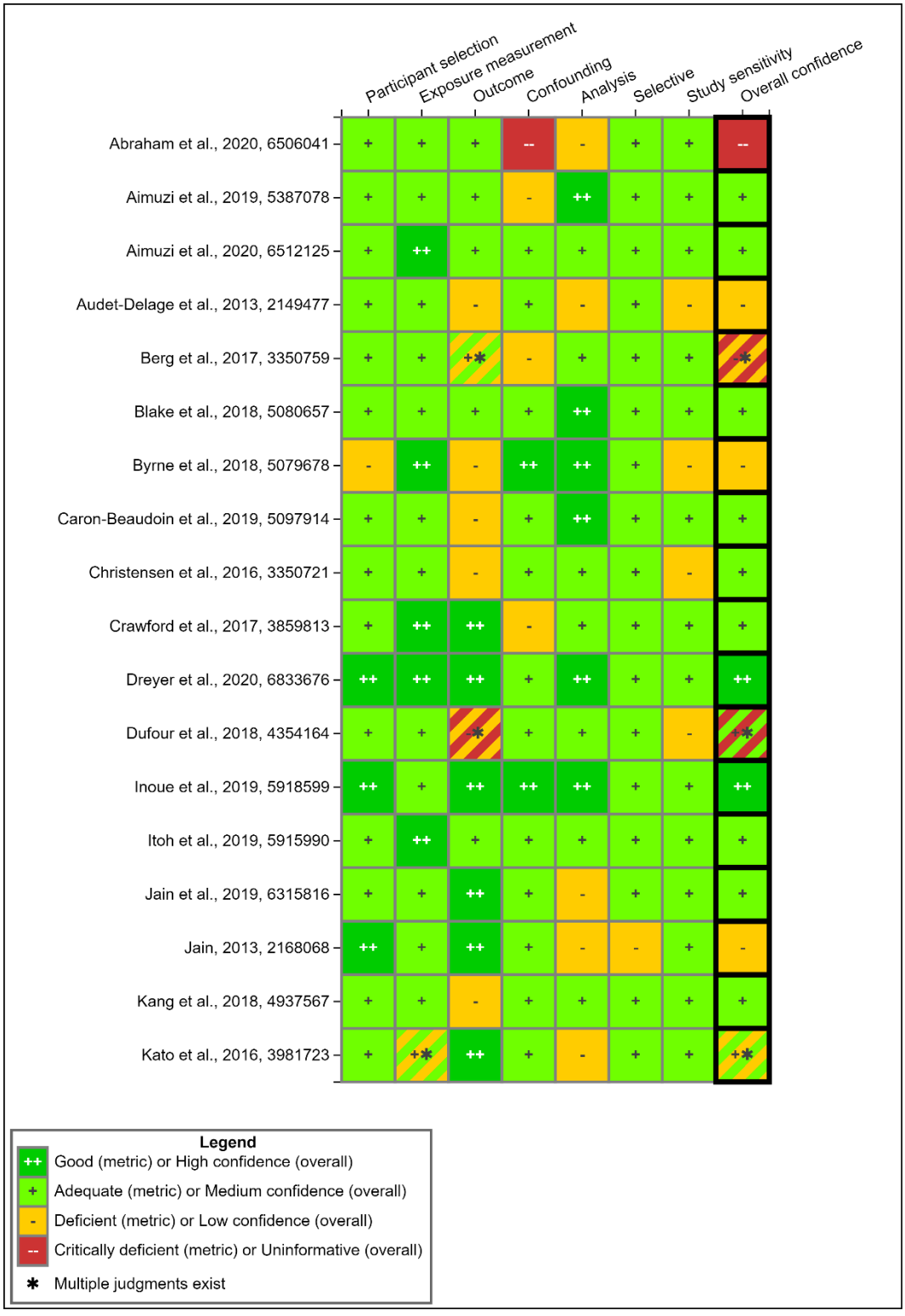


Figure C-13. Summary of Study Evaluation for Epidemiology Studies of PFOS and Endocrine Effects

Interactive figure and additional study details available on HAWC.

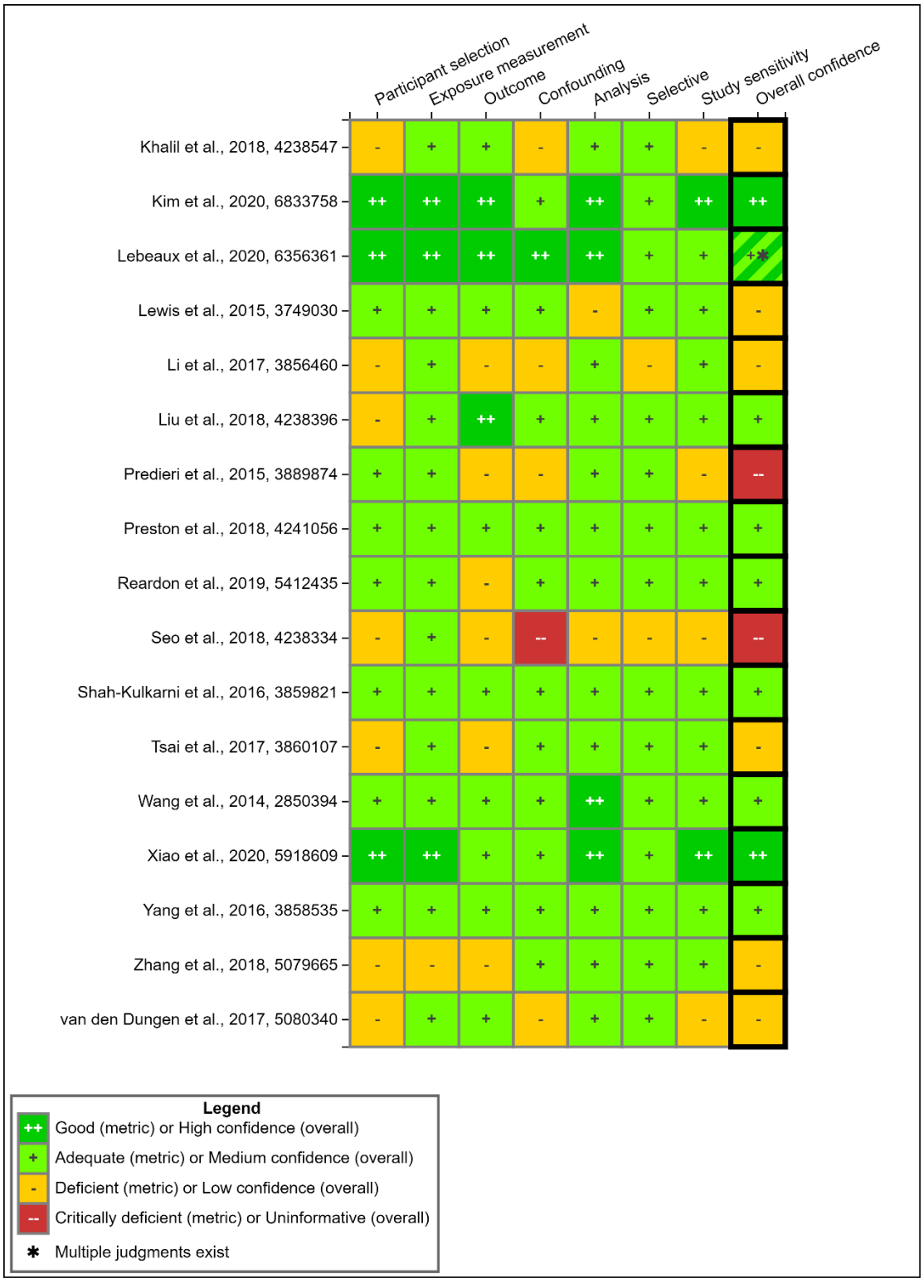


Figure C-14. Summary of Study Evaluation for Epidemiology Studies of PFOS and Endocrine Effects (Continued)

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, 2015, 3749030}, or use of statistical methods that did not account for confounding {Abraham, 2020, 6506041}. Case-control studies {Kim, 2016, 3351917; Predieri, 2015, 3889874} 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, 2020, 6833758} 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, 2020, 6506041;Aimuzi, 2019, 5387078;Caron-Beaudoin, 2019, 5097914;Dufour, 2018, 4354164;Itoh, 2019, 5915990;Kang, 2018, 4937567;Kato, 2016, 3981723;Khalil, 2018, 4238547;Kim, 2016, 3351917;Kim, 2020, 6833758;Lebeaux, 2020, 6356361;Predieri, 2015, 3889874;Preston, 2018, 4241056;Shah-Kulkarni, 2016, 3859821;Tsai, 2017, 3860107;Wang, 2014, 2850394;Xiao, 2019, 5918609;Yang, 2016, 3858535} and five observed significant effects (Appendix D). One *high* confidence study {Xiao, 2019, 5918609} 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, 2016, 3981723} 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, 2019, 5915990} 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, 2019, 5387078} 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, 2018, 4241056} 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, 2017, 3860107} 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, 2020, 6512125;Berg, 2017, 3350759;Inoue, 2019, 5918599;Itoh, 2019, 5915990;Reardon, 2019, 5412435;Shah-Kulkarni, 2016, 3859821} and five observed significant effects (Appendix D). One *high* confidence study {Xiao, 2019, 5918609} 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, 2019, 5412435} 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 post-partum. 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, 2018, 4241056} observed a significant inverse association for maternal TSH among TPOAb-positive mothers. One *low* confidence analysis {Kato, 2016, 3981723} 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, 2019, 5915990}. Another *low* confidence study {Berg, 2017, 3350759} 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, 2018, 4354164} 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 two-fold increases in serum PFOS concentrations {Dreyer, 2020, 6833676}. 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 {Audt-Delage, 2013, 2149477; Blake, 2018, 5080657; Byrne, 2018, 5079678; Christensen, 2016, 3350721; Crawford, 2017, 3859813; Jain, 2013, 2168068; Jain and Ducatman, 2019, 6315816; Lebeaux, 2020, 6356361; Lewis, 2015, 3749030; Li, 2017, 3856460; Liu, 2018, 4238396; Seo, 2018, 4238334; van den Dungen, 2017, 5080340; Zhang, 2018, 5079665} and six observed significant effects (Appendix D). A *medium* confidence study {Blake, 2018, 5080657} 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, 2013, 2168068;Jain, 2019, 6315816;Lewis, 2015, 3749030}. One *low* confidence study {Lewis, 2015, 3749030} 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, 2168068), another *low* confidence study, did not find any significant effects among NHANES (2007–2008) participants. A *medium* confidence follow-up study {Jain, 2019, 6315816} examined effects on thyroid hormones stratified by 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, 2018, 5079678} 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, 2017, 3856460} 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, 2018, 5079665} 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, 2016, 3603365} 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.

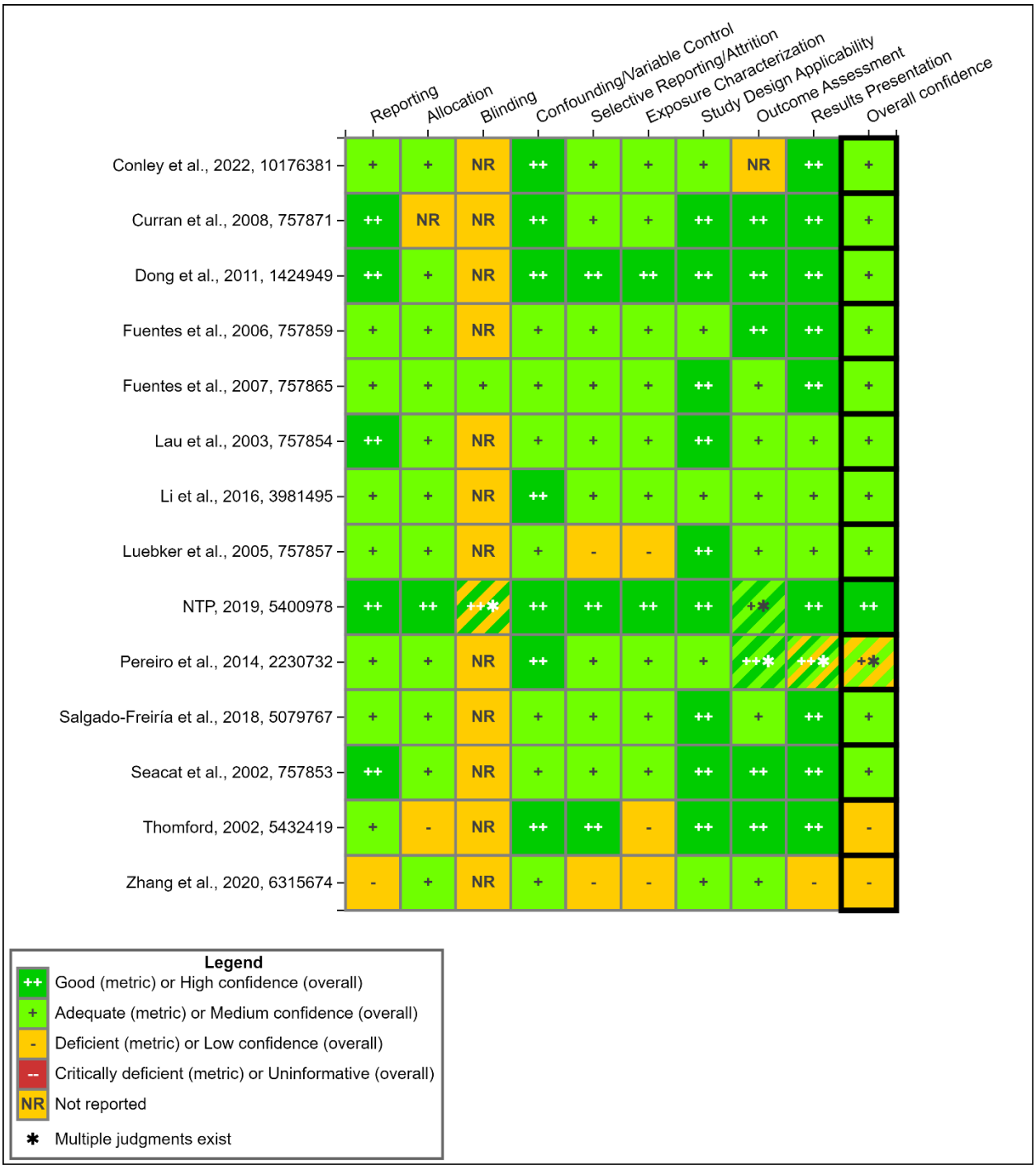


Figure C-15. Summary of Study Evaluation for Toxicology Studies of PFOS and Endocrine Effects

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, 2002, 757853} 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 PFOS Main Document). Moreover, reductions were observed in thyroid hormone levels, including total and free thyroxine (TT4 and FT4) and total and free triiodothyronine (TT3 and FT3) {Luebker, 2005, 757857; NTP, 2019, 5400978; Lau, 2003, 757854}, as well as reductions in adrenocorticotropic hormone (ACTH), corticosterone, and/or corticotropin releasing hormone (CRH) {Pereiro, 2014, 2230732; Salgado-Freiría, 2018, 5079767}. Absolute and relative adrenal gland weights were reduced in rats {NTP, 2019, 5400978}, however adrenal glands subject to histopathologic examination appeared normal {Chang, 2009, 757876; Luebker, 2005, 757857; Pereiro, 2014, 2230732} (see PFOS Main Document).

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, 2008, 757871}. 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, 2008, 757871}. 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, 5400978}. 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, 5400978}. Yu et al. (2009, 757872) 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, 2009, 757872}.

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, 757854) 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 to controls {Lau, 2003, 757854}. In a cross-fostering study conducted by Yu et al. (2009, 757880), 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, 2009, 757880}. 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, 2009, 757876}.

Luebker et al. (2005, 757857) 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–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 vs. postnatal effects of PFOS on thyroid hormones were not clear {Luebker, 2005, 757857}. 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, 2005, 757857}. Conley et al. (2022, 10176381) 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%–34% in TT3 and 3%–24% in TT4 were observed in dams exposed to doses below 10 mg/kg/day. Fuentes et al. (2006, 757859) 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 11–57% in TT3, 36%–57% in FT3, and 42%–67% in FT4. Conversely, increases in TT4 levels ranged from 158%–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, 757854) 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. Due to 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 to control animals {Lau, 2003, 757854}. 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, 2002, 757853}. 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, 2002, 757853}.

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, 757853)[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, 757859)[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. (2022, 10176381)[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, 757854)[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, 757871)[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
					3.73	1.17 ± 0.15*	−60.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					7.58	1.27 ± 0.36*	−56.5
NTP (2019, 5400978)[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. (2009, 757872)[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, 757854)[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. (2005, 757857)[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
					1.6	0.01 ± 0.0	−98.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					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. (2009, 757880)[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 & 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 & 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 & Lactation)	4.23 ± 0.22*	−37.3
Free Thyroxine (FT4)							
NTP (2019, 5400978)[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
					2.5	0.00036 ± 0.00005*	−85.8

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL)[a]	Percent Change
					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. (2009, 757872)[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, 757859)[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, 757854)[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. (2005, 757857)[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, 757859)[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. (2005, 757857)[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, 757859)[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. (2022, 10176381)[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, 757853)[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, 5400978)[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. (2009, 757872)[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, 757854)[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. (2005, 757857)[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. (2009, 757880)[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 & 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 & 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 & 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. (2009, 757880)[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 & 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 & 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 & Lactation)	0.02 ± 0.001	0.0
Thyroid Stimulating Hormone (TSH)							
Seacat et al. (2002, 757853)[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, 5400978)[c]	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. (2009, 757872)[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, 757876)[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, 757854)[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. (2005, 757857)[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: F = female; F_1 = first generation; GD = gestation day; LD = lactation day; M = male; NA = not applicable; P_0 = parental generation; PND = postnatal day.
*Statistically significant at $p \leq 0.05$.
[a] Values were converted to µg/dL for Seacat et al. (2002, 757853) (ng/dL TT3, FT3, FT4; uU/mL TSH); Curran et al. (2008, 757871) (nmol/L T4; nmol/L TT3); NTP (2019, 5400978) (ng/dL FT4, ng/dL TT3; ng/mL TSH); Yu et al. (2009, 757872) (µg/L TT4; µg/L FT4; µg/L TT3; µg/L TSH); Lau et al. (2003, 757854) (ng/mL TT4; pg/mL FT4; ng/mL TT3; ng/mL TSH); Luebker et al. (2005, 757857) (ng/dL FT4; pg/mL FT3; ng/dL TT3; ng/mL TSH); Yu et al. (2009, 757880) (ng/mL TT4; ng/mL TT3; ng/mL rT3); Chang et al. (2009, 757876) (ng/mL TSH); Conley et al. (2022, 10176381) (ng/mL TT3, TT4); Fuentes et al. (2006, 757859) (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, 5079767) and Pereiro et al. (2014, 2230732) 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, 2016, 3981495}. 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 {Fuentes, 2006, 757859; Fuentes, 2007, 757865; Dong, 2011, 1424949}. Fuentes et al. (2006, 757859) 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, 2007, 757865}. 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, 2011, 1424949}. 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, 2230732) reported on the same data as Salgado-Freiría et al. (2018, 5079767) 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, 5400978}. In a longer-term study conducted by Yu et al. (2009, 757872), 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, 2009, 757872}. 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, 2008, 757871}.

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, 5400978}. 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, 5400978}. Additionally, relative adrenal gland weight was decreased in male rats treated at doses of ≥ 0.5 mg/kg/day for 28 days {Pereiro, 2014, 2230732}. Curran et al. (2008, 757871) observed significant trends towards 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, 757853) 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, 2002, 757853}. 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, 3981583) (see PFOS Main Document).

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, 5400978)	Sprague-Dawley rat	28 days	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, 5400978)	Sprague-Dawley rat	28 days	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
Adrenal Weight Absolute	Curran et al. (2008, 757871)	Sprague Dawley rat	28 days	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, 757871)	Sprague Dawley rat	28 days	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, 757871)	Sprague Dawley rat	28 days	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, 2230732)	Sprague-Dawley rat	28 days	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, 757853)	Cynomolgus monkeys	182 days	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, 757853)	Cynomolgus monkeys	182 days	0, 0.03, 0.15, 0.75 mg/kg/day	M	n.s.
					F	n.s.
Thyroid Weight, Absolute	NTP (2019, 5400978)	Sprague-Dawley rat	28 days	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.
	Curran et al. (2008, 757871)	Sprague Dawley rat	28 days	0, 2, 20, 50, 100 mg/kg/day	M	n.s
	Yu et al. (2009, 757872)	Sprague-Dawley rat	91 days	0, 1.7, 5.0, or 15 mg/L	M	n.s.
Thyroid Weight, Relative to Body Weight	NTP (2019, 5400978)	Sprague-Dawley rat	28 days	0, 0.312, 0.625, 1.25, 2.5, 5 mg/kg/day	M	n.s.
					F	n.s.
	Curran et al. (2008, 757871)	Sprague Dawley rat	28 days	0, 2, 20, 50, 100 mg/kg/day	M	n.s.
	Yu et al. (2009, 757872)	Sprague-Dawley rat	91 days	0, 1.7, 5.0, or 15 mg/L	M	n.s.
	Seacat et al. (2002, 757853)	Cynomolgus monkeys	182 days	0, 0.03, 0.15, 0.75 mg/kg/day	M	n.s.
					F	n.s.
Thyroid weight,	Curran et al. (2008, 757871)	Sprague Dawley rat	28 days	0, 2, 20, 50, 100 mg/kg/day	M	n.s.

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
Relative to Brain Weight						

Notes: F = female; M = male; n.s. = nonsignificant.

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, 2009, 757876}. On GD 20, female fetuses had a significantly higher number of thyroid follicular epithelial cells compared to 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, 2009, 757876}. Luebker et al. (2005, 757857) 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, 2005, 757857}.

Pereiro et al. (2014, 2230732) 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, 2014, 2230732}. In contrast, NTP (2019, 5400978) 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, 2002, 757853}.

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, 2016, 3603365}. 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 Tableau.

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 HESD for PFOS {U.S. EPA, 2016, 3603365} 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, 2020, 6833758} and the other was classified as *uninformative.* The most consistent effects were for TSH in children. Three *medium* confidence studies {Xiao, 2019, 5918609; Kato, 2016, 3981723; Itoh, 2019, 5915990} reported elevated TSH among infants with increasing PFOS exposure, but other studies found the opposite effect {Aimuzi, 2019, 5387078}. 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, 2019, 5080657} and T3 {Byrne, 2019, 5079678}. 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, 2019, 5387078} 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 multi-pollutant 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	
Adrenocortical hormones	Mixed effects on corticosterone levels were	• *High* and *medium* confidence studies	• No factors 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**	
5 *Medium* confidence studies[a]	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).			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 gland following exposure to male and female mice, rats, and non-human primates (3/3).	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes		

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, 2230732) reported on the same data as Salgado-Freiría et al. (2018, 5079767) 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 multiple judgments 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 Main PFOS Document).

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, 2015, 2857764}, 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, 2009, 1290820}. Another study reported no association with metabolic syndrome or glucose homeostasis parameters {Fisher, 2013, 2919156}. 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, 5080655) measured exposure to PFOS using biomarkers in blood and in semen) Shapiro et al. (2016, 3201206) 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 (1 in children, 9 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. Due to 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, 2016, 3603365} 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.

Based on 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, 2018, 4238396} 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 {Predieri 2015, 3889874; Huang, 2018, 5024212; Jiang, 2014, 2850910}, lack of fasting measures for glucose measurements {Jiang, 2014, 2850910}, and treating PFOS as an outcome instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination {Predieri, 2015, 3889874; Jain 2020, 6833623}. Other concerns leading to an *uninformative* rating included inadequate reporting of population selection {Jiang, 2014, 2850910}, small sample size, and narrow ranges for exposure {Predieri, 2015, 3889874}.

The most common reason for a *low* confidence rating was potential for residual confounding, particularly by SES {Christensen, 2016, 3858533; Fassler, 2019, 6315820; Heffernen, 2018, 5079713; Koshy, 2017, 4238478; Lin, 2013, 2850967; Convertino, 2018, 5080342; Khalil, 2018, 4238547}, by adiposity {Lin, 2013, 2850967}, by age {Koshy, 2017, 4238478}, or by diabetes

status {Lind, 2014, 2215376}. *Low* confidence studies presented concerns with the outcome measures including potential for outcome misclassification {Christensen, 2016, 3858533; He, 2018, 4238388; Zong, 2016, 3350666}, failing to account for diabetes status {Lind, 2014, 2215376} or use of medications that would impact insulin levels or beta-cell function {He, 2018, 4238388; Fleisch, 2017, 3858513}, analytical methods {Koshy, 2017, 4238478}, and failure to establish temporality between PFOS exposure and diabetes {Lind, 2014, 2215376}. Other concerns included selection bias {Fassler, 2019, 6315820; van Den Dungen, 2017, 5080340}, which resulted from self-selection {Christensen, 2016, 3858533}, failure to provide information on control group selection {Heffernan, 2018, 5079713}, or differential recruitment for cases and controls {Lin, 2013, 2850967}. Small sample size was also a concern in some studies {Christensen, 2016, 3858533; Heffernan, 2018, 5079713; Khalil, 2018, 4238547; van den Dungen, 2017, 5080340}. 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.

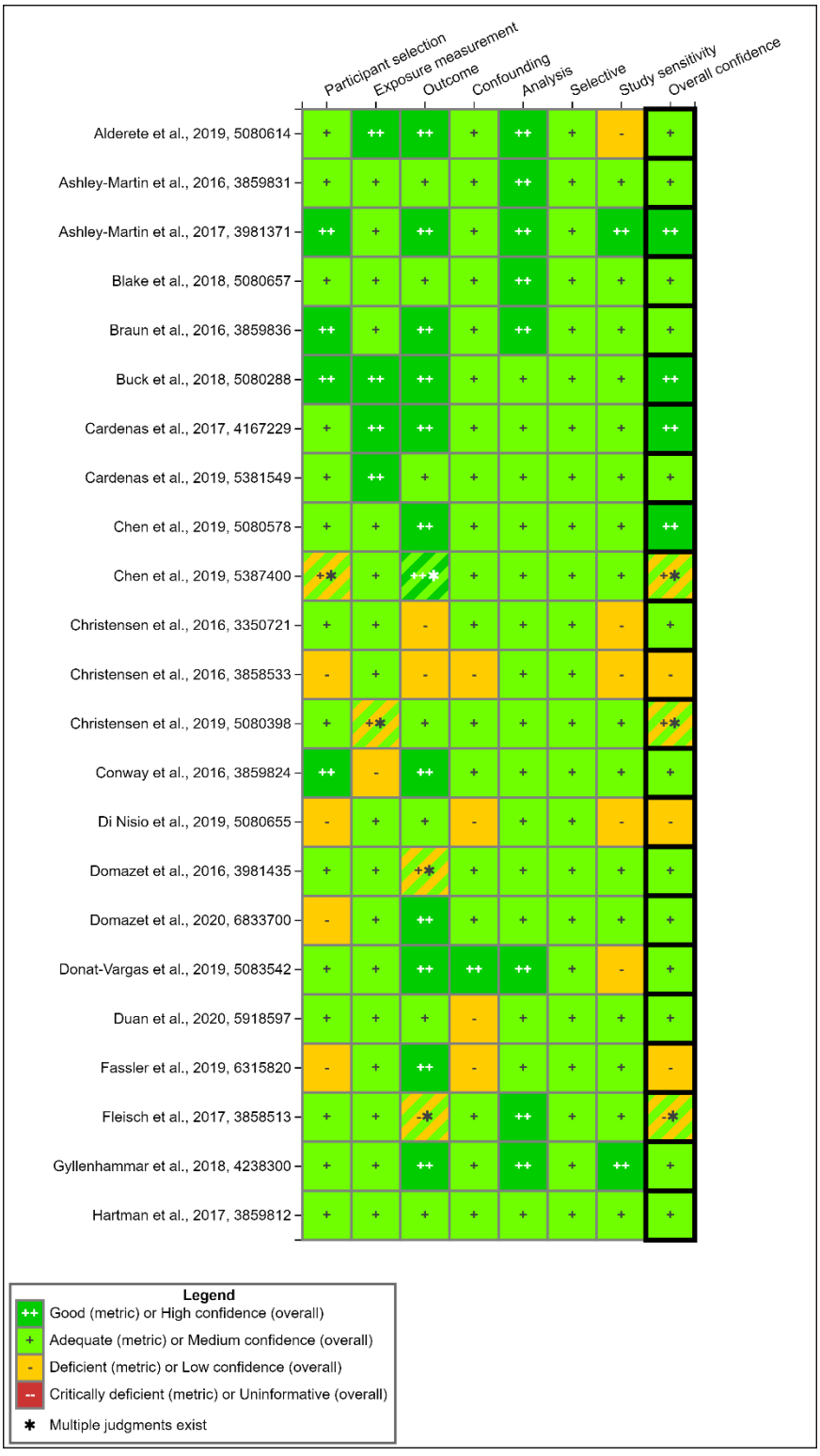


Figure C-18. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic Effects

Interactive figure and additional study details available on HAWC.

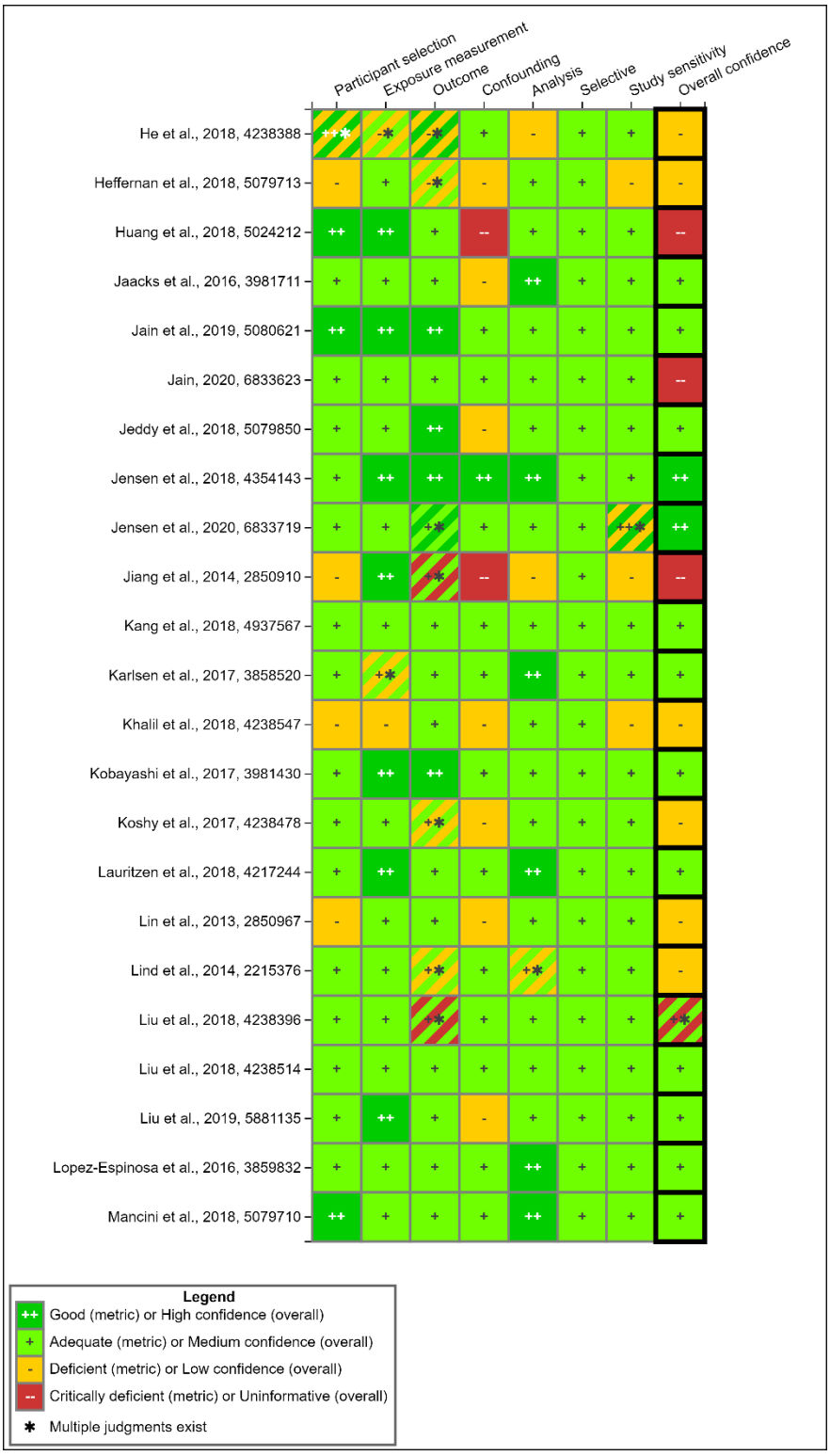


Figure C-19. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic Effects (Continued)

Interactive figure and additional study details available on HAWC.

Figure C-20. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic 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 {Domazet, 2016, 3981435; Kang, 2018, 4937567} 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, 2019, 5080614}, and two of *low* confidence {Khalil, 2018, 4238547; Fassler, 2019, 6315820}. Alderete et al. (2019, 5080614) also reported a positive, non-significant association with 2-hour glucose {Alderete, 2019, 5080614}. (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, 3858513) 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, 2019, 5080614; Fassler, 2019, 6315820; Koshy, 2017, 4238478; Khalil, 2018, 4238547; Domazet, 2016, 3981435}. 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, 2016, 3981435}.

Three studies examined fasting insulin levels. All three of these studies reported negative, non-significant associations with fasting insulin {Domazet, 2016, 3981435; Khalil, 2018, 4238547; Fassler, 2019, 6315820}.

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, 2019, 6315820}.

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, 2016, 3859832}. 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, 2016, 3859832}.

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, 2016, 3981435}.

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, 2017, 3981691}. Three other studies (one *high* and two *medium* confidence studies) reported positive, non-significant associations with adiponectin {Buck, 2018, 5080288; Domazet, 2020, 6833700; Fleisch, 2017, 3858513}. Buck et al. (2018, 3981371)

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, 2020, 6833700; Fleisch, 2017, 3858513; Minatoya, 2017, 3981691}. Minatoya et al. (2017, 3981691) 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, 2018, 5080288}.

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, 2018, 3859812}. One study observed non-significant negative associations with body fat percentage {Braun, 2016, 3859836}, and two studies observed a non-significant negative association with body fat mass {Jeddy, 2018, 5079850; Domazet, 2020, 6833700}.

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, 2019, 5080578}. 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, 2017, 3859823}.

Eleven studies examined BMI and related measures with mixed results. In the European Youth Heart Study (EYHS) study, Domazet et al. (2016, 3981435) 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, 2016, 3981435}. Additionally, two *medium* confidence studies observed significant positive associations with children's BMI {Lauritzen, 2018, 4217244; Mora, 2017, 3859823}. Mora et al. (2017, 3859823) 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, 2017, 3859823}. 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, 2017, 3859823}.

Significant negative associations were observed between maternal serum PFOS levels and BMI of girls from the ALSPAC study {Hartman, 2017, 3859812} and between serum PFOS levels and BMI of girls from the Breast Cancer and Environment Research Program (BCERP) study {Fassler, 2019, 6315820}. Three studies (one of *high* confidence and two of *low* confidence) reported negative, non-significant associations with BMI {Koshy, 2017, 4238478; Khalil, 2018, 4238547; Chen, 2019, 5080578}. In a sex-stratified analysis, Chen et al. (2019, 5080578)

observed a negative, non-significant association among girls, but a positive non-significant association among boys.

Di Nisio et al. (2019, 5080655) reported no difference between BMI between Italian male high school students exposed to PFOS pollution compared to 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, 2017, 3981430}.

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, 2017, 3858520}. In children from the POPUP study, Gyllenhammar et al. (2018, 4238300) 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 {Mora, 2017, 3859823; Manzano-Salgado, 2017, 4238509; Jensen, 2020, 6833719}. In an age-stratified analysis, Jensen et al. (2020, 6833719) 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, 2017, 4238478; Braun, 2016, 3859836}.

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, 2020, 6311645}. Another *medium* confidence study observed significantly increased odds of being overweight with increasing maternal PFOS among 5-year-old children {Lauritzen, 2018, 4217244}. A *medium* confidence study of mother-child pairs in the Faroe Islands reported a significantly increased risk of being overweight at 18 months {Karlsen, 2017, 3858520}. Two *medium* confidence studies observed an increased, non-significant risk of being overweight {Mora, 2017, 3859823; Manzano-Salgado, 2017, 4238509}. Manzano-Salgado et al. (2017, 4238509) 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, 2017, 4238478; Braun, 2016, 3859836}. Braun et al. (2016, 3859836) 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, 2016, 3981435}. Two studies, one *high* confidence and one *low* confidence observed negative, non-significant associations with waist

circumference {Chen, 2019, 5080578; Mora, 2017, 3859823}. After stratification by sex, Mora et al. (2017, 3859823) 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, 2017, 3859812}. In a tertiles analysis, Braun et al. (2016, 3859836) 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 to non-exposed children {Di Nisio, 2019, 5080655}.

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, 2020, 6833719; Manzano-Salgado, 2017, 4238509}. Manzano-Salgado et al. (2017, 4238509) 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. (2020, 6833719) 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, 2019, 6315820}.

A *high* confidence study of children from the Shanghai Prenatal Cohort observed negative, non-significant associations with waist-to-height ratio {Chen, 2019, 5080578}. 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, 2017, 4238509}.

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, 2019, 6315820}, while a null association was observed in a *medium* confidence study {Mora, 2017, 3859823}. After stratification by sex, Mora et al. (2017, 3859823) 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, 2016, 3981435}. 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, 2018, 4217244}.

Mora et al. (2017, 3859823) 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, 2017, 3859823}.

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, 2020, 6833657; Wang, 2018, 5080352; Liu, 2019, 5881135; Matilla-Santander, 2017, 4238432}. 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, 2019, 5024206}.

Four *medium* and one *low* confidence studies reported inverse, non-significant associations with gestational diabetes {Xu, 2020, 6833677; Wang, 2018, 5079666; Valvi, 2017, 3983872; Zong, 2016, 3350666; Shapiro, 2016, 3201206}. With the exception of the *low* confidence study {Zong, 2016, 3350666}, gestational diabetes was determined through standard clinical methods. The nested case-control study conducted by Xu et al. (2020, 6833677) 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, 3201206) 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, 2018, 5080352}. Two *high* confidence studies and one *medium* confidence study reported negative, non-significant associations with fasting glucose {Starling, 2017, 3858473; Jensen, 2018, 4354143; Liu, 2019, 5881135}. In contrast, two *medium* confidence studies reported positive, non-significant associations with fasting glucose among pregnant women {Ren, 2020, 6833646; Wang, 2018, 5079666}.

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., 2020, 6833657}. Additionally, a *medium* confidence study reported a significant association with 1-hour glucose levels among pregnant women in the Shanghai-Minhang Birth Cohort {Ren, 2020, 6833646}. Three studies observed positive, non-significant associations with oral glucose tolerance test results {Wang, 2018, 5080352; Jensen, 2018, 4354143; Liu, 2019, 5881135}.

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, 2017, 4238432}. 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, 2020, 6833657; Shapiro, 2016, 3201206}.

Two *high* confidence studies evaluated associations between plasma PFOS levels and hyperglycemia or HbA1c among members of Project Viva. Preston et al. (2020, 6833657) reported a positive, non-significant association with hyperglycemia. Conversely, Mitro et al. (2020, 6833625) 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, 2018, 4354143; Wang, 2018, 5079666}. These studies evaluated members of the OCC in Denmark with high risk of gestational diabetes {Jensen, 2018, 4354143} and women in China in early pregnancy {Wang, 2018, 5079666}. Jensen et al. (2018, 4354143) 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, 2018, 4354143}.

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, 2020, 6833625} while another *medium* confidence study reported a positive, non-significant association with adiponectin {Ashley-Martin, 2017, 3981371}. After stratification by age during pregnancy, Mitro et al. (2020, 6833625) 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, 2020, 6833625}, while the other reported a negative, non-significant association {Ashley-Martin, 2017, 3981371}.

Three *medium* confidence studies examined gestational weight gain, with mixed results.

Jaacks et al. (2016, 3981711) 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, 2016, 3981711}.

Ashley-Martin et al. (2016, 3859831) 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 1kg increase in gestational weight gain and IQR increase in gestational weight gain {Ashley-Martin, 2016, 3859831}.

Marks et al. (2019, 5381534) observed a negative, non-significant association with gestational weight gain. However, a significant interaction was observed between PFOS and pre-pregnancy BMI {Marks, 2019, 5381534}.

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, 2020, 6833625}.

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, 2020, 6833625}.

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, 2016, 3860116}. In a quartile analysis, odds of type 2 diabetes significantly increased with increasing quartiles of PFOS {Su , 2016, 3860116}. 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, 2018, 4241053}. 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, 2018, 5079710} (Appendix D).

Three *low* confidence studies reported non-significant positive associations with diabetes {Lind, 2014, 2215376; Christensen, 2016, 3858533; He, 2018, 4238388} and prediabetes {Christensen, 2016, 3858533}.

Significant decreased odds of type 1 and type 2 diabetes were observed among 6889 participants in the C8 Health Project {Conway, 2016, 3859824}. 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, 2016, 3859824}. 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, 2017, 4167229}. 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, 2017, 4167229}. Two other *medium* confidence study reported non-significant negative associations with type 2 diabetes {Donat-Vargas, 2019, 598342; Cardenas, 2019, 5381549}.

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. (2019, 5387400) observed a positive non-significant association with risk of Metabolic syndrome 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 metabolic syndrome. Liu et al., 2018 observed adults aged 20 and older from the 2013–2014 NHANES cycle and Christensen et al. (2019, 5080398) 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, 5080398) reported non-significant increased odds of

metabolic syndrome 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 metabolic syndrome for participants with serum PFOS > 1.90 ng/mL compared to those with serum PFOS ≤ 1.90 ng/mL {Yang, 2018, 4238462}. 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, 2020, 5918597}. Additionally, a study using NHANES 1999–2014 data observed a significant positive correlation between fasting glucose and serum PFOS {Huang, 2018, 5024212}. Su et al. (2017, 3860116) 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. (2018, 4238514) 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, 2018, 5079713}, 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, 2018, 4238462}.

Two studies (one *high* confidence and one *medium* confidence) observed non-significant positive associations with 2-hour glucose {Cardenas, 2017, 4167229; Su, 2016, 3860116} and 30-minute glucose {Cardenas, 2017, 4167229}. Another *medium* confidence study reported a negative, non-significant association with 2-hour glucose {Liu, 2018, 4238514}.

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, 2016, 3860116}. In the POUNDS-Lost clinical trial, a positive, non-significant correlation was observed between PFOS and glucose levels {Liu, 2018, 4238514}.

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, 2019, 5080398}. A *low* confidence study reported a negative association with blood glucose levels {van den Dungen, 2017, 5080340}. 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, 2018, 4238396}. 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, 2018, 4238396}. 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, 2018, 4238396}. In a sex-stratified analysis, average resting metabolic rate significantly decreased with increasing tertiles of PFOS among men and women {Liu, 2018, 4238396}.

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, 2017, 4167229}. Two *low* confidence reported non-significant positive associations with fasting insulin {Chen, 2019, 5387400; Sun, 2018, 4241053}, and one reported a non-significant negative association (He et al., 2018, 4238388). One *medium* confidence study reported a positive, non-significant association with insulin levels {Liu, 2018, 4238514}.

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, 2017, 4167229}. A *medium* confidence study of 1871 adults in NHANES observed a non-significant positive association with HOMA-IR {Liu, 2018, 4238514}. However, Donat-Vargas et al. (2019, 5083542) 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, 2019, 5083542}.

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 {Lind, 2014, 2215376; Chen, 2019, 5387400; Lin, 2013, 2850967}. 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, 2018, 4238388}. These studies were of *low* confidence due to concerns with the statistical analysis (not accounting for design of NHANES) {He, 2018, 4238388}, failure to account for diabetes status {Lind, 2014, 2215376} or medications that could affect insulin levels {Chen, 2019, 5387400}, and concerns for residual confounding and selection bias {Lin, 2013, 2850967}.

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, 2017, 4167229}.

In a *high* confidence study, Cardenas et al. (2017, 4167229) 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, 2017, 4167229}.

In a *low* confidence study, a non-significant positive association was reported for the ratio of proinsulin to insulin and PFOS {Lind, 2014, 2215376}. 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, 4167229) 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, 2018, 4238514} and {Chen, 2019, 5387400}. A *medium* confidence studies reported negative, non-significant associations with HOMA-B {Donat-Vargas, 2019, 5083542}.

Four studies examined adiponectin, and none reported significant associations. Two *high* confidence studies reported non-significant positive associations with adiponectin {Buck, 2018, 5080288; Ashley-Martin, 2017, 3981371}. In contrast, a non-significant negative association with adiponectin was observed among 945 adults in the Diabetes Prevention Program {Cardenas, 2017, 4167229}. A *medium* confidence study reported a negative non-significant correlation between PFOS and plasma adiponectin {Sun, 2018, 4241053}.

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, 2018, 5080288}, and the other reported a non-significant negative association {Ashley-Martin, 2017, 3981371}. 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, 2018, 4238396}.

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, 2017, 4167229}. 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, 2020, 5918597}. Two *medium* confidence studies observed positive correlations with HbA1c; one was non-significant {Sun, 2018, 4241053} and the other was significant {Huang, 2018, 5024212}. Another *medium* confidence cross-sectional study assessed the association between plasma PFOS and HbA1c in adults aged 20–60 {Su, 2016, 3860116}. 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, 2016, 3860116}.

In the POUNDS-Lost trial, a negative, non-significant correlation was observed between PFOS and HbA1c {Liu, 2018, 4238396}. Additionally, a *medium* confidence study of 1871 adults from NHANES reported a non-significant negative association with HbA1c {Liu, 2018, 4238514}.

One *low* confidence study reported a non-significant negative association with HbA1c {Heffernan, 2018, 5079713}. Another *low* confidence study observed a non-significant positive association between PFOS and HbA1c {Chen, 2019, 5387400}. Concerns with measurement of confounders and inclusion of medications that could affect insulin levels {Chen, 2019, 5387400}, as well as concerns with case selection and residual confounding {Heffernan, 2018, 5079713} 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, 2018, 4238396}. A significant increase in average weight gain during months 6–24 of the trial was observed with increasing tertiles of PFOS {Liu, 2018, 4238396}.

Two studies evaluated being overweight, one of which reported an association. A *medium* confidence study reported significantly greater serum PFOS among obese adults compared to non-obese adults {Jain, 2019, 5080621}. 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, 2020, 6311645}.

One *low* confidence study observed significant increased odds of being overweight or obese {Tian, 2019, 5080586}. Another *low* confidence study reported non-significant negative associations with being overweight and obese {Yang, 2018, 4238462}.

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, 2017, 3859812}. A negative, non-significant association was observed between maternal plasma PFOS and body fat percentage {Hartman, 2017, 3859812}.

Three *medium* confidence studies reported positive, non-significant associations with body fat measures {Mora, 2017, 3859823; Braun, 2016, 3859836; Liu, 2019, 5881135}.

Two *medium* confidence studies evaluated fat mass; one reported a non-significant negative association with fat mass among children {Jeddy, 2018, 5079850} and a non-significant positive association with fat mass among overweight and obese adults {Liu, 2019, 5881135}.

11 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, 2016, 3859836}. Negative, non-significant associations with BMI z-score were

observed in the second and third tertile of maternal PFOS exposure {Braun, 2016, 3859836}. Liu et al. (2018, 4238396) 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 {Cardenas, 2017, 4167229; Chen, 2019, 5387400; Blake, 2017, 5080657}.

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, 2017, 3859812}. 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, 2018, 5080657}. In the single *low* confidence study, Tian et al. (2019, 5080586) 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, 2019, 5080586}. 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, 2020, 6833719} 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, 2018, 4217244}.

Five studies examined waist circumference. Two single *medium* confidence studies observed a negative, non-significant association with waist circumference {Liu, 2018, 4238396; Liu, 2018, 4238514}. One *low* confidence study reported a non-significant positive association with waist circumference {Tian, 2019, 5080586}. 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, 2019, 5080586}. 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, 2019, 5080398}.

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 3 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 4 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 4 studies are shown in Figure C-21.

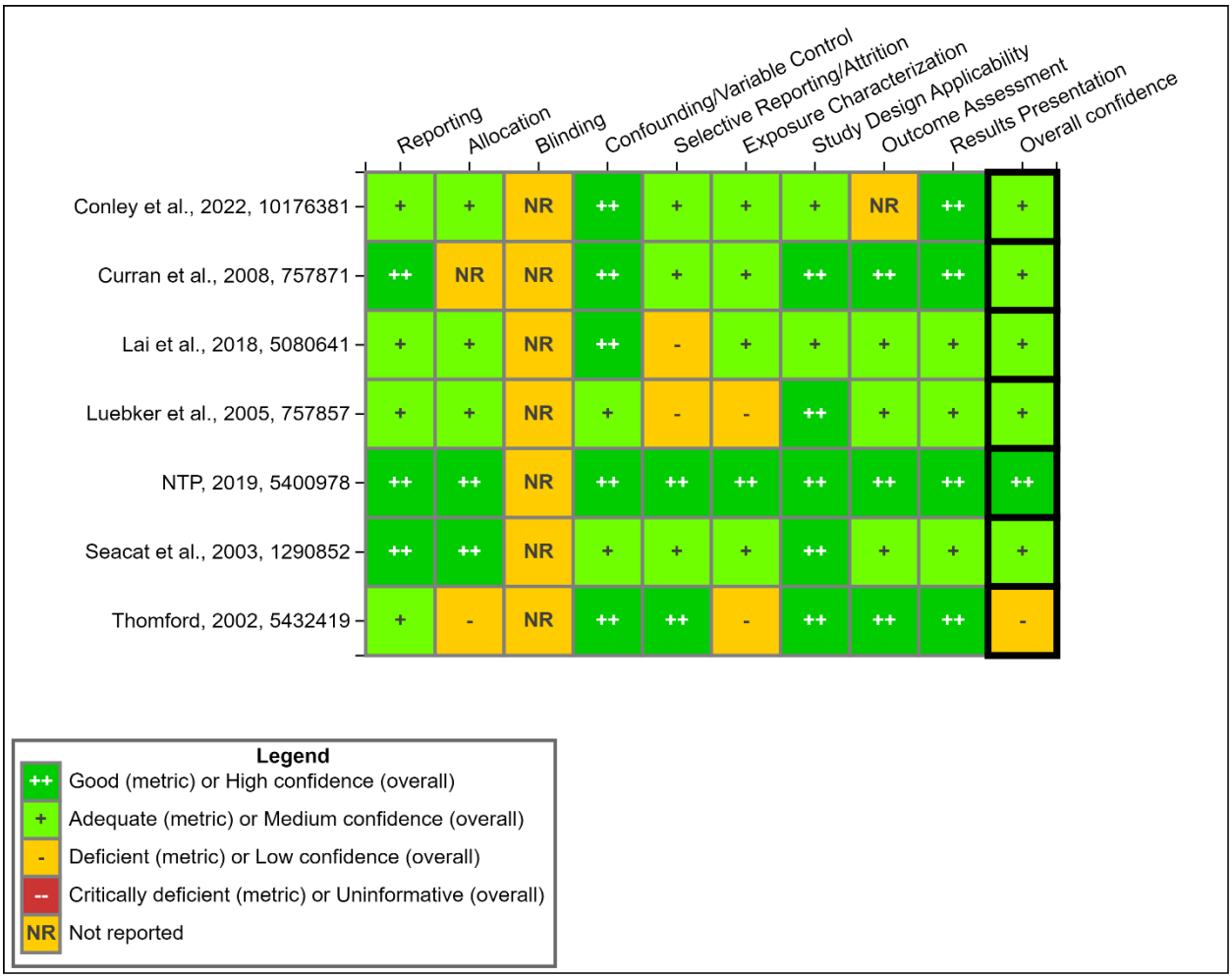


Figure C-21. Summary of Study Evaluation for Toxicology Studies of PFOS and Metabolic Effects

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, 5400978) 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, 1290852) observed a significant decrease in serum glucose in adult male Sprague Dawley rats compared to 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. (2005, 757857) 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, 2850947) 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, 2013, 2850947}.

Wan et al. (2014, 2850405) 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, 2014, 2850405}. 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 to the high-fat diet control group. However, the HOMA-IR indices were significantly higher for the high-fat diet groups compared to the standard diet groups within a specific PFOS treatment group and sex. In contrast, Ngo et al. (2014, 2850267) 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, 5080641) 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 to 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, 2016, 3603365} 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-24.

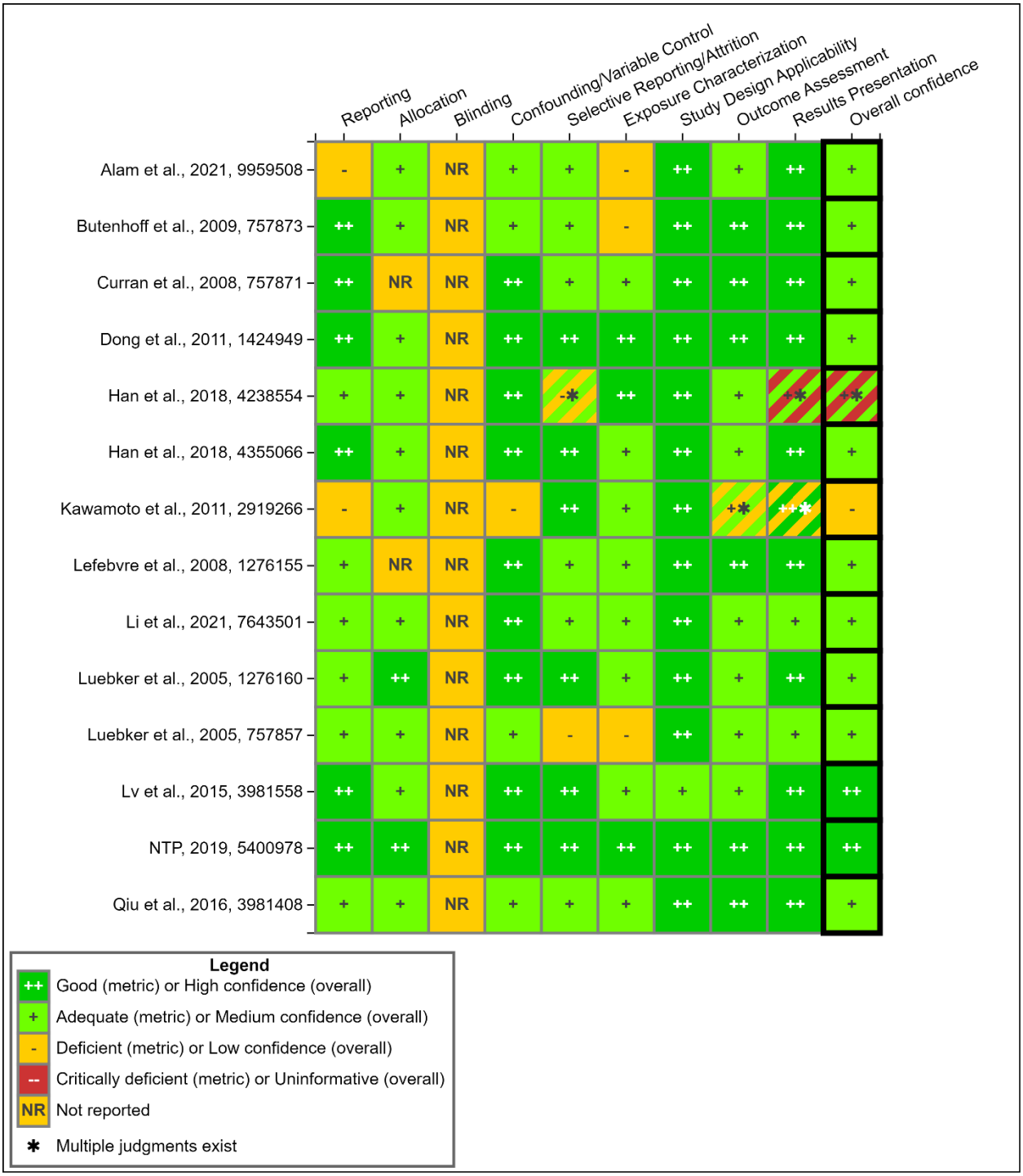


Figure C-22. Summary of Study Evaluation for Toxicology Studies of PFOS and Systemic Effects[a]

Interactive figure and additional study details available on HAWC.

[a] Lefebvre et al. (2008, 1276155) reported on the same animals as Curran et al. (2008, 757871).

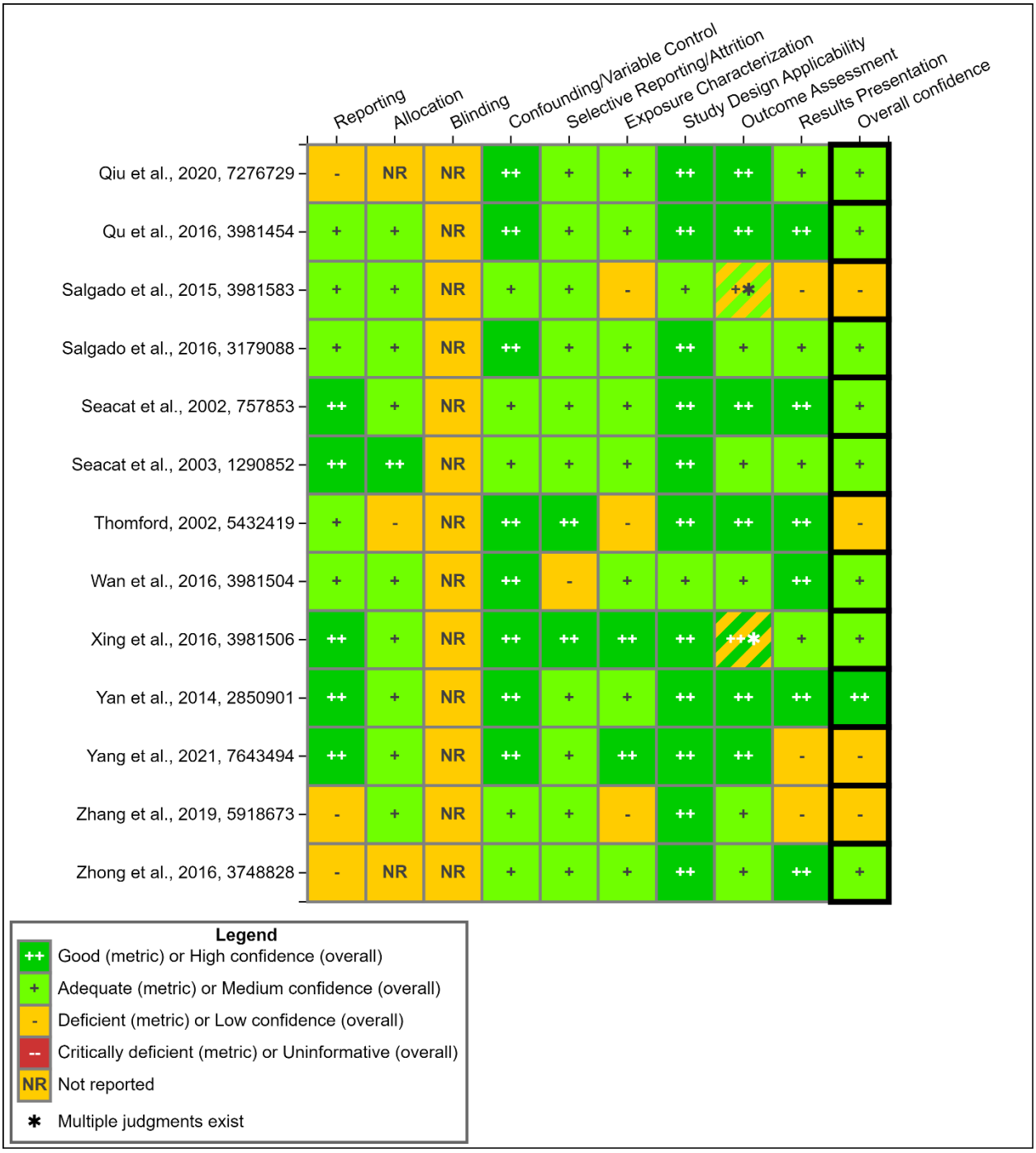


Figure C-23. Summary of Study Evaluation for Toxicology Studies of PFOS and Systemic Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

[a] Lefebvre et al. (2008, 1276155) reported on the same animals as Curran et al. (2008, 757871).

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, 2002, 757853}. 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, 2002, 757853}.

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, 2016, 3981454}. NTP (2019, 5400978) 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, 5400978}. There were no treatment-related clinical observations reported in male or female rats {NTP, 2019, 5400978}. Similarly, Alam et al. (2021, 9959508) 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, 3981506) 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, 2016, 3981506}. 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, 2005, 1276160}. 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, 2005, 1276160; Luebker, 2005, 757857} (see PFOS Main Document).

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, 2016, 3981506}. All dose groups had a significant difference in body weight gain when compared to the control with the 10 mg/kg/day group having a 31% reduction in body weight over the study period compared to 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, 2016, 3981506}. 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, 2016, 3981454}. In a separate study, although reductions in body

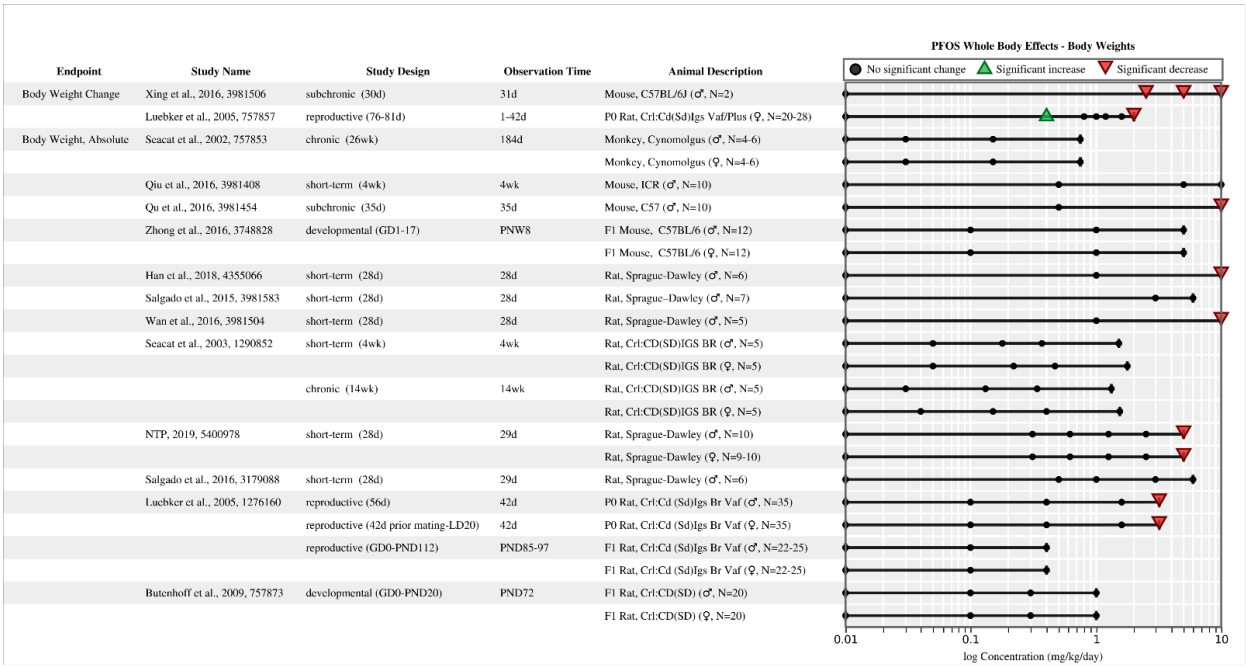
weight were observed in male BALB/c mice after 1 week of exposure to 10 mg/kg/day PFOS via gavage, this effect was attenuated at the end of the exposure period at 3 weeks {Lv, 2015, 3981558}. Additionally, a significant increase in body weight was observed in 2.5 mg/kg/day exposure group at the end of the 3-week exposure period {Lv, 2015, 3981558}. Food consumption was not reported in these studies {Qu, 2016, 3981454; Lv, 2015, 3981558}. 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, 2016 3981408}.

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. (2018, 4355066) and Wan et al. (2016, 3981504) 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, 5400978) 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. (2018, 4355066), and food consumption was not reported in the other studies {Wan, 2016, 3981504; NTP, 2019, 5400978}. Two studies by Salgado et al. (2015, 3981583; 2016, 3179088) 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, 2005, 1276160}. 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, 2005, 757857}. 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, 2003, 1290852}.

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, 2002, 757853}. 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, 2003, 1290852}. 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, 2003, 1290852}.



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Body Weight Change	Xing et al., 2016, 3981506	subchronic (30d)	31d	Mouse, C57BL/6J (♂, N=2)
	Luebker et al., 2005, 757857	reproductive (76-81d)	1-42d	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=20-28)
Body Weight, Absolute	Seacat et al., 2002, 757853	chronic (26wk)	184d	Monkey, Cynomolgus (♂, N=4-6)
				Monkey, Cynomolgus (♀, N=4-6)
	Qiu et al., 2016, 3981408	short-term (4wk)	4wk	Mouse, ICR (♂, N=10)
	Qu et al., 2016, 3981454	subchronic (35d)	35d	Mouse, C57 (♂, N=10)
	Zhong et al., 2016, 3748828	developmental (GD1-17)	PNW8	F1 Mouse, C57BL/6 (♂, N=12)
				F1 Mouse, C57BL/6 (♀, N=12)
	Han et al., 2018, 4355066	short-term (28d)	28d	Rat, Sprague-Dawley (♂, N=6)
	Salgado et al., 2015, 3981583	short-term (28d)	28d	Rat, Sprague–Dawley (♂, N=7)
	Wan et al., 2016, 3981504	short-term (28d)	28d	Rat, Sprague-Dawley (♂, N=5)
	Seacat et al., 2003, 1290852	short-term (4wk)	4wk	Rat, Crl:CD(SD)IGS BR (♂, N=5)
				Rat, Crl:CD(SD)IGS BR (♀, N=5)
		chronic (14wk)	14wk	Rat, Crl:CD(SD)IGS BR (♂, N=5)
				Rat, Crl:CD(SD)IGS BR (♀, N=5)
	NTP, 2019, 5400978	short-term (28d)	29d	Rat, Sprague-Dawley (♂, N=10)
				Rat, Sprague-Dawley (♀, N=9-10)
	Salgado et al., 2016, 3179088	short-term (28d)	29d	Rat, Sprague-Dawley (♂, N=6)
	Luebker et al., 2005, 1276160	reproductive (56d)	42d	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♂, N=35)
		reproductive (42d prior mating-LD20)	42d	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=35)
		reproductive (GD0-PND112)	PND85-97	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂, N=22-25)
				F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=22-25)
	Butenhoff et al., 2009, 757873	developmental (GD0-PND20)	PND72	F1 Rat, Crl:CD(SD) (♂, N=20)
				F1 Rat, Crl:CD(SD) (♀, N=20)

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 PFOS Main Document). However, the effects on body weight may not persist into adulthood. No change was observed in adult body weight (PND 85–PND 97) compared to 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, 2005, 1276160}. 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, 2016, 3748828}. 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, 2009, 757873}.

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, 2016, 3981506}. 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,

2005, 1276160}, and in female Crl:Cd(Sd)lgs Vaf/Plus rats following a 6 week exposure to 2.0 mg/kg/day {Luebker, 2005, 757857} (see PFOS Main Document).

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, 2003, 4355066}, 3 or 6 mg/kg/day {Salgado, 2015, 3981583}, nor 0.5 mg/kg/day, 1 mg/kg/day, 3 mg/kg/day, or 6 mg/kg/day {Salgado, 2016, 3179088} for 28 days. Seacat et al. (2003, 1290852) 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, 2003, 1290852}. 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, 2016, 3603365}. 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 Tableau.

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 Tableau.

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 suggests a potential association between PFOS exposure and adiponectin in children, but not adults. Findings for an association between PFOS exposure and metabolic syndrome were mixed in four general population epidemiological studies identified since 2016: two reported negative associations with metabolic syndrome, 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, 5400978) and Seacat et al. (2003, 1290852) 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, 1290852) and Curran et al. (2008, 757871) 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, 2850947) 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, 2850405) 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 metabolic syndrome, 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-hour 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 metabolic syndrome, 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), and 5 reported imprecise associations (5/8). The 3	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effect • *Imprecision* of findings • Potential for *outcome misclassification*, self-selection, residual confounding by SES,		

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**	
	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.*		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 generally imprecise findings for measures of insulin resistance.	• *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**	
	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 differences in direction of effect between men and women. Findings for BMI in children were	• *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**	
	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 over-weight).				
Metabolic syndrome 4 *Medium* confidence studies 1 *Low* confidence study	In adults, findings for metabolic syndrome 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.3.2.1 and Section C.3.2.2)					
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, and 4 reported significant effects with inconsistent directionality. Reduced glucose levels were	• *High* and *medium* confidence studies	• *Inconsistent direction* and *magnitude* of effects across study designs and sex • *Limited number* of studies examining outcomes	⊙⊙⊙ *Indeterminate* Alterations related to glucose homeostasis were reported in 6 *high* or *medium* confidence studies were inconclusive	

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 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).			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		
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,	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes • *Qualitative* and *subjective* data reporting		

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 localized areas of partial alopecia.				
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; 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 multiple judgments 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 Main PFOS Document).

C.4.1 Human Evidence Study Quality Evaluation and Synthesis

C.4.1.1 Introduction

The 2016 Health Assessment {U.S. EPA, 2016, 3603365} 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, 2014, 2852208)}. 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, 2010, 1291112}. 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, 2008, 1290822}. 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, 2011, 758428}. 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 {Hoyer, 2015, 2851038}. Data interpretations within these studies were limited in some cases by use of a cross-sectional study design {Fei, 2008, 1290822; Hoffman, 2010, 1291112}, potential random misclassification error resulting from using current PFOS levels as proxy measures of etiologically relevant exposures {Hoffman, 2010, 1291112}, outcomes defined by parental report {Fei, 2008, 1290822; Fei, 2011, 758428; Hoyer, 2015, 2851038; Hoffman, 2010, 1291112}, and limited sample sizes in some countries {Hoyer, 2015, 2851038}.

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, 2020, 6364693}. One publication {Vuong, 2020, 356876} was conducted in pregnant women. The remainder were conducted in the general population. Study designs included 3 case-control {Ode, 2014, 2851245; Long, 2019, 5080602; Shin, 2020, 6507470}, 2 nested case-control {Liew, 2015, 2851010; Lyall, 2018, 4239287}, 26 cohort, and 5 cross-sectional studies (Appendix D). The studies measured PFOS in different

matrices including blood, serum, plasma, cord blood, breast milk {Forns, 2015, 3228833; Lenters, 2019, 5080366}, maternal serum, maternal plasma, and amniotic fluid {Long, 2019, 5080602}. Several studies {Braun, 2014, 2345999; Vuong, 2016, 3352166; Vuong, 2018, 5079675; Vuong, 2018, 5079693; Vuong, 2019, 5080218; Vuong, 2020, 6356876; Vuong, 2020, 6833684; Zhang, 2018, 4238294} were conducted on subsets of data from the HOME study. Two studies {Forns, 2015, 3228833; Lenters, 2019, 5080366} utilized data from the Norwegian Human Milk Study (HUMIS). Two studies {Liew, 2015, 2851010; Liew, 2018, 5079744} 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)[5] from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} 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, 2019, 5381527; Oulhote, 2016, 3789517; Harris, 2018, 4442261} 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, 2014, 2713574} 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, 2015, 3228833}. 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, 3860112) 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, 2020, 6718530. Finally, limitations in Ode et al. (2014, 2851245) 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.

[5] Vuong et al. (2018, 5079675) reports score trajectories for the same population and test as Vuong et al. (2016, 3352166). Vuong et al. (2020, 6833684) reports on an overlapping population with the same test as Zhang et al. (2018, 4238294).

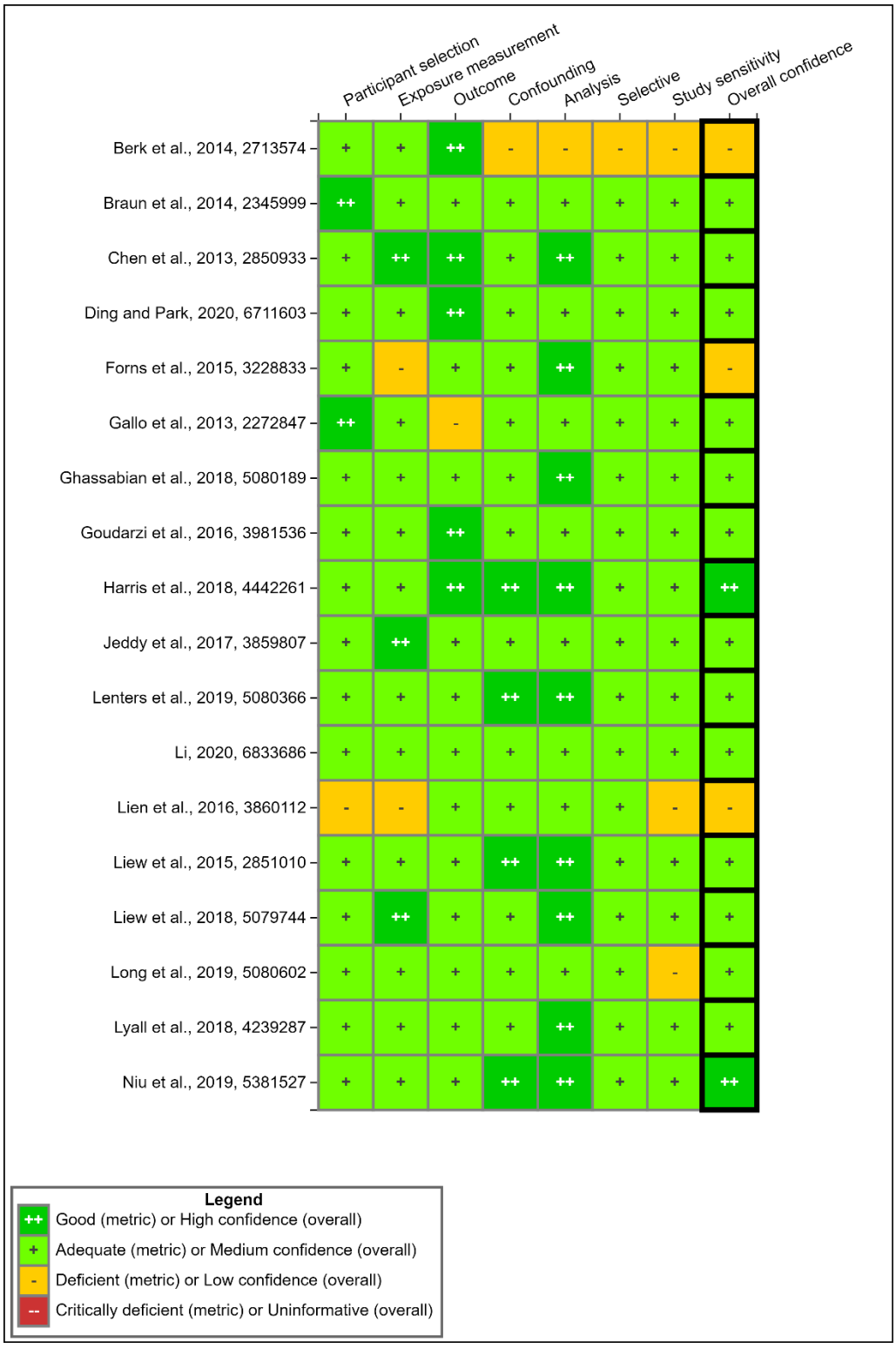


Figure C-27. Summary of Study Evaluation for Epidemiology Studies of PFOS and Neurological Effects

Interactive figure and additional study details available on HAWC.

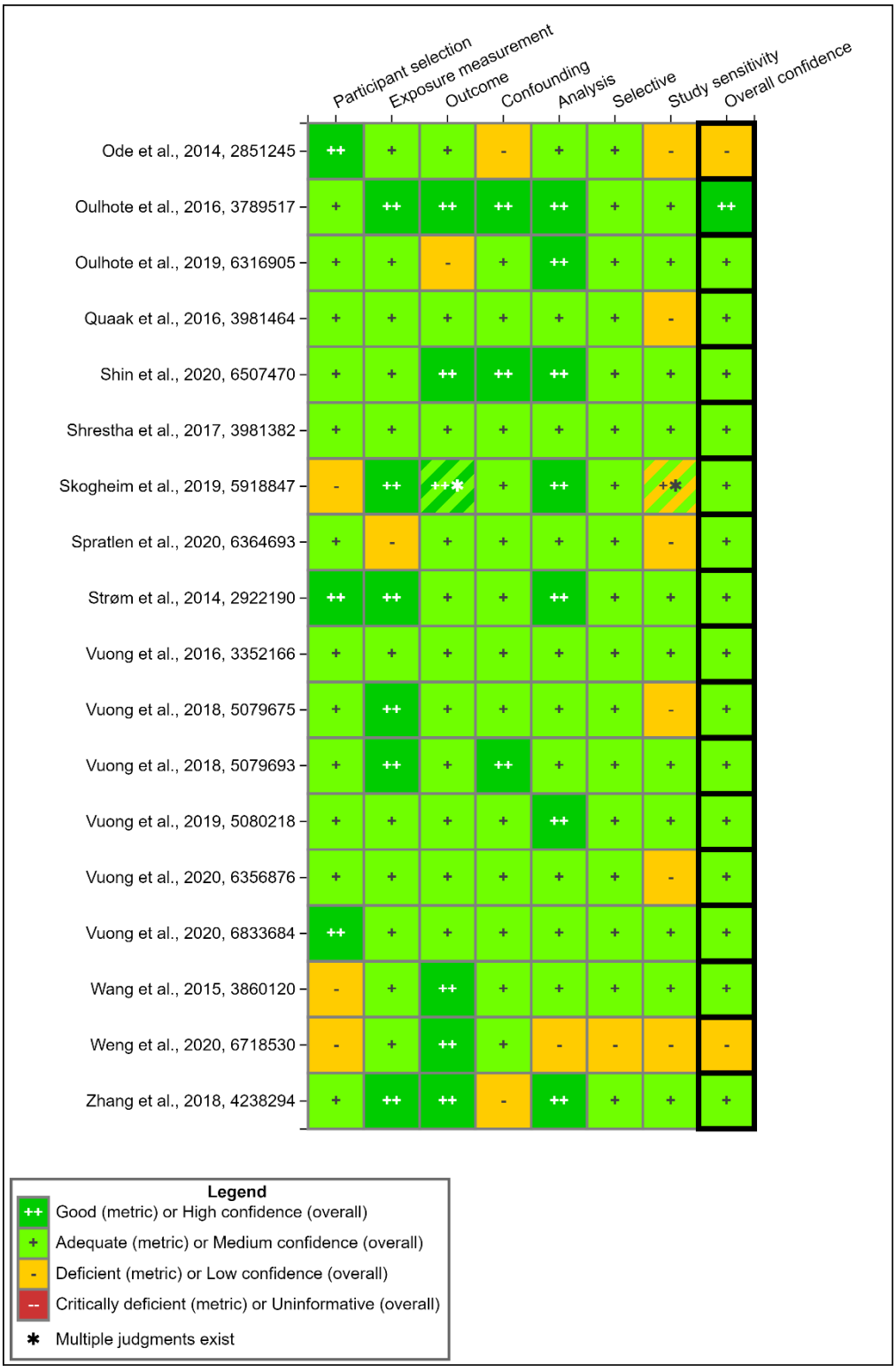


Figure C-28. Summary of Study Evaluation for Epidemiology Studies of PFOS and Neurological Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.4.1.3 Findings from Children and Adolescents

Six cohort studies {Goudarzi, 2016, 3981536; Chen, 2013, 2850933; Jeddy, 2017, 3859807; Forns, 2015, 3228833; Niu, 2019, 5381527; Shrestha, 2017, 3981382}, and one high-exposure community study {Spratlen, 2020, 6364693| examined developmental outcomes in children. In a *high* confidence study {Niu, 2019, 5381527} from the Shanghai-Minhang Birth Cohort Study (SMBCS), 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, 2013, 2850933} 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, 2017, 3859807} 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, 2016, 3981536} 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, 2020, 6364693}.

Ten studies evaluated cognitive function and IQ measures among children, with most conducted within the general population {Vuong, 2020, 6833684; Zhang, 2018, 4238294; Strom, 2014, 2922190; Harris, 2018, 4442261; Oulhote, 2019, 6316905; Liew, 2018, 5079744; Vuong, 2019, 5080218; Wang, 2015, 3860120; Skogheim, 2019, 5918847}, and one within a high-exposure community {Spratlen, 2020, 6364693}. 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 non-verbal IQ scores, although dose-response patterns appeared non-linear {Harris, 2018, 4442261}. 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, 2020, 6833684} 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, 2018, 4238294}. 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, 2014, 2922190}. 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, 2019, 6316905}.

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 to males {Spratlen, 2020, 6364693}. 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, 2018, 5079744}. Consistent adverse associations with age eight cognitive development as assessed by IQ were not observed in an additional *medium* confidence study {Vuong, 2019, 5080218}. Similarly, utilizing data from participants within the Taiwan Maternal and Infant Cohort Study, a *medium* confidence prospective cohort study by Wang {Wang, 2015, 3860120} 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 non-verbal 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, 2019, 5918847}.

Six studies assessed the relationship between PFOS and behavioral development problems and behavioral regulation problems {Quaak, 2016, 3981464; Vuong, 2018, 5079693; Oulhote, 2019, 6316905; Ghassabian, 2018, 5080189; Oulhote, 2016, 3789517; Weng, 2020, 6718530}. 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, 2016, 3781464}. 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 two-fold 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, 2016, 3789517}. 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, 2019, 6316905}. 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, 2018, 5080189}. 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, 2018, 5079693}. 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, 2020, 6718530}.

One *medium* confidence study {Strom, 2014, 2922190} 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, 2016, 3352166; Vuong, 2018, 5079675; Shrestha, 2017, 3981382} 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, 2016, 3352166; Vuong, 2018, 5079675} 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, 2016, 3352166}. Vuong et al. (2018, 5079675) 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 {Strøm, 2014, 2922190; Liew, 2015, 2851010; Quaak, 2016, 3981464; Skogheim, 2019, 5918847; Lenters, 2019, 5080366}. One *medium* confidence study {Lenters, 2019, 5080366} 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, 2922190) 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, 2015, 2851010} 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, 3981464) 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 {Ode, 2014, 2851245; Lien, 2016, 3860112} examined PFOS exposures in relation to ADHD. Ode et al. (2014, 2851245) 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, 2016, 3789517}and five *medium* confidence studies since the 2016 assessment evaluated PFOS exposures in relation to autism, autistic behaviors, and ID {Braun, 2014, 2345999; Liew, 2015, 2851010; Long, 2019, 5080602; Lyall, 2018, 4239287; Shin, 2020, 6507470}. A two-fold 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, 2016, 3789517}. 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, 2014, 2345999}. 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, 2015, 2851010}. 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, 2019, 5080602}. 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, 2018, 4239287}. 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, 2020, 6507470}.

The effects on visuospatial performance were evaluated in one *high* confidence study of participants of Project Viva {Harris, 2018, 4442261}. 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, 2018, 5079693}.

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, 2020, 6356876}.

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, 2017, 3981382} 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, 2017, 3981382}, 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 {Shrestha, 2017, 3981382; Vuong, 2020, 6356876}. 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, 2017, 3981382}. 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, 2020, 6356876}. One *low* confidence study (Berk, 2014, 2713574) 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, 2017, 3981382}. 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. {Gallo, 2013, 2272847; Shrestha, 2017, 3981382}. Statistically significant inverse associations between PFOS and memory impairment were reported in a medium confidence study of adults in the C8 Health Project {Gallo 2013, 2272847}. No adverse effects of PFOS on memory impairment were again reported in a separate *medium* confidence study of older adults {Shrestha, 2017, 3981382}.

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, 6711603) observed no significant associations.

C.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 3 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} 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.

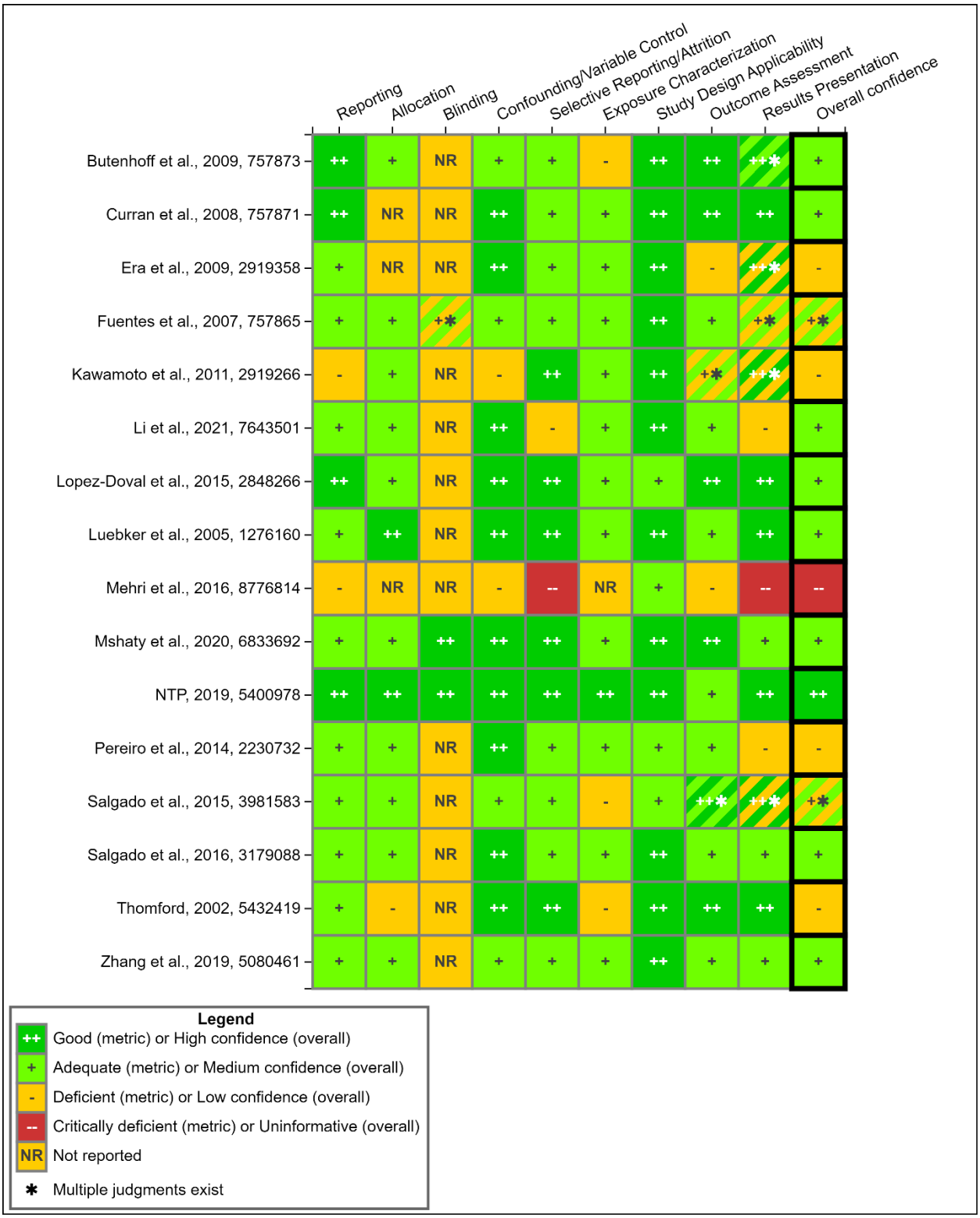


Figure C-29. Summary of Study Evaluation for Toxicology Studies of PFOS and Nervous Effects

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, 2009, 757873}. 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, 2008, 757871}. 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, 2016, 3179088}; 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 {Butenhoff, 2009, 757873; NTP, 2019, 5400978}. 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, 2021, 7643501}.

One developmental {Mshaty, 2020, 6833692}, one short-term {Fuentes, 2007, 757865}, and one subchronic study {Long, 2013, 2850984} in mice and several reproductive {Luebker, 2005, 1276160} and developmental studies {Butenhoff, 2009, 757873; Wang, 2015, 2851030; Johansson, 2008, 1276156; Fuentes, 2007, 757863} in rats assessed the neurobehavioral effects associated with PFOS. Mshaty et al. (2020, 6833692) 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, 2013, 2850984}. 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, 2007, 757865}. 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, 2015,

2851030}. 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 {Luebker, 2005, 1276160; Butenhoff, 2009, 757873}. In a two-generation study, Luebker et al. (2005, 1276160) also reported no effects on learning, memory, and short-term retention, as measured in a passive avoidance paradigm, and Butenhoff et al. (2009, 757873) 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, 2009, 757873}. 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, 2008, 1276156}; 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, 2007, 757863}. 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, 2007, 757865}. 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, 2007, 757863}. 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, 2008, 1276156}.

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. (2007, 757863)[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, 6833692)[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, 1276156)[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. (2007, 757865)[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, 2850984)[d]	Subchronic exposure (3 months) 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. (2015, 2851030)[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, 757873)[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. (2005, 1276160)[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, 2016, 3603365}.

Several short-term studies in mice and rats {Salgado, 2015, 3981583; Salgado, 2016, 3179088; Lopez-Doval, 2015, 2848266}, one developmental study in mice {Mshaty, 2020, 6833692}, and one subchronic study in mice {Long, 2013, 2850984} 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, 2020, 6833692}. 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, 2015, 2848266}. The effect of PFOS on dopamine and/or gamma-aminobutyric acid (GABA) in various brain regions was examined in three studies {Mshaty, 2020, 6833692; Long, 2013, 2850984; Salgado, 2015, 3981583}. 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, 2013, 2850984}. 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, 2020, 6833692}. 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, 2015, 3981583}. 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, 2016, 3179088}. 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, 2015, 3981583; Salgado, 2016, 3179088}. No changes in dopamine levels were seen in the mediobasal hypothalamus {Salgado, 2015, 3981583}. 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, 2013, 2850984}. 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, 2020, 6833692}. 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, 2850984) study compared to increases seen at a lower dose in the Mshaty et al. (2020, 6833692) 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, 6833692)[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, 2850984)[b]	Subchronic exposure (3 months) 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, 3981583)[a]	Short-term exposure (28 days) 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, 3179088)[a]	Short-term exposure (28 days) 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, 2848266)[a]	Short-term exposure (28 days) 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: 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, 2019, 5080461}. Zhang et al. (2019, 5080461) 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, 2016, 3603365}. 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 Tableau.

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 {Chen, 2013, 2850933; Jeddy,

2017, 3859807; Niu, 2019, 5381527}, cognitive development {Harris, 2018, 4442261; Oulhote, 2019, 6316905}, and executive function {Vuong, 2016, 3352166} 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, 6833686}, 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, 2016, 3789517; Oulhote, 2019, 6316905; Ghassabian, 2018, 5080189}, however overall results were mixed. Of the multiple studies examining associations between PFOS and ADHD, only one {Lenters, 2019, 5080366} 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, 2018, 4239287}. There was an indication of a potential relationship between PFOS and autistic behaviors or ASD diagnosis in some studies {Braun, 2014, 2345999; Oulhote, 2016, 3789517; Shin, 2020, 6507470}. 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, 6833692) 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, 6833692). 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, 2850984) 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 reported. One *high*	• *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**	
	confidence study observed non-significantly increased non-verbal 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 correlation with the region of the brain	• *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**	
	associated with impulsive behavior.				
Depression 3 *Medium* confidence studies 1 *Low c*onfidence study	One *medium* confidence study reported positive but non-significant results for depression in general population adults. Another *medium* confidence study explored depression in children followed for 20 years, 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 reported no significant associations. A *medium*	• *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**	
	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 to males. Findings from the five *medium* confidence studies were mixed. Two studies observed positive associations, with one	• *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**	
	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 significant effects were reported from the	• *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**	
	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	

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**	
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 region-specific decreases of GABA (1/1) and dopamine (2/2).	• *Medium* confidence studies • *Coherence* of findings in neurobehavior endpoints • *Dose-response* relationship	• *Limited number* of studies examining outcome • *Biological significance* of the magnitude of effect is unclear	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 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		

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**	
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: 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 2 were considered *uninformative* (Section C.5.1). Of the animal studies, 2 were classified as *high* confidence, 8 as *medium* confidence, and 2 were considered *low* confidence (Section C.5.2). Studies may have multiple judgments 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 Main PFOS Document).

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, 2016, 3603365}. 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 HESD for PFOS {U.S. EPA, 2016, 3603365} 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, 2011, 2919232; Steenland, 2010, 1290810} and two on children {Geiger, 2014, 2851286; Watkins, 2013, 2850974}; 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 {Geiger, 2013, 2919148; Kataria, 2015, 3859835; Khalil, 2018, 4238547; Predieri, 2015, 3889874; Qin, 2016, 3981721}, one study in pregnant women {Nielsen, 2020, 6833687}, one study in occupational workers {Rotander, 2015, 3859842}, 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, 2015, 3889874}, and three cohorts {Blake, 2018, 5080657; Conway, 2018, 5080465; Nielsen, 2020, 6833687} (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, 2019, 5080583; Zeng, 2019, 5918630}. Among the studies investigating populations in the United States, five studies utilized data from the NHANES {Geiger, 2013, 2919148; Jain, 2019, 5080378; Jain, 2019, 5381566; Kataria, 2015, 3859835; Scinicariello, 2020, 6833670}. Outcomes evaluated in these studies including 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, 2017, 3981432}.

There are 19 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} 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* {Predieri, 2015, 3889874; Seo, 2018, 4238334}. 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 {Khalil, 2018, 4238547; Nielsen, 2020, 6833687}. 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, 3889874) included a small sample size and narrow ranges of exposures which contributed to an *uninformative* rating. Seo et al. (2018, 4238334) 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.

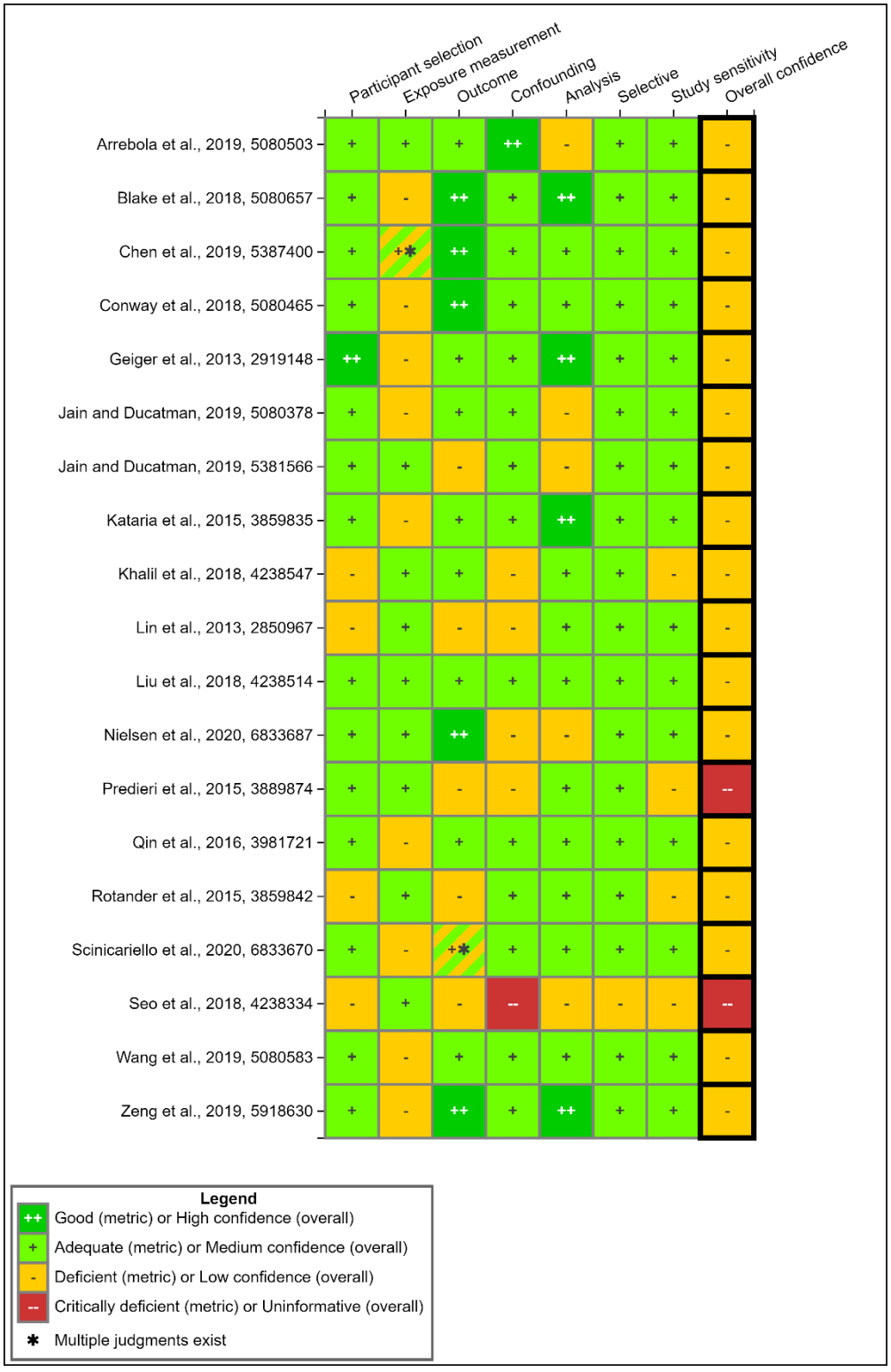


Figure C-31. Summary of Study Evaluation for Epidemiology Studies of PFOS and Renal Effects

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 {Geiger, 2013, 2919148; Qin, 2016, 3981721; Kataria, 2015, 3859835} with two also reporting on hyperuricemia {Geiger, 2013, 2919148; Qin, 2016, 3981721}, 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, 2919148) 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, 2015, 3859835} 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 to the lowest PFOS quartile (< 7.9 ng/mL). Qin et al. (2016, 3981721) 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, 2015, 3859835} 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 to the lowest quartile.

Two *low* confidence studies and one *uninformative* study investigated serum creatinine among children and adolescents {Kataria, 2015, 3859835; Khalil, 2018, 4238547; Predieri, 2015, 3889874}. One *low* confidence study {Kataria, 2015, 3859835} 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, 2018, 4238547} 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 {Conway, 2018, 5080465; Wang, 2019, 5080583} 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. (2019, 5080583) 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, 2020, 6833670} 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 {Arrebola, 2019, 5080503; Chen, 2019, 5387400; Jain, 2019, 5080378; Lin, 2013, 2850967; Scinicariello, 2020, 6833670; Zeng, 2019, 5918630} and one *low* confidence occupational study {Rotander, 2015, 3859842} examined PFOS and uric acid levels, and three of those studies evaluated uric acids as they pertained to hyperuricemia {Arrebola, 2019, 5080503; Scinicariello, 2020, 6833670; Zeng, 2019, 5918630}.

A *low* confidence NHANES (2009–2014) study {Scinicariello, 2020, 6833670} 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 to 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, 2019, 5918630} 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 (2019, 5080378) 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, 2019, 5387400}, or in adolescents and young adults aged 12–30 years in the Young Taiwanese Cohort Study {Lin, 2013, 2850967}. Another *low* confidence study {Arrebola, 2019, 5080503} using pooled cohort data (the BIOAMBIENT.ES study) observed a non-s ignificant 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, 2015, 3859842}. No significant association was observed for serum uric acid and increasing PFOS exposure.

Two general population studies evaluated PFOS and eGFR {Blake, 2018, 5080657; Wang, 2019, 5080583}. A *low* confidence study {Blake, 2018, 5080657} 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, 2019, 5080583} 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, 2020, 6833687} 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, 2019, 5387400; Jain, 2019, 5381566}. 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, 2019, 5387400} did not observe significant associations with plasma creatinine in Croatian adults ages 44–56 years.

One *low* confidence study, Liu et al. (2018, 4238514) 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 4 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 8 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.

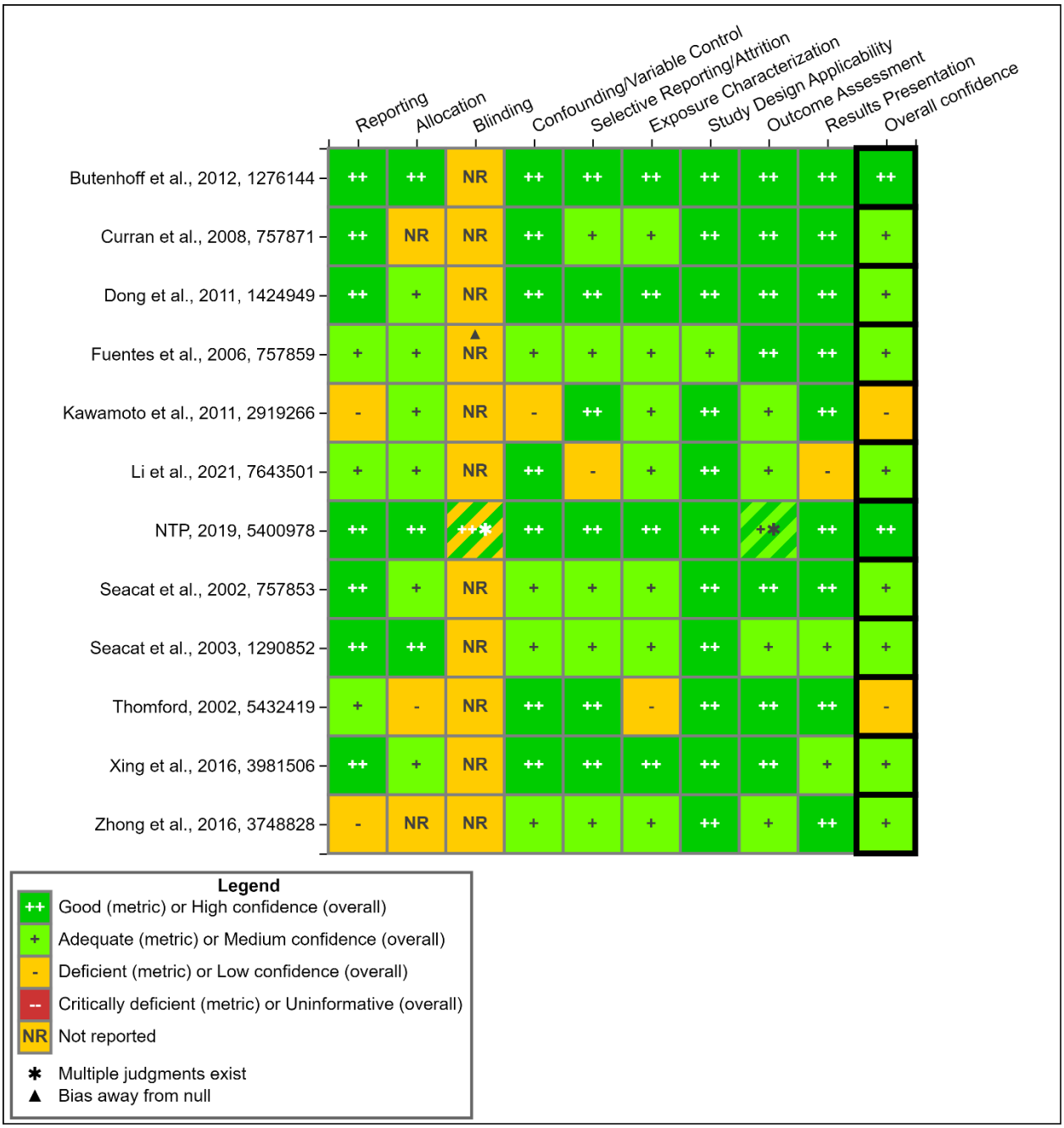


Figure C-32. Summary of Study Evaluation for Toxicology Studies of PFOS and Renal Effects

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 {Seacat, 2002, 757853; Seacat, 2003, 1290852; Fuentes, 2006, 757859; Peden-Adams, 2008, 1424797; Yahia, 2008, 2919381; Dong, 2011, 1424949; Zhong, 2016, 3748828; Li, 2021, 7643501}. 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, 2016, 3981506} 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, 2009, 1424951}. 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, 2006, 757859}.

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, 5400978}, 5 mg/kg/day {Cui, 2009, 757868}, 6 mg/kg/day {Goldenthal, 1978, 1291068}, and 6.34 mg/kg/day {Curran, 2008, 757871}. NTP (2019, 5400978) 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, 757868) 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, 1291068), Cui et al. (2009, 757868), and Curran et al. (2008, 757871). Curran et al. (2008, 757871) 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, 5400978) 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, 757871) 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, 2012, 1276144}.

Cui et al. (2009, 757868) 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, 757868), all other studies reported no treatment-related changes in kidney histopathology {Seacat, 2003, 1290852; Curran, 2008, 757871; Yahia, 2008, 2919381; Butenhoff, 2012, 1276144; Xing, 2016, 3981506; NTP, 2019, 5400978; Li, 2021, 7643501}.

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, 1290852) and NTP (2019, 5400978) (males only) both reported significant increases in BUN in rats after 14-week and 28-day exposures, respectively. Similarly, Curran et al. (2008, 757871) 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, 1290852) study, Butenhoff et al. (2012, 1276144) 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 {Seacat, 2003, 1290852; Curran, 2008, 757871; Butenhoff, 2012, 1276144; NTP, 2019, 5400978}; NTP (2019, 5400978) and Butenhoff et al. (2012, 1276144) 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, 2016, 3981506} or in male or female monkeys treated with up to 0.75 mg/kg/day PFOS for 26 weeks {Seacat, 2002, 757853}. Other clinical chemistry endpoints, including creatine kinase {Seacat, 2002, 757853; Curran, 2008, 757871; NTP, 2019, 5400978}, uric acid {Curran, 2008, 757871}, urinary N-acetyl-b-glucosaminidase (NAG) {Xing, 2016, 3981506}, and urinalysis parameters including urine pH {Seacat, 2002, 757853; Seacat, 2003, 1290852; Curran, 2008, 757871; Butenhoff, 2012, 1276144}, 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, 2016, 3603365}. There are 3 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 Tableau.

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 HESD for PFOS {U.S. EPA, 2016, 3603365} 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 {Conway, 2018, 5080465; Wang, 2019, 5080583}. 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 {Blake, 2018, 5080657; Wang, 2019, 5080583}. 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, 2015, 3859835}. 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.6.1)					⊕⊙⊙ ***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.6.2)					
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 • Changes in body weight may limit ability to interpret these responses	⊙⊙⊙ *Indeterminate* Evidence was based on 10 *high* and *medium*	

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.			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 weeks 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.

C.6 Hematological

EPA identified 8 epidemiological and 5 animal studies that investigated the association between PFOS and hematological effects. Of the epidemiological studies, 3 were classified as *medium* confidence, 2 as *low* confidence, and 3 were considered *uninformative* (Section C.6.1). Of the animal studies, 1 was classified as *high* confidence, 3 as *medium* confidence, 1 was considered *low* confidence (Section C.6.2). Studies may have multiple judgments 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 Main PFOS Document).

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, 2020, 6506041; Chen, 2019, 5387400; Jain, 2020, 6333438}. PFOS has been implicated in endocrine disruption, which may affect vitamin D homeostasis {Etzel, 2019, 5043582}. It could also alter epigenetics via DNA methylation {van den Dungen, 2017, 5080340}. 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 HESD for PFOS {U.S. EPA, 2016, 3603279}. 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 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 {Etzel, 2019, 5043582; Jain, 2020, 6333438; Jain, 2020, 6833623}. Etzel et al. (2019, 5043582) used 2003–2010 NHANES data for adolescents and adults 12 years and older, and Jain (2020, 6333438) and Jain (2020, 6833623), used 2003–2016 NHANES data for adults 20 years and older. Also in the United States, Khalil et al.(2018, 4238547) included 48 obese children 8–12 years old from a hospital lipid clinic in Dayton, Ohio. Abraham et al.(2020, 6506041) 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, 2850910) recruited 141 pregnant women in Tianjin, China. Chen et al.(2019, 5387400) 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, 5080340) 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, 2020, 6506041}. The blood matrix (whole blood vs. 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, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures.

There are 8 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and hematological effects. Study quality evaluations for these 8 studies are shown in Figure C-34.

Based on 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, 4238547) 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, 5080340) 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 (2020, 6833623), the use of PFOS as the dependent variable and health outcomes as the independent (predictive) variable rendered the study uninformative for hazard assessment {Jain, 2020, 6833623}. Abraham et al. (2020, 6506041) and Jiang et al. (2014, 2850910) only performed unadjusted correlation analyses and therefore did not consider the influence of potential confounding factors.

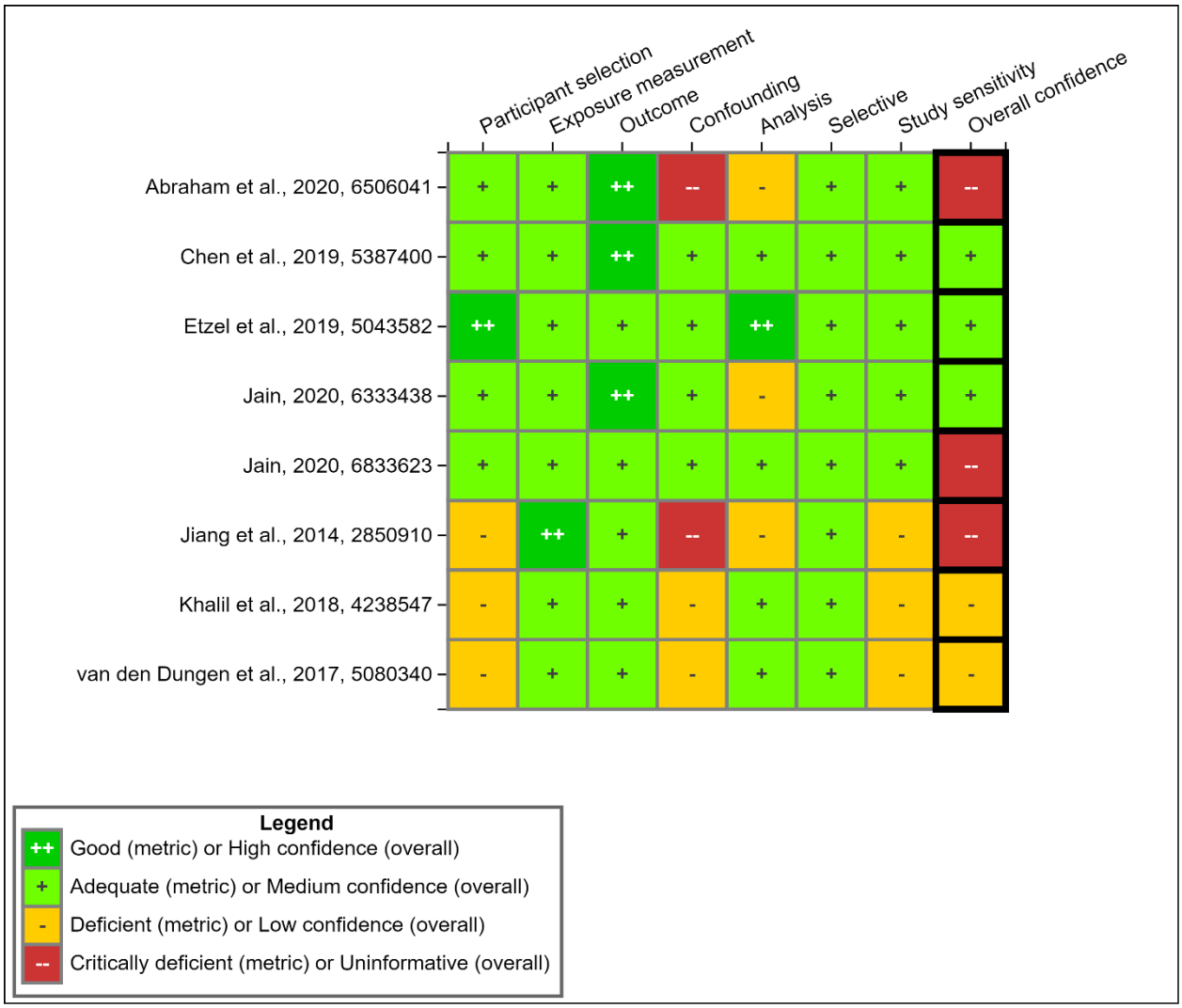


Figure C-34. Summary of Study Evaluation for Epidemiology Studies of PFOS and Hematological Effects

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, 2019, 5043582}. In adolescents and adults from NHANES (2003–2010), Etzel et al.(2019, 5043582) 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, 4238547) also observed a decrease in 25-hydroxy vitamin D levels, but it did not reach significance.

In adults from NHANES (2003–2016), Jain(2020, 6333438) 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 (2020, 6333438) also evaluated 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, 2014, 2850910}. 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, 2017, 5080340}, observed non-significant decreases in hemoglobin and hematocrit levels, and non-significant increases in retinol.

Chen et al.(2019, 5387400) 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 3 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 2 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 5 studies are shown in Figure C-35

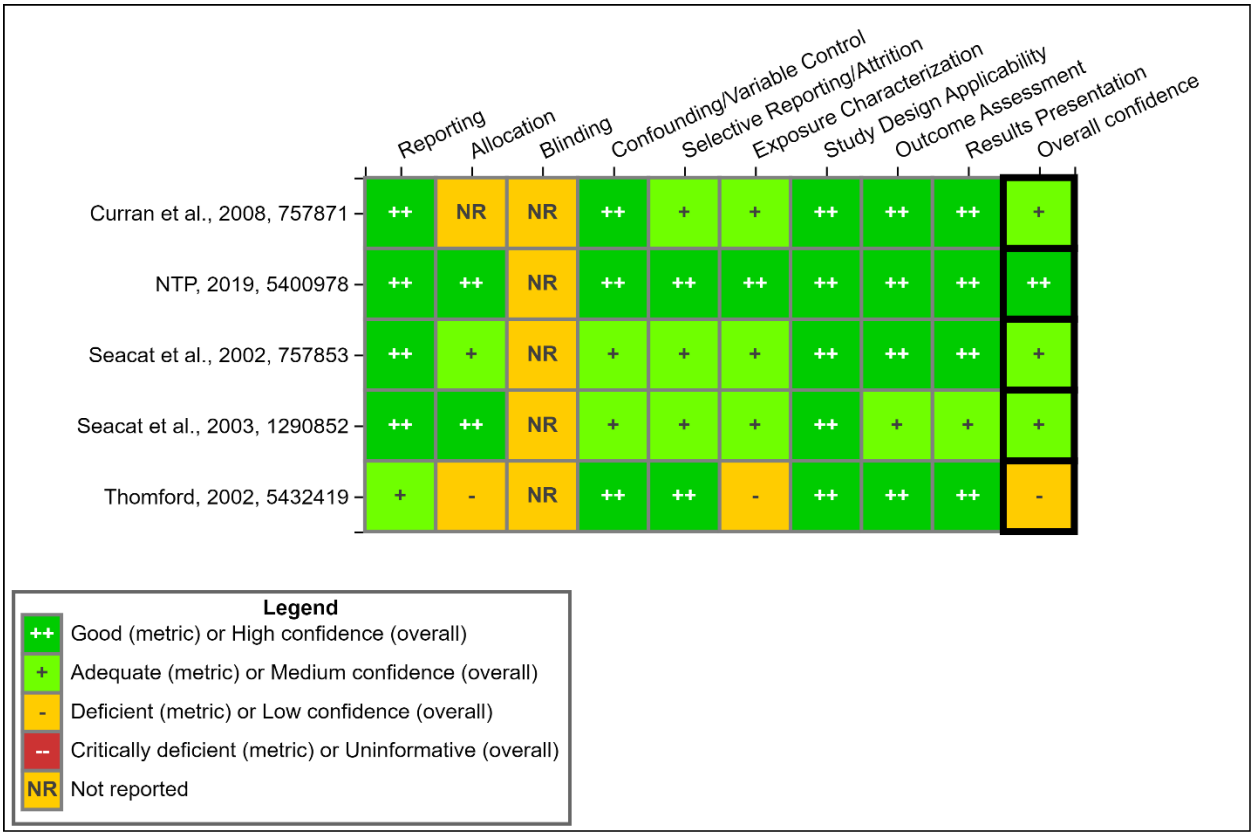


Figure C-35. Summary of Study Evaluation for Toxicology Studies of PFOS and Hematological Effects

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 PFOS Main Document).

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, 5400978}. 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 PFOS Main Document). NTP (2019, 5400978) 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, 5400978) 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, 2003, 1290852}. 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, 2008, 757871}. 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, 2008, 757871}.

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, 2003, 1290852}. 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, 757853) 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, 2016, 3603365}. There are 2 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 Tableau.

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, 2020, 6333438}, particularly among anemic adults in a large NHANES study. Increases in hemoglobin and RBC may also affect pregnant women {Jiang, 2014, 2850910}. 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, 5400978). These reductions in reticulocyte counts support histopathological changes in the spleen (splenic extramedullary hematopoiesis) that have been identified as notable immune endpoints (see PFOS Main Document). 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 at the highest dose tested following short-	• *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	

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**	
	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 short-term exposure to PFOS. No significant exposure-related changes were observed in platelet count (3/3).			hematological 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**	
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 5 epidemiological and 5 animal studies that investigated the association between PFOS and respiratory effects. All 5 of the epidemiological studies were classified as *medium* confidence (Section C.7.1). Of the animal studies, 1 was classified as *high* confidence, 3 as *medium* confidence, and 1 was considered *low* confidence (Section C.7.2). Studies may have multiple judgments 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 Main PFOS Document).

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, 2016, 3603365} 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, 2019, 5043613; Impinen, 2018, 4238440; Manzano-Salgado, 2019, 5412076}, one was a cross-sectional case-control study (cross-sectional analyses were performed in asthmatic cases and non-asthmatic controls) conducted in Taiwan {Qin, 2017, 3869265}; and one was a cross-sectional study of adolescents and young adults residing near the WTC {Gaylord, 2019, 5080201}. 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 20Hz, 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 5 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and respiratory effects. Study quality evaluations for these 5 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, 2019, 5080201}, reduced sensitivity because of low exposure levels and narrow ranges {Impinen, 2018, 4238440}, or concerns with potential bias in selection of non-asthmatic controls {Qin, 2017, 3869265}.

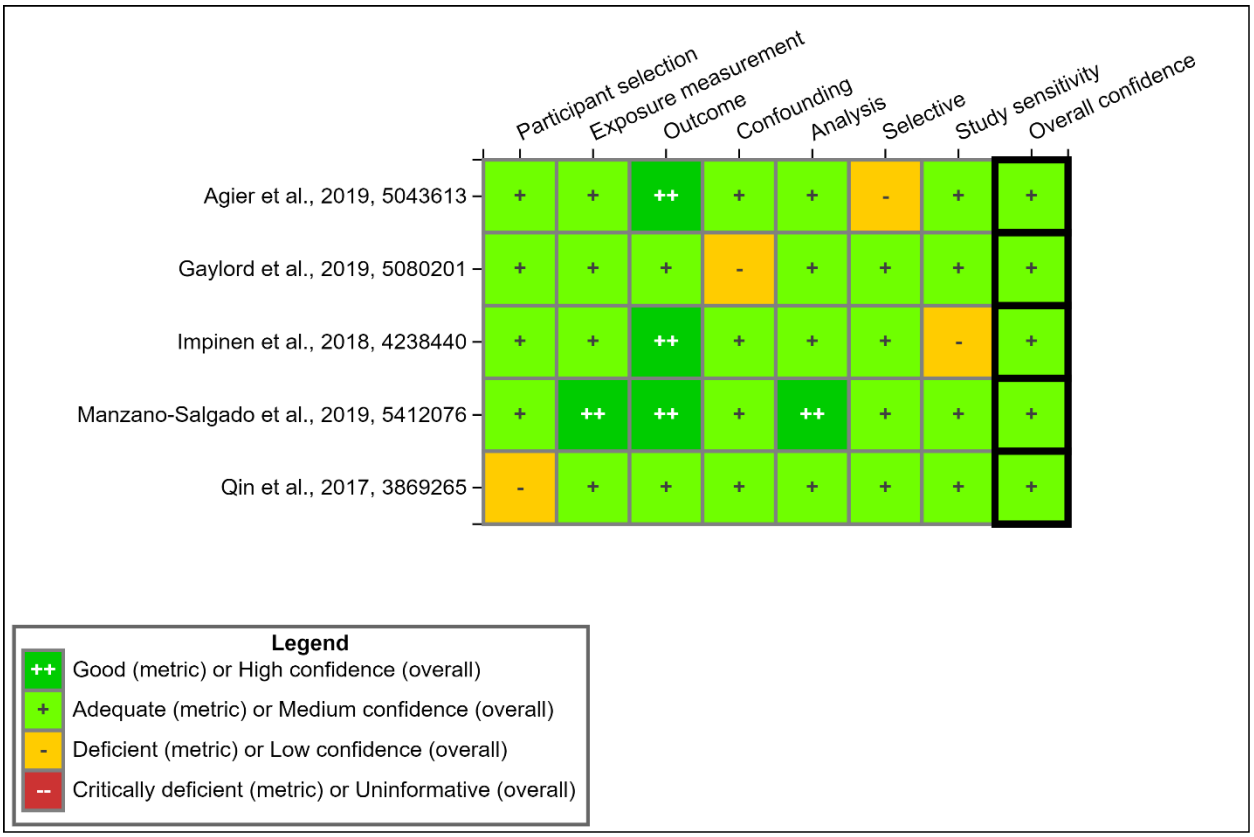


Figure C-37. Summary of Study Evaluation for Epidemiology Studies of PFOS and Respiratory Effects

Interactive figure and additional study details available on HAWC.

C.7.1.3 Findings in Children and Adolescents

Four studies examined respiratory health effects in children up to 15 years old {Agier, 2019, 5043613; Impinen, 2018, 4238440; Manzano-Salgado, 2019, 5412076; Qin, 2017, 3869265} and one examined adolescents and young adults ages 13–22 years {Gaylord, 2019, 5080201} (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, 3869265) 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, 2019, 5080201; Manzano-Salgado, 2019, 5412076} observing inverse associations and one study {Agier, 2019, 504613} reporting a positive association.

For other lung function measures examined there was also limited evidence of associations. Qin et al. (2017, 3869265) 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, 4238440) 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, 2019, 5080201; Manzano-Salgado, 2019, 5412076}.

C.7.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 5 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and respiratory effects. Study quality evaluations for these 5 studies are shown in Figure C-38.

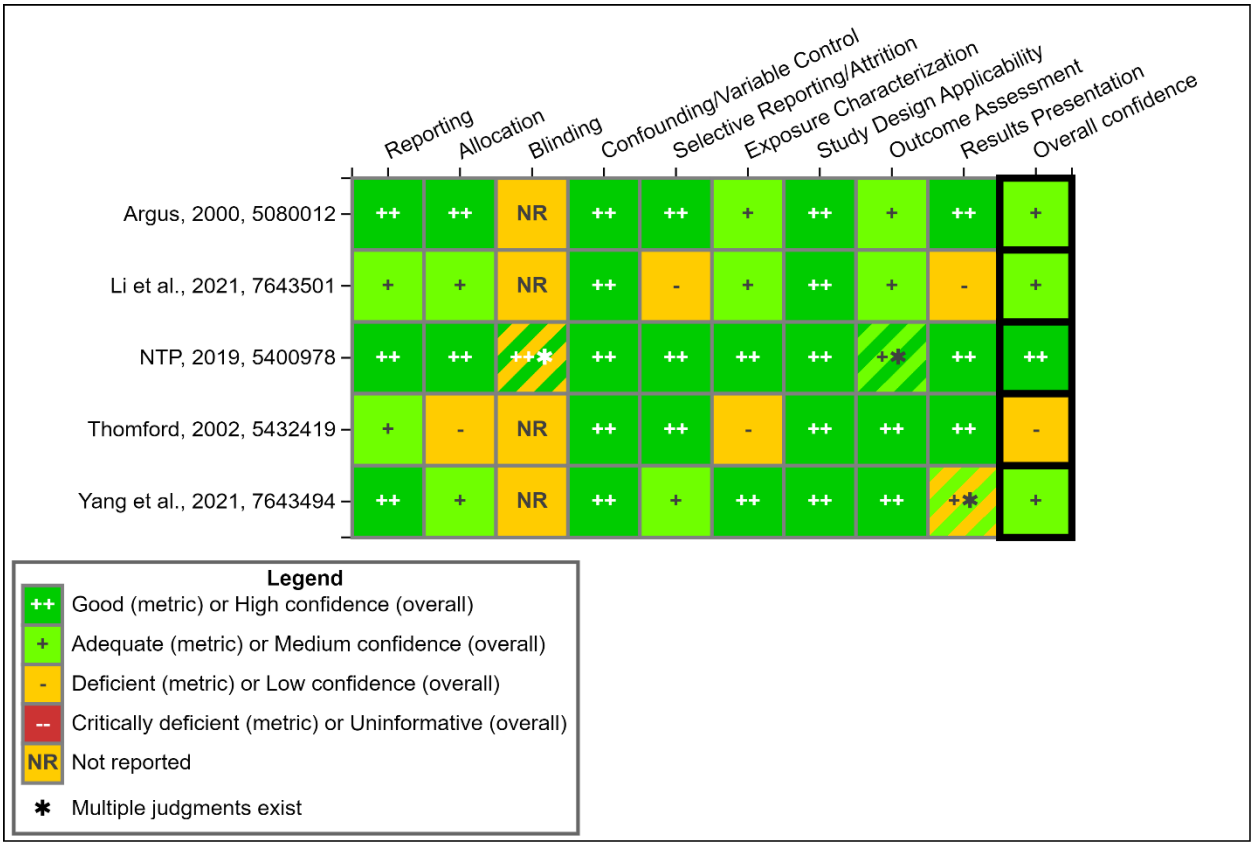


Figure C-38. Summary of Study Evaluation for Toxicology Studies of PFOS and Respiratory Effects

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 {Argus Research Laboratories, 2000, 5080012; Grasty, 2003, 1332670; Grasty, 2005, 2951495; Yahia, 2008, 2919381; Chen, 2012, 1276152; Ye, 2012, 2919212; U.S. EPA, 2016, 3603365}. There are also several available studies that reported pulmonary effects in adult mammalian models {Goldenthal, 1979, 9573133; Cui, 2009, 757868; NTP, 2019, 5400978; Li, 2021, 7643501; Yang, 2021, 7643494}.

Yahia et al. (2008, 2919381) 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 5 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, 2919381) 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, 2008, 2919381}.

Chen et al. (2012, 1276152) 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, 6988534) 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 to controls.

In an attempt to identify the prenatal window of susceptibility to PFOS in neonatal rats, Grasty et al. (2003, 1332670) 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, 2003, 1332670}. 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, 2951495) 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, 2951495) 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, 1332670), 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, 2919212) 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, 2003, 1332670; Grasty, 2005, 2951495}.

In a rabbit teratology study, Argus (2000, 5080012) 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 to 2/175 in controls). However, this increase was not statistically significant when analyzed by litter (4/19 litters compared to 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, 5080012) 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, 757868) 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, 9573133) 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, 5400978) 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, 7643494) 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 to the control group. The toxicological significance of the increase is unclear due to both low sample size with 6 animals per group and lack of report on body weight or absolute lung weight. Li et al. (2021, 7643501) 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, 7643494). 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 to 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, 2016, 3603365}. There are 3 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 Tableau.

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 {NTP, 2019 5400978; Li, 2021, 7643501}, but an exacerbated immune response appears to occur in the lung based on a *medium* confidence study {Yang, 2021, 7643494}. 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 PFOS Main Document), 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–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, 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***
Lung function measures 4 *Medium* confidence studies	Two studies (2/4) observed decreases in forced expiratory volume in 1s (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 FEF25-75% 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.	*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, 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.
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 was observed following	• *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.	

	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: FEF25-75% = forced expiratory flow at 25-75%. ; FEV1 = forced expiratory volume; FVC = forced vital capacity.

C.8 Musculoskeletal

EPA identified 6 epidemiological and 1 animal studies that investigated the association between PFOS and musculoskeletal effects. Of the epidemiological studies, 6 were classified as *medium* confidence and 2 as *low* confidence (Section C.8.1). The animal study was classified as *low* confidence (Section C.8.2). Studies may have multiple judgments 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 Main PFOS Document).

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 {Uhl, 2013, 1937226; Khalil, 2016, 3229485}.

The 2016 HESD for PFOS {U.S. EPA, 2016, 3603365} 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, 2019, 6315798}. All studies measured PFOS in blood components (i.e., blood, plasma, or serum), and one study {Di Nisio, 2019, 5080655} measured PFOS in semen. Three studies {Khalil, 2016, 3229485; Lin, 2014, 5079772; Uhl, 2013, 1937226} 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, 2019, 5412438}, the POUNDS-Lost clinical trial {Hu, 2019, 6315798}, and the ALSPAC {Jeddy, 2018, 5079850}. 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, 2019, 5412438; Jeddy, 2018, 5079850; Khalil, 2016, 3229485; Khalil, 2018, 4238547}. 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 8 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and musculoskeletal effects. Study quality evaluations for these 8 studies are shown in Figure C-40.

Based on the considerations mentioned, six studies were classified as *medium* confidence and two as *low* confidence. The two cross-sectional studies {Di Nisio, 2019, 5080655; Khalil, 2018, 4238547} 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.

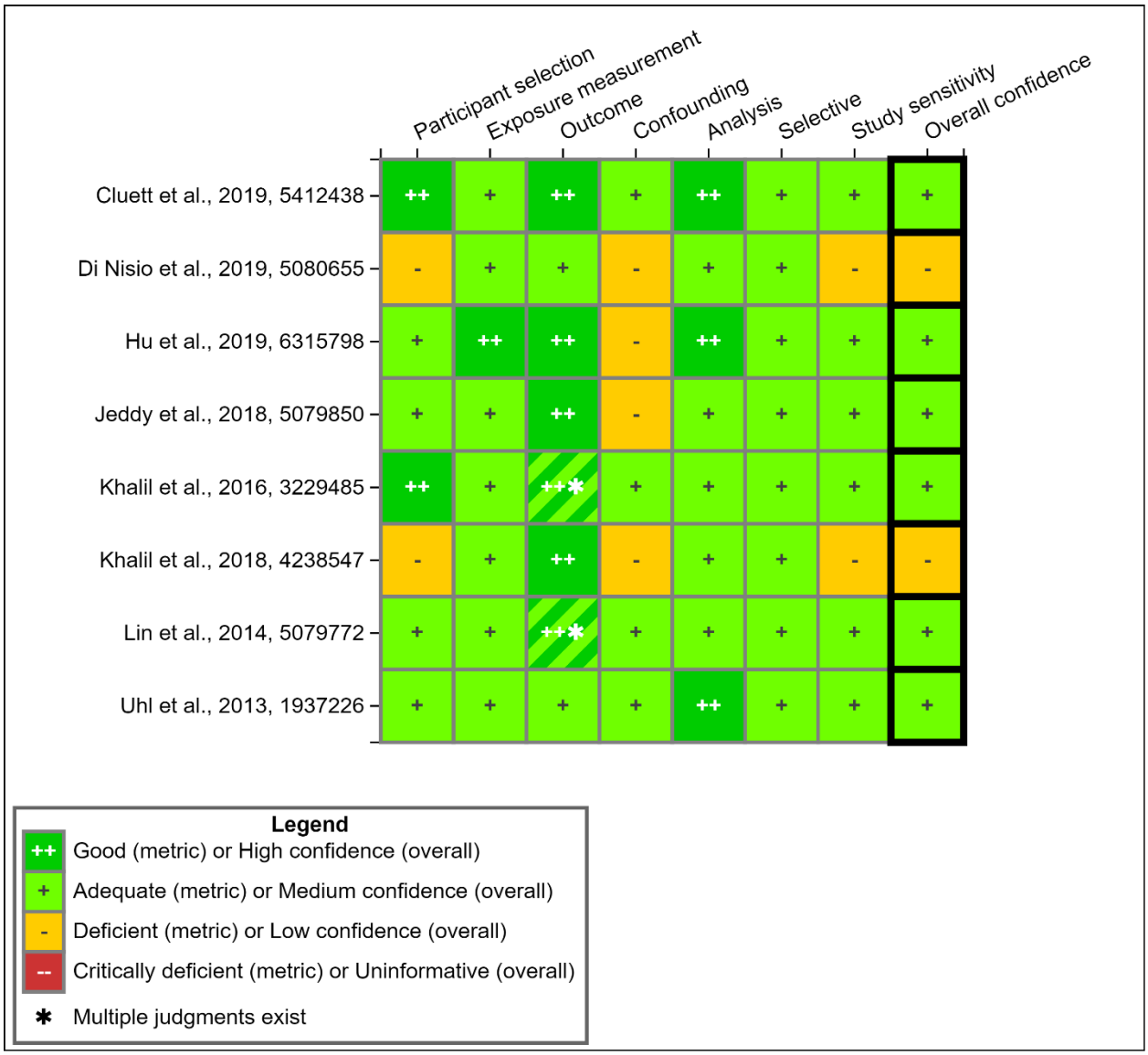


Figure C-40. Summary of Study Evaluation for Epidemiology Studies of PFOS and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

C.8.1.3 Findings from Children and Adolescents

Three studies {Cluett, 2019, 5412438; Jeddy, 2018, 5079850; Khalil, 2018, 4238547} 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, 5412438) 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 BMD in boys and in girls with bone mineral content (BMC) z score. Jeddy et al. (2018, 5079850) 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 bone mineral content 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, 2018, 4238547} 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 {Khalil, 2016, 322948; Uhl, 2013, 1937226; Lin, 2014, 5079772; Hu, 2019, 6315798; Di Nisio, 2019, 5080655} 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, 1937226) 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, 2014, 5079772}, observed decreased total lumbar spine bone mineral density 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, 3229485) observed a statistically significant inverse association with bone mineral density 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, 6315798) observed small but statistically significant inverse associations with bone mineral density (or two-year change in bone mineral density) 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, 2019, 5080655} did not find evidence of associations between PFOS exposure and arm span.

C.8.2 Animal Evidence Study Quality Evaluation and Synthesis

There is 1 study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and musculoskeletal effects. Study quality evaluation for this 1 study is shown in Figure C-41.

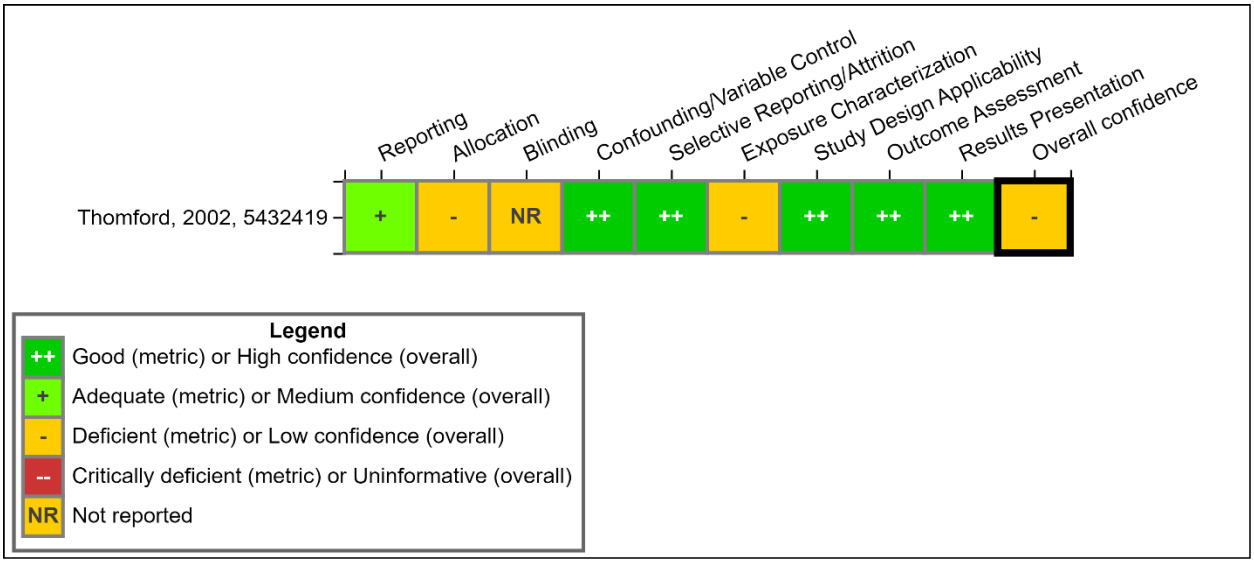


Figure C-41. Summary of Study Evaluation for Toxicology Studies of PFOS and Musculoskeletal Effects

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 PFOS Main Document). 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, 2016, 3603365} 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, 2016, 3603365}. There are 6 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 Tableau.

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 bone mineral density 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 HESD for PFOS {U.S. EPA, 2016, 3603365}.

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 bone mineral density (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 PFOS Main Document). 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 bone mineral density 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 bone mineral content (BMC) were observed in two studies (2/6), with significant decreases observed among female children. Reductions in bone mineral density (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 bone mineral density 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 years 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 span were largely	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome • *Low* confidence study		

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**	
	imprecise in a study on male high school students.				
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.

C.9 Gastrointestinal

EPA identified 4 epidemiological and 2 animal studies that investigated the association between PFOS and gastrointestinal effects. Of the epidemiological studies, 3 were classified as *medium* confidence and 1 as *low* confidence (Section C.9.1). Of the animal studies, 1 was classified as *high* confidence, and 1 was considered *low* confidence (Section C.9.2). Studies may have multiple judgments 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 Main PFOS Document).

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 HESD for PFOS, 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, 1937228) 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, 2013, 1937228}.

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 {Steenland, 2018, 5079806; Xu, 2020, 6315709}. GI outcomes only assessed in the context of immune system health, including ulcerative colitis and Crohn's disease, are discussed (see PFOS Main Document). However, some research suggests an overall immunosuppressive effect of PFOS could reduce the efficiency of routine childhood immunizations {Dalsager, 2016, 3858505} 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, 2019, 8776815}.

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, 3858505) 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, 8776815) 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. (2020, 6315709) 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, 6833710) 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, 2020, 6315709}. 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, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures.

There are 4 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and gastrointestinal effects. Study quality evaluations for these 4 studies are shown in Figure C-43.

Based on the considerations mentioned, one study was considered *medium* confidence {Timmermann, 2020, 6833710} and three as *low* confidence {Dalsager, 2016, 3858505; Hammer, 2019, 8776815; Xu, 2020, 6315709}. The *medium* confidence study {Timmermann, 2020, 6833710} 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, 2020, 6315709}. Another concern was potential for outcome misclassification or underreporting due to inconsistent participation and adherence to the parent reporting mechanism {Dalsager, 2016, 3858505}. Another common reason for *low* confidence was a serious risk for residual confounding by SES {Hammer, 2019, 8776815}. Exposure misclassification was also a concern in Xu et al. (2020, 6315709), due to use of residential history as a proxy. Deficiencies in multiple domains contributed to an overall *low* confidence rating.

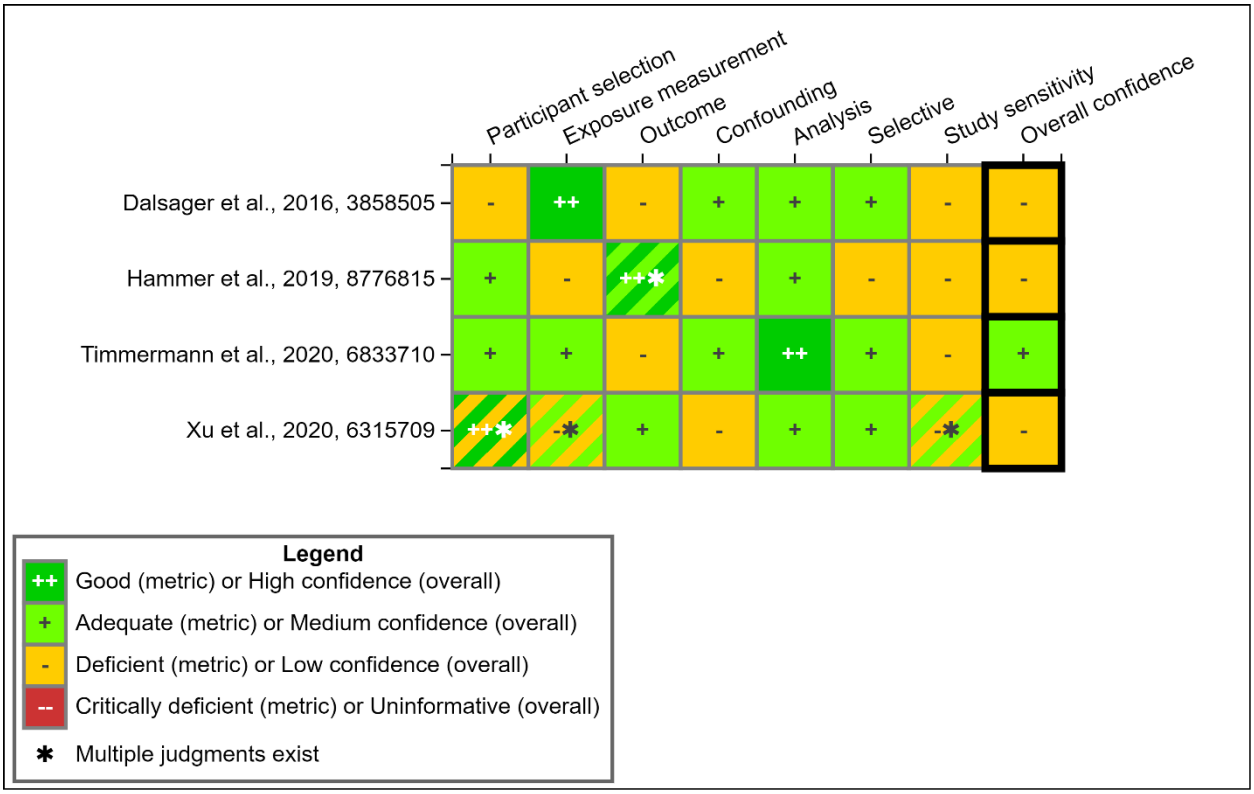


Figure C-43. Summary of Study Evaluation for Epidemiology Studies of PFOS and Gastrointestinal Effects

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, 6833710) observed increased odds of diarrhea in very young children (up to 9 months old) in Guinea-Bissau. Dalsager et al. (2016, 3858505) 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, 8776815) observed a non-significant decrease in incidence of IBD in Faroese children and adults. Xu et al. (2020, 6315709) 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 2 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the

association between PFOS and gastrointestinal effects. Study quality evaluations for these 2 studies are shown in Figure C-44.

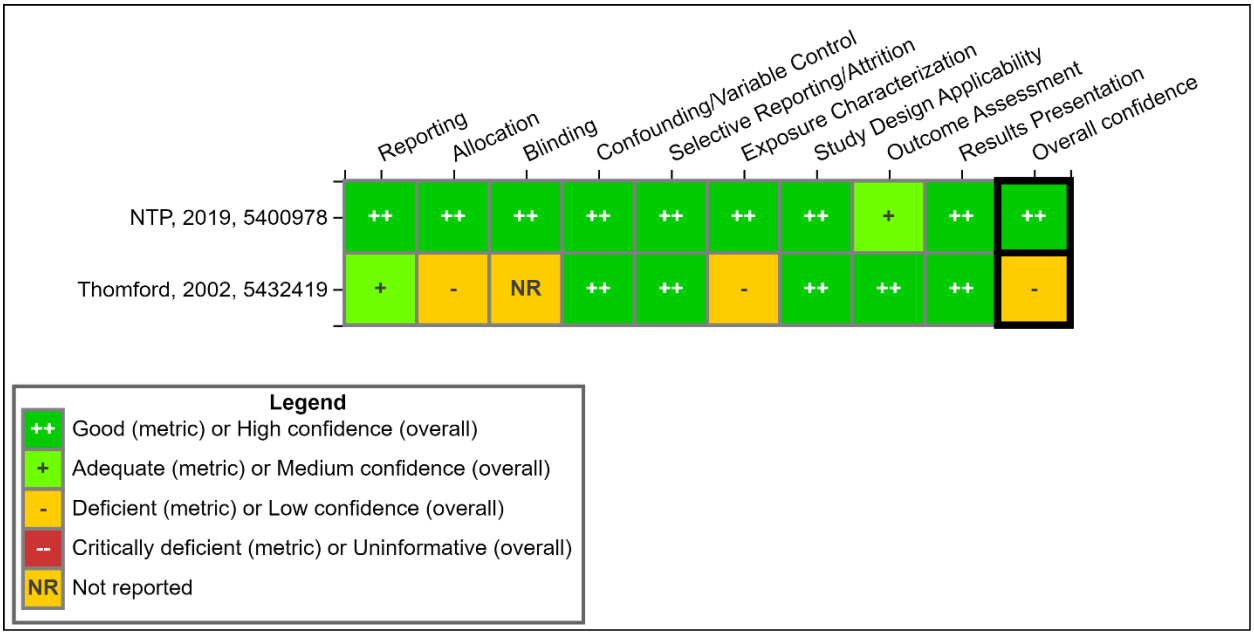


Figure C-44. Summary of Study Evaluation for Toxicology Studies of PFOS and Gastrointestinal Effects

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, 5400978), 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, 5400978}.

The 2016 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. Based on the findings, the acute oral LD_{50} was 233 mg/kg in males, 271 mg/kg in females, and 251 mg/kg combined {Dean, 1978, 9579905}.

The 2016 HESD also identified a sub-acute study in rhesus monkeys in which Goldenthal et al. (1979, 9573133) exposed 2 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, 1979, 9573133}.

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, 2016, 3603365}. 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 Tableau.

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 HESD for PFOS, 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 years of age. One study observed inconsistent non-significant associations with vomiting across exposure tertiles in children ages 1–4 years. 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 years 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 Stream Summary and Interpretation					Evidence Integration Summary Judgment
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.

C.10 Dental

EPA identified 2 epidemiological studies that investigated the association between PFOS and dental effects. No animal studies were identified. The 2 epidemiological studies were both classified as *medium* confidence (Section C.10.1). Studies may have multiple judgments 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 Main PFOS Document).

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, 2019, 5080517}. 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, 2019, 5386081}.

For this updated review, two studies examined the association between PFOS exposure and dental caries {Puttige Ramesh, 2019, 5080517; Wiener, 2019, 5386081}. 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, 2019, 5080517}. 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. (2019, 5080517} assessed data from 2,869 12–19-year-old adolescents included in 1999–2012 NHANES and Wiener and Waters (2019, 5386081) 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, 2019, 5386081}. 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, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures.

There are 2 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and dental effects. Study quality evaluations for these 2 studies are shown in Figure C-46.

Based on 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, 2019, 5080517; Wiener, 2019, 5386081}. Puttige Ramesh et al. (2019, 5080517) 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.

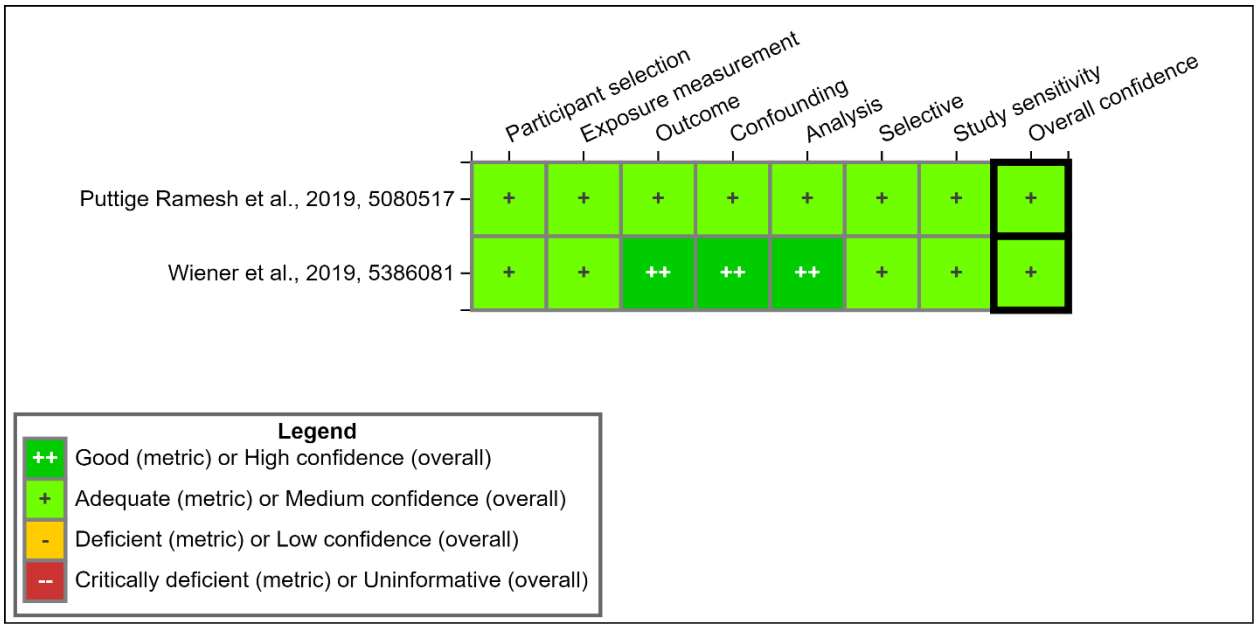


Figure C-46. Summary of Study Evaluation for Epidemiology Studies of PFOS and Dental Effects

Interactive figure and additional study details available on HAWC.

C.10.1.3 Findings

The two studies observed mixed effects {Puttige Ramesh, 2019, 5080517; Wiener, 2019, 5386081}. Wiener and Waters (2019, 5386081) 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, 5080517) observed increased odds of dental caries only in the third quartile of exposure, but decreased odds in the second and highest quartiles compared to 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, 2016, 3603365}. 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 HESD for PFOS {U.S. EPA, 2016, 3603365}. 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, 2019, 5080517; Wiener, 2019, 5386081}. 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 1 epidemiological and 2 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, 1 was classified as *high* confidence, and 1 was considered *low* confidence (Section C.11.2). Studies may have multiple judgments 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 Main PFOS Document).

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, 2020, 6315698}.

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 1 study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and ocular effects. Study quality evaluation for this 1 study is shown in Figure C-47.

Zeeshan et al. (2020, 6315698) 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 if exposure occurred at an etiologically relevant time period to reflect changes in ocular function.

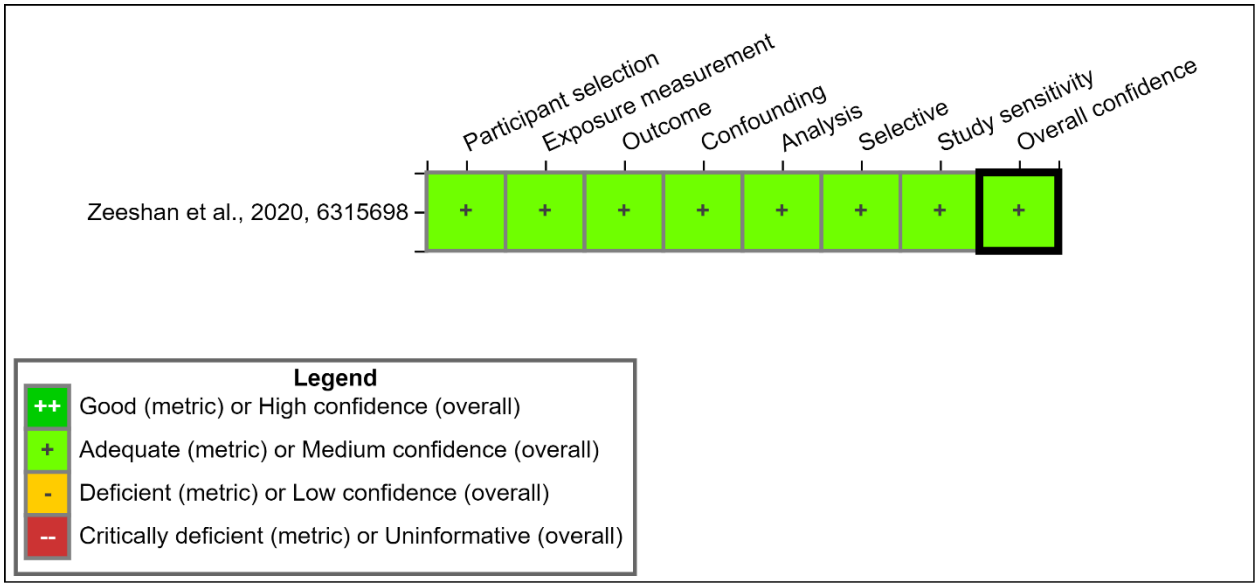


Figure C-47. Summary of Study Evaluation for Epidemiology Studies of PFOS and Ocular Effects

Interactive figure and additional study details available on HAWC.

C.11.1.3 Findings

Zeeshan et al. (2020, 6315698) 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 2 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and ocular effects. Study quality evaluations for these 2 studies are shown in Figure C-48.

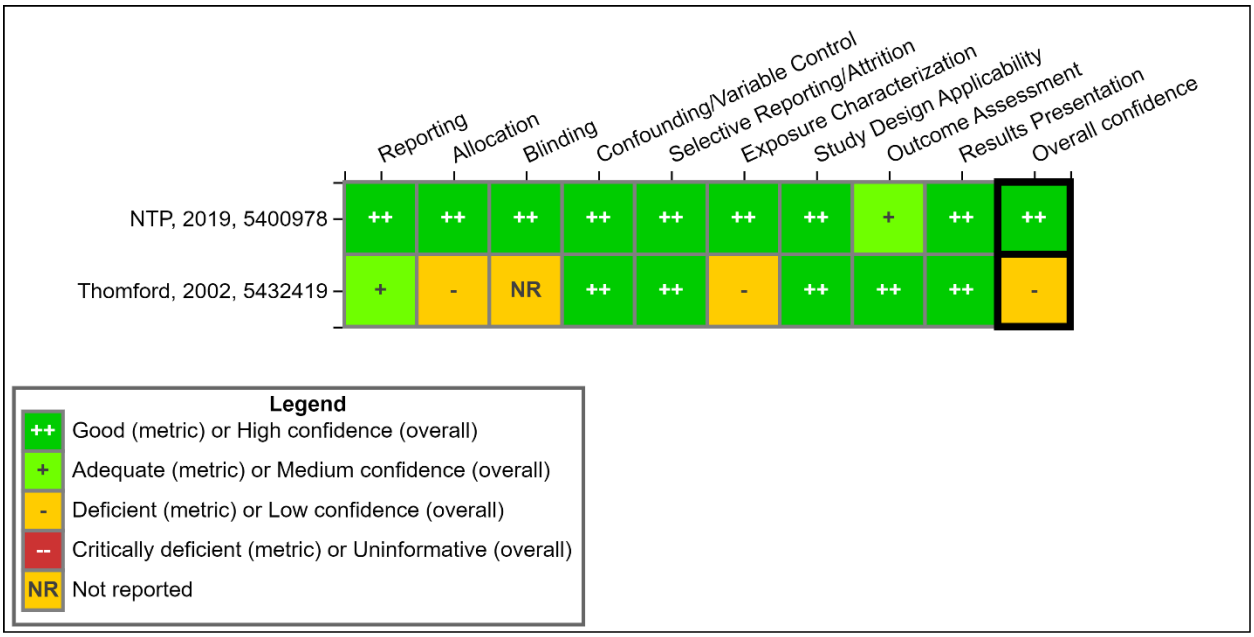


Figure C-48. Summary of Study Evaluation for Toxicology Studies of PFOS and Ocular Effects

Interactive figure and additional study details available on HAWC.

An eye irritation study in rabbits suggests that PFOS acts as an ocular irritant {Biesemeier, 1974, 4467668}; 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, 5400978}.

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, 2016, 3603365}. There is 1 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 Tableau.

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 years.	• *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 years.	
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.	

C.12 Dermal

EPA identified 1 epidemiological and 2 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, 1 was classified as *high* confidence, and 1 was considered *low* confidence (Section C.12.2). Studies may have multiple judgments 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 Main PFOS Document).

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, 5080529) examined the association between prenatal PFOS exposure and pubertal development. Mother-child pairs were recruited for the DNBC from 1996–2002, 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 1 study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and dermal effects. Study quality evaluation for this 1 study is shown in Figure C-50.

Ernst et al. (2019, 5080529) 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.

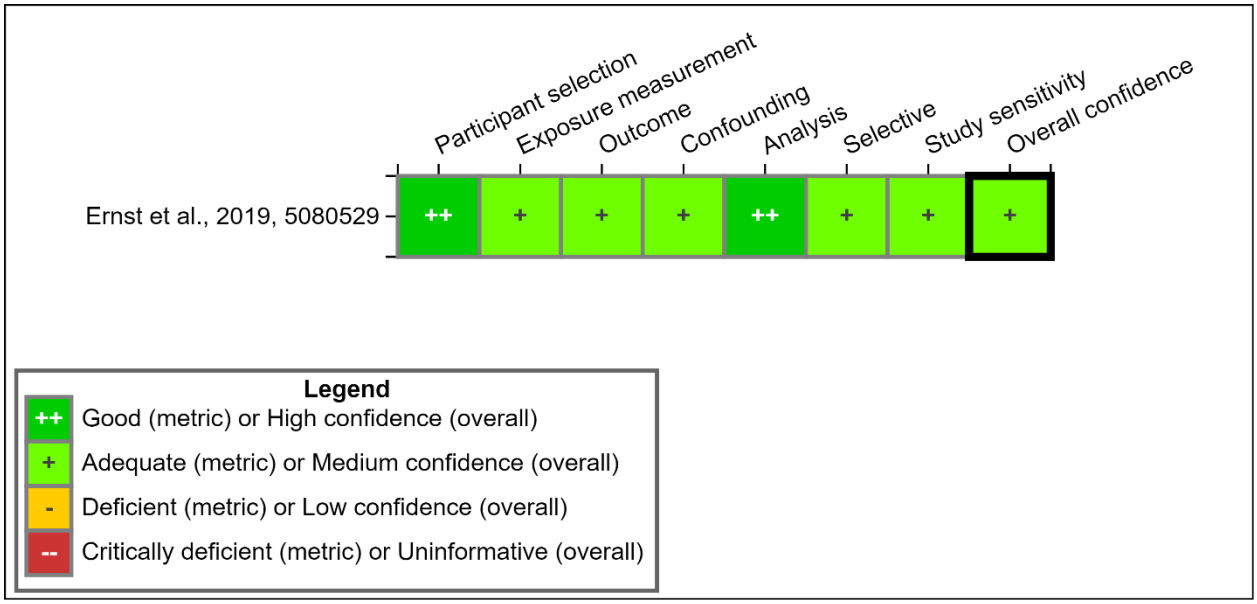


Figure C-50. Summary of Study Evaluation for Epidemiology Studies of PFOS and Dermal Effects

Interactive figure and additional study details available on HAWC.

C.12.1.3 Findings

Ernst et al. (2019, 5080529) 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 to the lowest (β: 0.09; 95% CI: −4.69, 4.87) {Ernst, 2019, 5080529}. Associations in boys were negative and non-significant (Appendix D).

C.12.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 2 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and dermal effects. Study quality evaluations for these 2 studies are shown in Figure C-51.

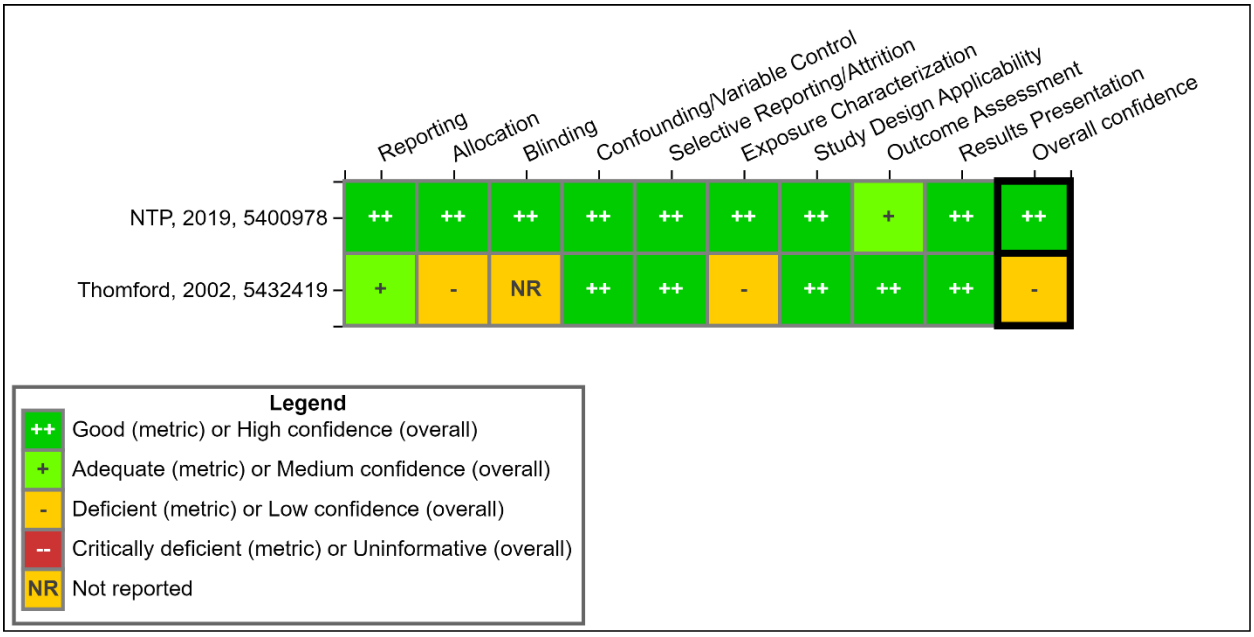


Figure C-51. Summary of Study Evaluation for Toxicology Studies of PFOS and Dermal Effects

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, 5400978}.

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, 2016, 3603365}. 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 HESD {U.S. EPA, 2016, 3603365}, 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, 2019, 5080529} 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

D.1.1 Forest Plots

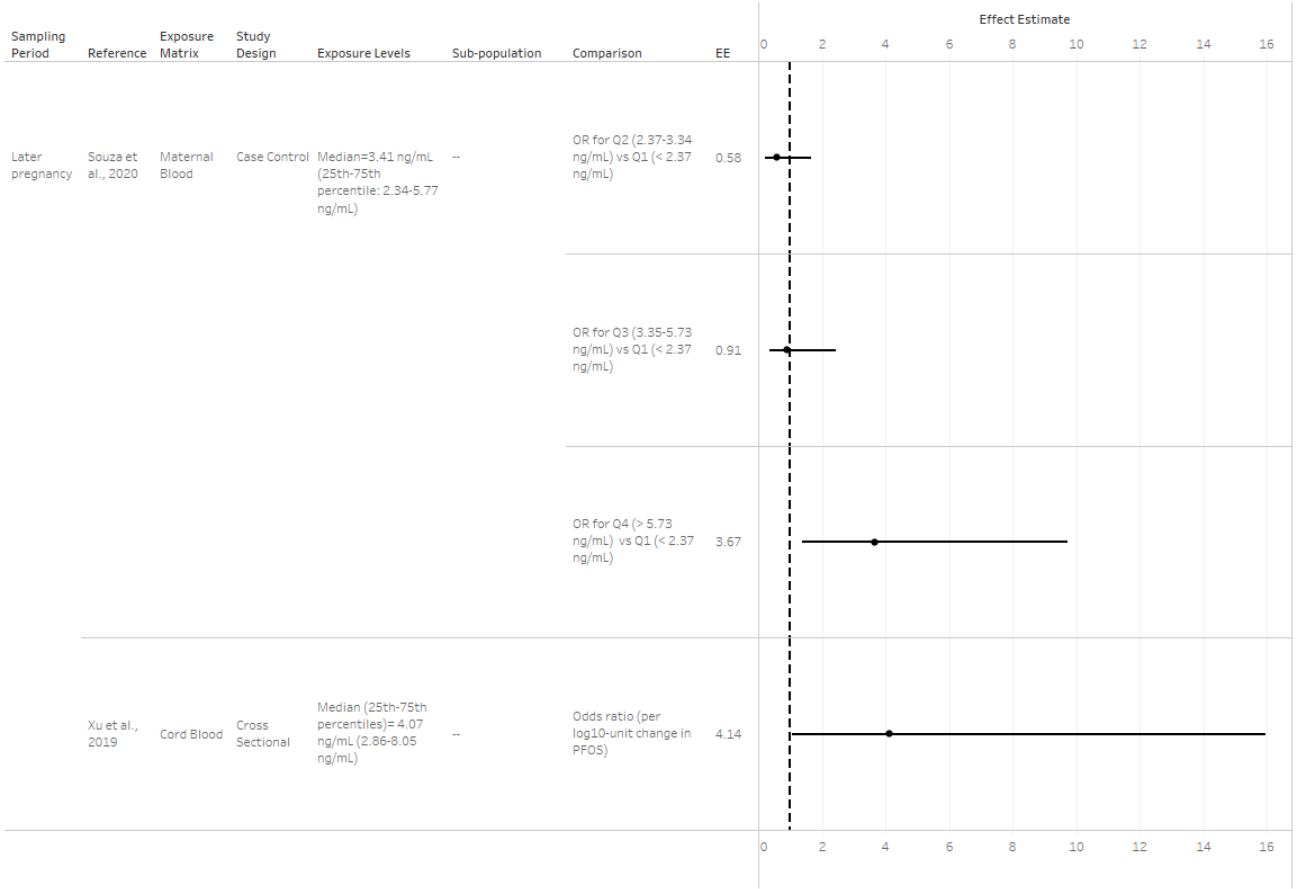


Figure D-1. Odds of Small-for-gestational-age in Children from Low Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.
Souza et al. (2020, HERO 6833697) reports the odds of the fetal growth ratio < 0.85.

D.1.2 Tables

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, 3981371 High	Canada, 2008–2011	Cohort	Pregnant women (enrolled if <14 weeks gestation, ≥18 years 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 (MIREC) **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, 3981534 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: –37 (–141, 67) 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, 5041287 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							S: 0.03 (–0.19, 0.24) 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, 5083648 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, 5016992 High	United States, 2009–2013	Cohort	Pregnant women (age range 18–40 years) 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 log-PFOS	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 body mass index, 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, 6315711 High	China, 2013	Cohort	Pregnant women (aged 18–45 years) 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, 5388081 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 weeks, 20 weeks, and 34 weeks	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, 2850966 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 weeks 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, 7102797 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 weeks 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, 7021199 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 weeks	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 weeks 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, 3230364 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Huo et al., 2020, 6835452 High	China, 2013–2016	Cohort	Mothers (aged ≥ 20 years) 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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Lauritzen et al., 2017, 3981410 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 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 US 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, height, pre-pregnancy BMI, education, parity, smoking status at conception, interpregnancy interval, offspring sex						
Lind et al., 2017, 3858512 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 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, 9959610 High for BW	China 2017–2019	Cohort	Mother-newborn pairs N = 224	Maternal blood and cord blood within three days of delivery	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Medium for birth length and ponderal index				5.01 (3.32, 7.62)			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., 2017, 4238465 High	Spain, 2003–2008	Cohort	Mother (aged ≥16 years)-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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 3981691 High	Japan 2002–2005	Cohort	Pregnant women and their children from the Sapporo Cohort (Hokkaido Study) 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 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, 4238310 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, race/ethnicity, education, pre-pregnancy BMI, and parity, black carbon, prenatal smoking						
Sagiv et al., 2018, 4238410 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 weeks **Results:** Lowest quartile used as reference. **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, 4619944 High	United States, 2003–2006; follow-up 4 weeks to 2 years from recruitment	Cohort	Pregnant women (aged ≥18 years) and their children at birth, 4 weeks and 2 years 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-	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					length (z-score)		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 weeks and 2 years. **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, 3858473 High	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 years) 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) 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, 5412449 High	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 years) and infants from Healthy Start assessed up to 5 months	Maternal serum 2.2 (1.4–3.4)	Adiposity (%), weight-for-age z-score (WAZ), weight-for-length z-score (WLZ), WAZ	Regression coefficient per ln-unit increase in PFOS and by tertiles	Adiposity at 5 months −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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 415 (202 girls, 213 boys)		and WLZ growth from birth to 5 months, rapid growth in WAZ or WLZ	Rapid growth: OR per ln-unit increase in PFOS	WAZ at 5 months: −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 months: −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 months, rapid growth: No statistically significant associations
	Outcome: Rapid growth defined as change in WAZ or WLZ >0.67 between birth and 5 months **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, 3983872 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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							−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., 2012, 2349577 High	Norway 2003–2004	Cohort	Pregnant women and their children from MoBa PTB, LGA, SGA N = 901 BW N = 838	Maternal plasma around 17 weeks 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) Q4: −0.18 (0.41, 0.05) p-trend = 0.12
	MoBa = Norwegian Mother and Child Cohort Study **Outcome:** PTB defined as GA <37 weeks. 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, 6311677	Sweden 2007–2010	Cohort	Infants exposed prenatally to PFAS	Maternal serum Early pregnancy	BW (g), BW-SDS, SGA	Regression coefficient	BW Per increase: −46 (−88, −3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
High			from the SELMA study N = 1533 (732 girls, 801 boys)	5.38 (3.97–7.60)		(BW, BW-SDS) and OR (SGA) per ln-unit increase in PFOS or by quartiles	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 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 7413606 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, 5918609 High	Denmark 1994–1995	Cohort	Pregnant women and their children N = 171	Maternal blood Later pregnancy GM = 20.8 µg/g (range: 6.9–47.6 µg/g)	Z-scores for BL, BW, and cranial circumference	Regression coefficient per log2-unit increase in PFOS	BL z-score −0.33 (−0.69, 0.03) Girls: −0.23 (−0.75, 0.30) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 9960202 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, 5080619 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-length (WFL) z-score	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 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 months or about 0.67 for 12 months. **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, 1429893 Medium	Denmark, 1996–2002	Cohort	Pregnant women and their children followed up at birth, 5 months, and 12 months from DNBC N at birth = 1114 (552 boys, 562 girls)	Maternal plasma from first and second trimester 33.4 (6.4, 106.7)	BW (g, z-score), BMI at 5 and 12 months, height at 5 and 12 months (cm), weight at 5 and 12 months (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 g: −3.2 (−6.0, −0.3), p-value <0.05 BMI at 5 months 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BMI at 12 months 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 months 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 months 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 months z-score: −0.001 (−0.005, 0.003)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 months 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 months included BW, models for length at 5 or 12 months included birth length, and models for body mass index at 5 or 12 months included birth body mass index."; adjusted models were used for all results. **Confounding:** Maternal age, parity, pre-pregnancy BMI, smoking, socioeconomic status, GA at blood drawing, breastfeeding. Additional confounding for BMI and 5 and 12 months: birth BMI. Additional confounding height at 5 and 12 months: birth height. Additional confounding for weight at 5 and 12 months: BW.						
Apelberg et al., 2007, 1290833 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 6356900 Medium	Canada, 2008–2011	Cohort	Pregnant women (age range = 17–42 years) and their infants from MIREC N = 205	Maternal blood 4.50 µg/L (3.30–6.10 µg/L)	Anoclitoris distance (ACD, mm), anofourchette distance (AFD, mm), anopenile distance (APD, mm), anoscrotal distance (ASD, mm)	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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, weight-for-length Z-score, and recruitment site						
Chang et al., 2022 Medium 9959688	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., 2012, 1332466 Medium	Taiwan, 2004–2005	Cross-sectional	Mother-infant pairs from TBPS N = 429	Cord blood at birth GM (SD) = 5.94 (1.95)	BW (g), BL (cm), GA (weeks), HC (cm), LBW, ponderal index (g/cm^3), PTB, SGA	BW: Regression coefficient per 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	BW 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
						PFOS, or by quartile	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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 weeks. 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., 2017, 3981292 Medium	Taiwan, 2004–2005	Cohort	Mother-infant pairs from the Taiwan Birth Panel Study (TBPS) N = 429	Cord blood 5.7 (IQR = 5.0)	BMI (z-score, kg/m^2), height (z-score, cm), weight (z-score, kg)	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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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: –0.0.05 (–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) 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 months **Confounding:** Maternal age, pre-pregnancy BMI, education level, ln-cord blood cotinine, infant sex, PTB, postnatal ETS exposure, breastfeeding						
Chen et al., 2021, 7263985 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 2850274 Medium	United States, Recruitment: 2005–2006, Follow-up: 2008–2011	Cohort	Pregnant women with known PFAS exposure (ages ≥20 years) 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) **Confounding:** Maternal age, educational level, smoking status, BMI, self-reported diabetes, time between conception, serum measurement.						
de Cock et al., 2014, 2713590 Medium	The Netherlands Recruitment: 2011–2013 Follow-up at 1, 2, 4, 6, 9, and 11 months 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, 3045435 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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., 2008, 1290822 Medium	Denmark Recruitment: 1996–2002, Assessment 6–18 months later	Cohort	Pregnant women and their children at 6 and 18 months from the DNBC Total N = 1,400 18-month 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) 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 child not using word-like sounds to tell what he/she wants. **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., 2008, 2349574 Medium	Denmark 1996–2002	Cohort	Pregnant women and their newborns from the DNBC Placental weight N = 1,337	Maternal plasma between 4–14 weeks gestation 33.4 (26.1–43.3)	Placental weight (g), HC (cm), BL (cm), abdominal	Regression coefficient per unit increase in PFOS	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			Birth length N = 1,376 HC N = 1,347 Abdominal circumference N = 1,325		circumference (cm)	Mean difference by quartile	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) 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, 4567442 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Gyllenhammar et al., 2018, 4238300 Medium	Sweden, 1996–2011 and follow-up at 5 years 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, 1290814 Medium	Canada Recruitment: 2005–2006 Follow-up at delivery: 2006–2007	Cohort	Pregnant women (≥18 years of age) and their singleton children delivered at or after 22 weeks gestation N = 252	Maternal serum collected at 15–16 weeks 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 SGA, PTB: Relative risk by tertile Length of gestation: Regression coefficient per ln-unit increase in PFOS and by tertile	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) 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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 weeks **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., 2020, 5880849 Medium	Greenland, Recruitment: 2010–2011, 2013–2015	Cohort	Pregnant women (≥18 years 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 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., 2020, 6833719 Medium	Denmark, 2010–2012 and follow-up at 18 months of age	Cohort	Pregnant women and infants at 3 and 18 months 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 months: –0.005 (–0.03, 0.016) 18 months: –0.003 (–0.03, 0.02) 3 and 18 months: 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Kashino et al., 2020, 6311632 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, 2850268 Medium	Japan, 2002–2005	Cross-section	Pregnant women (aged 28–34 years) and infants from the Hokkaido Study Females, N = 165 Males, N = 141	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) 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, 3981430 Medium	Japan, 2002–2005	Cross-sectional	Pregnant women at 22–35 weeks gestation and infants from	Maternal serum 5.3 (3.9–7.2)	BL (cm), BW (g)	Regression coefficient per ln-unit	Length: 0.32 (–0.19, 0.82) BW: –56 (–162.8, 50.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			Hokkaido Study on Environment and Children's Health N = 177			increase in PFOS	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, 10176408 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 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, 3858531 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 & 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Lenters et al., 2016, 5617416 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., 2016, 6387285 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, 3858527 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						
Maisonet et al., 2012, 1332465 Medium	Great Britain Recruitment: 1991–1992, followed-up until 20 months of age	Cohort	Pregnant women and their singleton girls assessed at birth, 9, and 20 months from ALSPAC BW N = 422 BL N = 356	Maternal serum during pregnancy (median 15 weeks) 19.6 (Range = 3.8–112.0)	BW (g), BL (cm), GA (weeks), ponderal index (g/cm^3), weight at 20 months (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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			GA N = 444 Ponderal index N = 360 Weight at 20 months N = 320 (106 upper tertile of BW, 107 middle tertile of BW, 107 lower tertile of BW)				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 months 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
	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 months (all tertiles): height at 20 months, 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., 2017, 4238509 Medium	Spain, 2003–2008	Cohort	Mother (aged ≥16 years)-child pairs from INMA	Maternal blood GM = 5.80 (4.52–7.84)	Weight gain z-score, rapid growth	Regression coefficient or RR per log2-	Weight gain z-score −0.02 (−0.11, 0.07) Girls: −0.09 (−0.21, 0.04) Boys: −0.05 (−0.08, 0.19)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			assessed at birth and 6 months N = 1,154 (568 girls, 586 boys)			unit increase in PFOS	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 months. **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, 4829851 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 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 body mass index, smoking during pregnancy, alcohol intake during pregnancy, study sample						
Ou et al., 2021, 7493134 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.						
Robledo et al., 2015, 2851197 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 1290816 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 N = 4,561	Maternal serum within 5 years 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) 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 weeks; 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.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Tian et al., 2019, 5390052 Medium	China 2012–2014	Cohort	Pregnant women and their sons at birth, 6 months, and 12 months from the S-MBCS Birth N = 439 6-month N = 322 12-month N = 301	Maternal serum 10.70 (7.61–15.71)	Weight gain z-score (0–6 months or 6–12 months), 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, 3102984 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 **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, 2850926 Medium	Denmark and Finland Recruitment 1997–2002, follow-up 3 months after birth	Nested case-control	Boys with (cases) or without (controls) cryptorchidism N = 215	Cord blood 9.1 (5^{th} – 95^{th} 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).						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Exposure Level: Denmark cases: 2.4 (5^{th} – 95^{th} percentile: 1.4–4.4); controls: 2.70 (5^{th} – 95^{th} percentile: 1.4, 4.0); Finland cases: 1.9 (5^{th} – 95^{th} percentile: 1.0–3.9); controls: 2.3 (5^{th} – 95^{th} percentile: 1.2–4.8) **Results:** Lowest tertile used as reference. **Confounding:** BW, GA, parity						
Wang et al., 2019, 5080598 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 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 body mass index, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain						
Woods et al., 2017, 4183148 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						
Yang et al., 2022, 10176806 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 3858524 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), ponderal index (g/cm3 x 100), proportion of optimal birth length (POBL), proportion of optimal HC (POHC)	Regression coefficient per ln-unit increase in PFOS	BW −69 (−231, 94) BL −0.22 (−1, 0.57) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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, 5080197 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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							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 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.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	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, 8442216 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–32nd weeks of gestation and were over 16 years 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, 7643497 Low	Sweden, Recruitment: 2007–2010, Follow-up at 7 years	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
	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, 7014743 Low	United States 2012–2014	Nested Case-control	Healthy and overweight 18-month-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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	StEP = Starting Early Program **Outcome:** Overweight defined as 18-month weight for length z-score ≥ 85th percentile **Confounding:** Maternal age, maternal education, maternal depressive symptoms, pre-pregnancy BMI, GA, parity, intervention status.						

Notes: BL = Birth Length; BMI = Body Mass Index; BW = Birth Weight; GA = gestational age; HC = head circumference; AC = Abdominal Circumference; FL = Femur Length; BPD = Biparietal Diameter; SGA = Small-for-Gestational-Age; LSM = least squares mean; GM = Geometric Mean; SD = Standard Deviation; SE = Standard Error; OR = Odds Ratio; PTB = Preterm Birth; RR = relative risk ratio; T2 = Tertile 2; T3 = Tertile 3

[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. (2020, 6311643) 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. (2017, 3858512) High	Denmark 2010–2012	Cohort	Infants from Odense child cohort	Maternal serum Total cohort: 8.1	Penile width (mm), anogenital distance	Regression coefficient per ln-unit increase in	AGDas Continuous: 1.2 (−0.4, 2.7) Q2: 0.9 (−0.9, 2.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			N = 649 (296 boys)		(scrotal, as; penile, ap) (mm)	PFOS, or by quartiles	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
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, weight for age z-score, pre-pregnancy BMI, parity, smoking						
Itoh et al. (2016, 3981465) Medium	Japan 2002–2005	Cohort	Infants from Sapporo Cohort of the Hokkaido study 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, least squares mean (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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							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) p-trend = 0.015 FSH, insulin-like 3, LH, prolactin, SHBG, testosterone, testosterone/SHBG: No statistically significant associations or trends
	Confounding: Age, parity, body mass index 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, 3859832) Medium	United States 2005–2006	Cross-sectional	Male children ages 6–9 years 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. (2017, 3981462) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							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, 5080529) 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-specific puberty indicator	Regression coefficient per log2-unit increase in first trimester maternal serum PFOS Puberty indicator: mean difference in age at puberty by tertiles	No statistically significant associations
	Confounding: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, prepregnancy body mass index, and daily number of cigarettes smoked in first trimester						
Tian et al. (2019, 5390052) Medium	China 2012–2013	Cohort	Male infants at birth, 6 months, and 12 months 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							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. **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 months of age)						
Wang et al. (2019, 5080598) 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 body mass index, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain						
Arbuckle et al. (2020, 6356900) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
	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, 3856472) 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						
Zhou et al. (2017, 3858488) 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, 5080655) 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	Anogenital distance (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 (%),	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					progressive motility (%), total sperm count (10^6), semen volume (mL), sperm concentration (10^6/mL), viability (%), FSH (U/L), testosterone (nmol/L)		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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							volume, sperm concentration, viability, FSH: No statistically significant associations after adjusting for comparison of medians
	Results: Values for each outcome are reported as median (25th–75th percentile). **Confounding:** Age						
				General Population			
Cui et al. (2020, 6833614) Medium	China 2015–2016	Cross-sectional	Adult men N = 651	Serum 9.94 Semen 0.15	Serum levels (ln-transformed) of E2 (pmol/L), FSH (IU/L), LH (IU/L), SHBG (nmol/L), free testosterone, total testosterone (nmol/L); free androgen index, total testosterone/LH ratio	Percent change per ln-unit increase in serum or semen PFOS, or by quartiles	SHBG Serum PFOS: −4.94 (−8.71, −1.02); p-value = 0.014 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							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						
Petersen et al. (2018, 5080277) 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 ($mL^{1/3}$),	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					sperm concentration ($(10^6/mL)^{1/3}$)		
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI groups, current smoking, time of sampling						
Kvist et al. (2012, 2919170) 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						
Leter et al. (2014, 2967406) 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, 6315783) 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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					velocity (VSL) (µm/s), DNA fragmentation index (DFI) (ln-%), high DNA stainability (HDS) (ln-%); semen volume (ln-mL)		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 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; FSH = follicle stimulating hormone; GEE = generalized estimating equation; HDS = high DNA stainability; LH = luteinizing hormone; LSM = least squares mean; MIREC = Maternal-Infant Research on Environmental Chemicals; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SHBG = sex hormone-binding globulin; VCL = curvilinear velocity; VSL = straight-line velocity.

[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. (2020, 6311643) High	Denmark, 2010–2012	Cohort	Female infants from the Odense Child Cohort, Age 4 months, N = 165	Maternal serum 8.07 (5th–95th 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. (2017, 3858512) 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, weight for age z-score, pre-pregnancy BMI, parity, smoking.						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Yao et al. (2019, 5187556) High	China, 2010–2013	Cross-sectional	Pregnant women (aged > 18 years) and female infants N = 171	Cord blood 1.39 (0.92, 2.01)	Testosterone (log10-ng/mL), Estradiol (log10-pg/mL), Testosterone to estradiol ratio (log10-transformed)	Regression coefficient per log10-unit increase in PFOS	Testosterone 0.15 (0.01, 0.29), p-value < 0.05 Estradiol 0.01 (−0.05, 0.07) 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, 5381537) 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: 0.01 (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, 5080529) 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 (10th–90th 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 (10th–90th 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						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Goudarzi et al. (2017, 3981462) Medium	Japan, 2002–2005	Cohort	Pregnant women and their infants from the Hokkaido Study on the Environment and Children's Health, N = 104	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)
	Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period						
Itoh et al. (2016, 3981465) Medium	Japan, 2002–2005	Cohort	Female infants from the Sapporo Cohort of the Hokkaido Study, 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. (2020, 6569227) 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)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	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, 3859832) Medium	United States, 2005–2006	Cohort	Females from the C8 Health Project, Ages 6–9, N = 1,123	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) 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. (2015, 3859841) 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 years. SHBG concentration included in testosterone model.						
Tsai et al. (2015, 2850160) 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)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Q4: 3.41 (SE = 0.44)
	Confounding: Age, BMI, high fat diet						
Wang et al. (2019, 5080598) 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-ng/mL), estriol (log10-ng/mL)	Regression coefficient per ln-unit increase in PFOS	Estrone 0.15 (0.04, 0.26), p-value = 0.007 β-estradiol −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; AMH = anti-Mullerian hormone; BMI = body mass index; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone-sulfate; FSH = follicle stimulating hormone; LH = luteinizing hormone; 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; ALSPAC = Avon Longitudinal Study of Parents and Children.

[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, 6505752) 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, 6833625) High	United States, Recruitment 1999–2002, outcome assessed 3-	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)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	years postpartum **Confounding**: Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity						
Borghese et al. (2020, 6833656) 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. (2019, 5083564) 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, 2850920) 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)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	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						
Romano et al. (2016, 3981728) 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 months postpartum), Breastfeeding termination (by 6 months postpartum)	RR by quartiles of PFOS	Termination at 3 months Q2: 1.08 (0.79, 1.46) Q3: 1.39 (1.04, 1.88) Q4: 1.32 (0.97, 1.79) Termination at 6 months 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, 6833607) 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. (2017, 3981439) 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, 3102984) 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	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

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
					(ln-nmol/L), testosterone (ln-nmol/L)		Androstenedione 0.15 (0.10, 0.21) T2: 8 (0, 17) 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.						
Wikstrom et al. (2019, 5387145) 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; 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.

[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, 6833612) 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, 3859813) 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. (2020, 6833596) 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, 3858516) 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, 2850160) 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, 3856459) 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; 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

D.3.1.1 Forest Plots

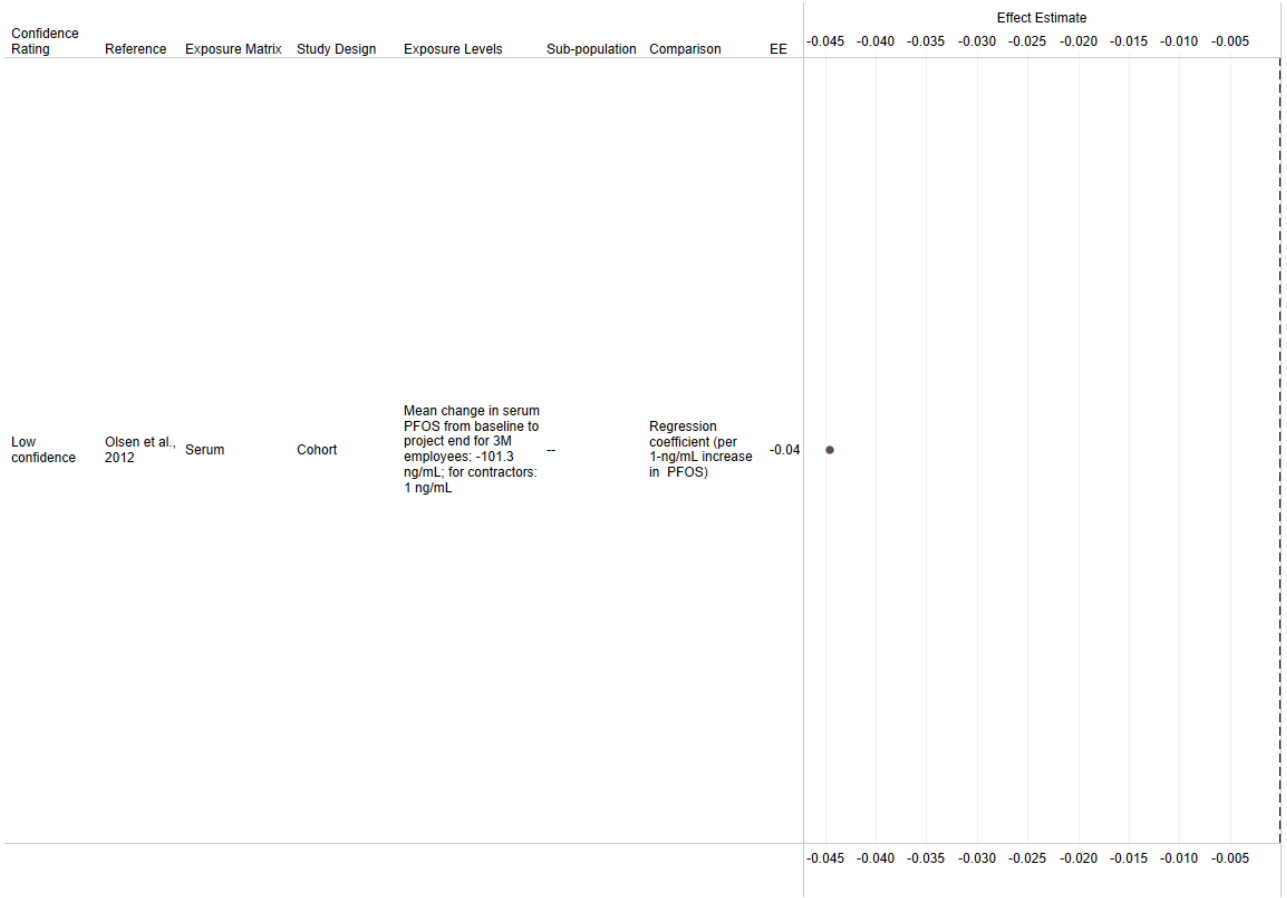


Figure D-1. Overall ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

D.3.1.2 Tables

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, 6988477) 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, 5381541) 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, GF-1: 0.011 GF-2: −0.013

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							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. (2018, 4238396) 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. (2018, 4238514)	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 (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Salihovic et al. (2018, 5083555) 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, 5080307) 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 ln-unit 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, 2850970) 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, 1276142) Medium	United States 2005–2006	Cross-sectional	Adults from the C8 Health Project, Ages ≥ 18 years, 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, 1291111) Medium	United States 1999–2000, 2003–2004	Cross-sectional	Adults from NHANES, Ages ≥ 18 years, 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, 5080340) Low	The Netherlands 2015	Cross-sectional	Men with habitual eel consumption, Ages 40−70, N = 37	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 0.19 (−0.17, 0.55)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Age, waist-to-hip ratio						
Olsen et al. (2003, 1290020) 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 to Q1 Elevated ($p < 0.05$) ALP for employees in Q3 and Q4 compared to Q1 No significant differences in mean AST or GGT by PFOS exposure quartile Females Elevated ($p < 0.05$) ALP for employees in Q4 compared to Q1 and Q2, and in Q3 compared to Q2 Elevated ($p < 0.05$) GGT for employees in Q4 compared to Q1 No significant differences in mean ALT or AST by PFOS exposure quartile
	Confounding: Sex						
Olsen et al. (2001, 10228462) Medium	United States, Belgium 1994–2000	Cohort	Male 3M fluorochemical plant workers in Antwerp, Belgium and Decatur, Alabama N = 175	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 x Years of observation interaction p-value < 0.001 AST 0.010 (SE = 0.011), p-value = 0.39 PFOS x Years of observation interaction p-value = 0.79

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							ALP 0.002 (SE = 0.009), p-value = 0.87 PFOS x Years of observation interaction p-value = 0.47 GGT –0.004 (SE = 0.020), p-value < 0.001 PFOS x Years of observation interaction p-value = 0.42
	Confounding: Years of observation, PFOS x Years of observation, age, BMI, drinks/day, cigarettes/day, location, entry period, baseline years worked, triglycerides						
Olsen et al. (2012, 2919185) 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, 3351439) 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, 2966740) Medium	United States 2007–2010	Cross-sectional	Adolescents from NHANES, Ages ≥ 12, N = 4,333	Serum 11.3 (7.0–18.0)	Levels of ALT (ln-U/L), GGT (ln-U/L), AST (ln-U/L), ALP (ln-U/L)	Regression coefficient per ln-unit increase in PFOS	ALT (0.013) (−0.009, 0.034) GGT 0.036 (0.001, 0.071) AST

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							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, 4239224) 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, 5412069) 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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							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, 4238547) 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, 6315720) 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) Nonalcoholic steatohepatitis 3.32 (1.40, 7.87), p-value < 0.05 Portal inflammation

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							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; 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, 1248827) 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 Postbooster (mean age 5.2) examination: N = 456	Maternal serum (prenatal) Geometric mean = 27.3 (23.2–33.1) Child serum (5 years) Geometric mean = 16.7 (13.5–21.1)	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 −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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Age 7 (mean age 7.5) examination: N = 464				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 −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 years						
Granum et al. (2013, 1937228) Medium	Norway 1999–2008	Cohort	Mother-infant pairs from MoBa at 3-year follow-up	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 56				
	Confounding: maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up.						
Mogensen et al. (2015, 3981889) Medium	Faroe Islands, Denmark 2002–2007	Cohort	Children aged 5–7 years 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]						
Grandjean et al. (2017, 3858518) Medium	Faroe Islands, Denmark Enrollment: 1997–2000	Cohort and cross-sectional	Children followed up at 7 years and 13 years N = 505 (13 years) N = 427 (7 years)	Serum 13 years: 6.7 (5.2–8.5) 7 years: 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. (2017, 4239492) Medium	Faroe Islands, Denmark 1997–2000 and 2007–2009 (year of birth)	Cross-sectional	Infants 2 weeks after expected term date, followed up at 18 months and 5 years All: N = 490, 18 months: N = 275, 5 years: N = 349	Serum 18 months: 7.1 (4.5–10.0) 5 years: 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							p-value = 0.21 Combined cohort 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, 6506041) Medium	Berlin, Germany Enrollment: 1997–1999	Cross-sectional	Children, 1 year 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, 6833710) Medium	Guinea-Bissau 2012–2015	Cohort	Infants enrolled at 4–7 months old (inclusion), followed up at	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-month visit

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			9 months and 2 years Inclusion: N = 236 9-month Unvaccinated controls: N = 100 Intervention: N = 133 2-year Unvaccinated controls: N = 100 Intervention: N = 91				Control (no measles vaccination): −27 (−44, −4) Intervention (1 measles vaccination): −21 (−37, −2) 2-year 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. (2022, 9416315) Medium	Greenland Recruitment: 1999–2005, Examination: 2012–2015	Cohort and cross-sectional	Vaccinated children ages 7–12 years 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 months, 12 months, >1 year). Additional confounding for child serum analyses: time since vaccine booster (only children with known vaccination date were included).						
Zeng et al. (2019, 5081554)	China 2013	Cohort	Infants from Guangzhou	Cord blood 3.17 (1.88–4.94)	HFMD antibody titers (CA16 or	Percent change or OR (below	CA16 Cord blood: −20.6 (−30.0, −9.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low			Birth Cohort Study at birth and 3 months Birth N = 194 (91 girls, 103 boys) 3-month N = 180 (89 girls, 91 boys)		EV71) in serum of cord blood or at 3 months	clinical protection) per doubling of PFOS	Girls: −14.0 (−27.5, 1.9) Boys: −24.7 (−37.6, −9.1) 3 months: −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 months: 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 months: −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) Boys: 2.01 (1.03, 3.90) p-value for interaction by sex = 0.265

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							3 months: 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. (2013, 2850913) 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 Q2: 0.03 (–0.19, 0.26), p-value = 0.78 Q3: 0.18 (–0.06, 0.41), p-value = 0.14

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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 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, 5080265) Medium for youth	United States 1999–2000	Cross-sectional	Adults and adolescents 12 years and older	Serum	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low for adult			Youths: N = 1,012 Adults: N = 542 women, 613 men	Women: mean = 22.1, SE = 0.9 Men: mean = 28.1 SE = 1.3			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, 7410156) Medium	Unites States 1999–2000, 2003–2016	Cross-sectional	NHANES adolescents and adults aged 12–49 years 12–19 years: N = 3,189 20–49 years: N = 5,589	Serum 12–19 years: GM (SE) = 7.54 (0.26) 20–49 years: GM (SE) = 8.67 (0.24)	Persistent infections of cytomegalovirus, Epstein-Barr virus, hepatitis C, hepatitis E, herpes simplex virus 1, herpes simplex virus 2, Toxoplasma gondii, and Toxocara species; pathogen burden	Persistent infections: Prevalence ratio per doubling in PFOS Pathogen burden: Relative difference per log2-unit increase in PFOS	Cytomegalovirus 12–19 years: 0.92 (0.77, 1.09), p-value = 0.36 20–49 years: 0.99 (0.92, 1.05), p-value = 0.70 Epstein-Barr virus 12–19 years: 1.01 (0.96, 1.05), p-value = 0.74 Hepatitis C virus 20–49 years: 0.96 (0.71, 1.29), p-value = 0.77 Hepatitis E virus

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							20–49 years: 1.00 (0.83, 1.20), p-value = 0.99 Herpes simplex virus 1 12–19 years: 1.05 (0.99, 1.11), p-value = 0.13 20–49 years: 1.04 (1.01, 1.06), p-value < 0.01 Herpes simplex virus 2 20–49 years: 1.04 (0.99, 1.09), p-value = 0.1 Toxoplasma gondii 12–19 years: 1.15 (0.90, 1.48), p-value = 0.27 20–49 years: 1.1 (0.97, 1.26), p-value = 0.15 Toxocara species 12–19 years: 1.12 (0.66, 1.91), p-value = 0.68 20–49 years: 1.57 (1.26, 1.96), p-value < 0.01 Pathogen burden 12–19 years: 1.30 (1.25, 1.36) 20–49 years: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, 7751049) Medium	United States 2005–2006, 2010	Cohort and cross-sectional	Adults from C8HP 2005–2006: N = 42,782	Serum 2005–2006: 19.7 (13.3–28.4)	Levels (ln-cells/µL or percentage of white blood	Counts: Precent 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			2010: N = 526	2010: 9.60 (6.10–14.9)	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	Percentages: Difference per IQR increase in PFOS	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, 9959487) Medium	Faroe Islands, Denmark Recruitment: 1986–1987, Follow-up through 2015	Cohort and cross-sectional	Faroe Island residents at birth, 7, 14, 22, and 28 years N = 399	Cord blood at birth 5.96 (IQR = 3.09) Serum 7 year: 31.89 (IQR = 13.37) 14 year: 31.29 (IQR = 9.62)	Levels (IU/mL) of hepatitis A antibody, hepatitis B antibody, diphtheria antibody, tetanus antibody; Hepatitis A	Percent change per log2-unit increase in PFOS	Hepatitis Type B Cord blood: −23.24 (−46.77, 10.69) 7-year serum: −4.65 (−45.87, 67.87) 14-year serum: 22.17 (−34.09, 126.46) 22-year serum: 15.26 (−22.88, 72.26) 28-year serum: 6.12 (−23.36, 46.93)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				22 year: 12.55 (IQR = 7.24) 28 year: 6.85 (IQR = 5.29)	antibody signal-to-cutoff ratio		Hepatitis Type A Cord blood: 0.11 (−0.36, 0.59) 7-year serum: 0.21 (−0.54, 0.96) 14-year serum: −0.14 (−1.01, 0.74) 22-year serum: −0.1 (−0.63, 0.44) 28-year serum: −0.23 (−0.66, 0.21) Diphtheria Cord blood: 28.26 (−5.7, 74.44) 7-year serum: 5.04 (−36.45, 73.59) 14-year serum: −3.5 (−42.87, 63.01) 22-year serum: 5.29 (−21.69, 41.56) 28-year serum: 6.91 (−14.26, 33.31) Tetanus Cord blood: 2 (−20.24, 30.44) 7-year serum: 8.91 (−25.85, 59.95) 14-year serum: −19.44 (−48.36, 54.7) 22-year serum: −9.1 (−28.42, 15.44) 28-year serum: −2.1 (−17.77, 16.56)
	Confounding: Sex						
Stein et al. (2016, 3860111) Low	United States 2010	Cohort	Adults enrolled at 18–49 years, followed up at day 30 Total population: N = 75, low	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			baseline Ab: N = 29				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, 6315718) 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 (HBsAg) (mIU-mL); HBsAb seronegative (< 10 mIU/mL)	Regression coefficient or OR (HBsAb seronegative) per log10-unit increase in linear or branched PFOS	HBsAb concentration Linear: −0.51 (−0.84, −0.18); p-value = 0.002 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						

Notes: Ab = antibody; C8HP = C8 Health Project; CI = confidence interval; GM = geometric mean; BMI = body mass index; HAI = hemagglutinin inhibition; ICH = immunohistochemistry; HFMD = hand, foot, and mouth disease; MoBa = Norwegian Mother and Child Cohort Study; OD = optical density; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SE = standard error; 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.

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; 1290805) Medium	Denmark, Recruitment: 1996–2003; Follow up: 2008	Cross-sectional and cohort	Mother infant pairs with follow-up to 11 years (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 **Confounding:** Maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period						
Gourdazi et al. (2017, 3859808) Medium	Hokkaido, Japan 2003–2009	Cohort	Children, early pregnancy followed up at 4 years	Maternal blood 4.93 (3.67–6.65)	Infectious diseases, total (including Otitis media,	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = ,1558 (793 boys, 765 girls)		Pneumonia, RS virus, Varicella)		p-value for trend = 0.036 Boys 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, breast-feeding period, and smoking during pregnancy[c]						
Manzano-Salgado et al. (2019, 5412076) Medium	Spain, 2003–2008	Cohort	Children ages 1.5, 4, or 7 years 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 years: 0.99 (0.83, 1.18) 4 years: 0.95 (0.79, 1.16) 7 years: 0.83 (0.57, 1.2) RR, 1.5–7 years 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, 6833636) Medium	Hokkaido, Japan Enrollment: 2003–2012	Cohort	Children, early pregnancy followed up at 7 years 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							RSV: OR: 0.72 (0.56, 0.91); p-value = 0.007 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, 6988475) Medium	China Recruitment: 2011–2013, Follow-up at 5 years	Cohort	Children ages 1–5 years 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, 7403067) Medium	Denmark 2020	Cross-sectional	Adults, ages 30–70 years, 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Dalsager et al. (2021, 7405343) Medium	Denmark Recruitment: 2010–2012, Follow-up until 2015	Cohort	Pregnant women and their children from the OCC, followed up to 4 years N = 1,472	Maternal serum 7.52 (0.49–27.5)	Hospitalization from infection (any infection, upper respiratory tract, lower respiratory tract, gastrointestinal, other)	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) 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, 7491706) 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, body mass index, diabetes, cardiovascular diseases, and urine albumin-to-creatinine ratio						
Wang et al. (2022, 10176501) Medium	China Recruitment: 2010–2013, Follow-up after 1 year	Cohort	Pregnant women and their children at 1 year 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							IRR: 0.76 (0.23, 2.46), p-value = 0.644 Diarrhea 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, 3858505) Low	Odense, Denmark 2010–2012	Cohort	Children, pregnancy followed up at 1–4 years 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, 4238440) Low	Oslo, Norway Recruited 1992–1993, followed up for 10 years	Cohort, Nested case-control	Infants followed up at 2 and 10 years of age N = 641	Cord blood 5.2 (4.0–6.6)	Common cold episodes from 0–2 years, LRTI episodes from 0–10 years	Regression coefficient per log2-unit increase in PFOS	Common cold 0–2 years −0.03 (−0.08, 0.01) p-value = 0.173 LRTI 0–10 years

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							0.5 (0.42, 0.57) p-value < 0.0001
	Confounding: Child sex						
Impinen et al. (2019, 5080609) Low	Oslo, Norway Enrollment: 1999–2008	Cohort	Pregnant women and their infants followed up at 3 and 7 years 0–3 years: N = 1,207 6–7 years: 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 years: 0.94 (0.92, 0.97); p-value < 0.05 Bronchitis/pneumonia 0–3 years: 1.20 (1.07, 1.34); p-value < 0.05 6–7 years: 0.77 (0.50, 1.19) Throat infection with strep 0–3 years: 0.90 (0.78, 1.04) Other throat infections 0–3 years: 0.90 (0.81, 1.01) Pseudocroup 0–3 years: 1.07 (0.96, 1.20) Ear infection 0–3 years: 0.88 (0.82, 0.94); p-value < 0.05 6–7 years: 1.13 (0.92, 1.40) Diarrhea/gastric flu 0–3 years: 0.98 (0.93, 1.03) 6–7 years: 1.12 (1.01, 1.24) Urinary tract infection 0–3 years: 0.78 (0.70, 0.87); p-value < 0.05 6–7 years: 0.91 (0.63, 1.31)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy						
Kvalem et al. (2020, 6316210) Low	Norway Enrollment: 1992–1993 Follow-up: 2002–2009	Cohort and cross-sectional	Children, 10 years, all: 378, boys: 193, girls: 185 Children, 10–16 years, all: 375, boys: 191, girls: 184 Children, 16 years, all: 330, boys: 170, girls: 160	Serum All: 19.4 (IQR: 9.23) Boys: 21.7 (IQR: 8.86) Girls: 17.52 (IQR: 8.02)	Common cold, LRTI	Colds: OR (reference: 1–2 colds) LRTI: RR per IQR increase in PFOS	Colds, 10–16 years 3–5 colds All: 1.26 (0.34, 4.55) p-value = 0.73 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 years 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 years 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 years, mother's education, physical activity level at 16 years						

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; SE = standard error; 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.

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, 1937230) Medium	Taiwan, 2009–2010	Case control and cross-sectional	Children from GBCA with (cases) or without (controls) asthma, ages 10–15 years, 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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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 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, 2851240) Medium	Unites States, 1999–2008	Cross-sectional	Adolescents, ages 12–19 years 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 months. Wheezing defined as history of wheezing in the past 12 months. **Confounding:** Sex, smoking, age, race/ethnicity, survey cycle, poverty income ratio, health insurance						
Smit et al. (2015, 2823268) Medium	Ukraine and Greenland, Exposure: 2002–2004, Outcome: 2010–2012	Cohort	Mother-child pairs with follow-up when the children were 5–9 years of age, N = 1,024	Maternal blood Ukraine: GM = 4.88 (P5–P95: 2.34–9.94)	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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Greenland: GM = 20.6 (P5–P95: 10.2–49.6)			
	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]						
Impinen et al. (2018, 4238440) Medium	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 and 10 years of age, N = 641	Cord blood 5.2 (4.0–6.6)	Asthma	OR per log2-unit increase in PFOS	Current asthma (10 y): 1.14 (0.84, 1.54); p-value = 0.392 Asthma ever (10 y): 1.32 (0.89,1.97); p-value = 0.167
	Confounding: Sex						
Beck et al. (2019, 5922599) Medium	Denmark, Enrollment: 2010–2012	Cohort	Children, early pregnancy to 5 years 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, 5080201) Medium	New York City, NY 2014–2016	Case-control	Children with (cases) or without (controls) asthma aged 13–22, N = 118 (cases), N = 169 (controls)	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)
	Comparison: Logarithm base not specified.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Sex, race/ ethnicity, age, BMI, tobacco smoke exposure						
Impinen et al. (2019, 5080609) 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 = 0.0.631 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, 5412076) Medium	Spain, 2003–2008	Cohort	Children, 4 years, N = 1,184 7 years, N = 1,068	Maternal blood 6.06 (4.52–7.82)	Asthma	OR or RR per log2-unit increase in maternal PFOS	4-year follow-up: OR = 0.72 (0.45, 1.13) 7-year follow-up: OR = 0.84 (0.57, 1.25) 4 and 7 years 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. (2019, 5412431) Medium	Shanghai, China, 2012–2015	Cohort	Enrolled in pregnancy, follow up at 5 years N = 358 (187 boys, 171 girls)	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 Girls: 0.17 (0.01, 4.15), p-value = 0.27
	Confounding: Child weight at age 5, gestational age, breastfeeding during the first 6 months, maternal education, maternal pre-pregnancy BMI, and annual household income						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Jackson-Browne et al. (2020, 6833598) Medium	NHANES, United States, 2013–2014	Cross-sectional	Children, ages 3–11 years, 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 y: 1.7 (1.0, 3.0) 6–11 y: 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, 6316210) Medium	Norway Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, 10 years N = 378 (193 boys, 185 girls) Children, 10–16 years N = 375 (191 boys, 184 girls) Children, 16 years	Serum 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 years All: 1.01 (0.86, 1.19) Boys: 1.06 (0.89, 1.26) Girls: 0.76 (0.52, 1.12) 10–16 years All: 0.94 (0.74, 1.20) Boys: 0.96 (0.71, 1.31) Girls: 0.85 (0.54, 1.31) 16 years All: 1.00 (0.79, 1.27) Boys: 1.01 (0.76, 1.36) Girls: 0.91 (0.60, 1.38)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 375 (191 boys, 184 girls)				
	Confounding: 10 y: Age at follow-up, physical activity, mothers' education; 16 y: BMI at 16 years, puberty status at 16 years, mothers' education, physical activity level at 16 years						
Huang et al. (2020, 6988475) Medium	China Recruitment: 2011–2013, Follow-up at 5 years	Cohort	Children ages 1–5 years 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. (2020, 6988472) Medium	United States 2007–2012	Cross-sectional	Adults from NHANES, ages 20–79 years N = 3,630	Serum Mean (SD) = 13.33 (12.92) µg/L	Fractional exhaled nitric oxide (ppb)	Percent change per doubling in 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. (2017, 3981296) 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	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]
			Control boys: 102 Control girls: 123				
	Confounding: Cases and controls were matched on age and sex						
Zhu et al. (2016, 3360105) 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. (2017, 3858488) 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. (2017, 3858497) Low	Faroe Islands, recruitment: 1997–2000	Cohort	Pregnant women and infants, follow up at ages 5, 7, and 13 years, 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, 5080647) 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, 5387046) 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; GBCA = Genetic and Biomarker study for Childhood Asthma; GM = geometric mean; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; MMR = measles, mumps, rubella; OR = odds ratio; RR = risk ratio; SD = standard deviation; TFF1 = Tromsø Fit Futures.

[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, 1424977)	Taiwan 2004	Cohort and cross-sectional	Pregnant women and	Cord blood	Atopic dermatitis, IgE	Atopic dermatitis:	Atopic dermatitis Q2: 0.68 (0.20, 2.3)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			their children at age 2 N = 244 (133 boys, 111 girls)	5.50 (0.11–48.36)	levels (log-KU/L)	OR by quartiles of PFOS IgE: Regression coefficient per ln-unit increase in PFOS	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, 1332477) Medium	Japan 2002–2005	Cohort	Pregnant women and children from the Hokkaido Study on Environment and Children's Health; follow up at 18 months 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 2.68 (0.39, 18.30) IgE: Linear regression

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							−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, breast-feeding period, environmental tobacco exposure, day care attendance and blood sampling period; for IgE: maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period						
Buser et al. (2016, 3859834) Medium	United States 2005–2016	Cross-sectional	Adolescents aged 12–19 years 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
	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. (2016, 3859523) Medium	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study 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, day-care attendance, environmental tobacco smoke exposure						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Timmermann et al. (2017, 3858497) Medium	Faroe Islands, Recruitment: 1997–2000	Cohort	Pregnant women and infants, follow up at ages 5, 7, and 13 years, 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 months, 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 months, 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 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, 4238440) Medium	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 years and 10 years 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 y 1.00 (0.72, 1.40); p-value = 0.983 Rhinitis, ever, 10 y 1.05 (0.74, 1.48); p-value = 0.775 Rhino-conjunctivitis, ever, 10 y 1.02 (0.72, 1.45); p-value = 0.905 Rhino-conjunctivitis, ever, spes IgE > 0.35, 10 y 1.02 (0.71, 1.47); p-value = 0.905 SPT, any pos, 10 y 0.87 (0.65, 1.17); p-value = 0.359 SPT + and/pr sIgE > 0.35, 10 y 0.91 (0.69, 1.19); p-value = 0.476
	Confounding: Sex						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Impinen et al. (2019, 5080609) 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 Allergy, inhaled, current All: 1.11 (0.0.72, 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, 6833636) Medium	Hokkaido, Japan, 2003–2012	Cohort	Early pregnancy to 7 years, N = 2,689	Maternal blood 5.12 (3.75–7.02)	Rhino-conjunctivitis	RR per ln-unit increase in PFOS, from	0.96 (0.79, 1.15); p-value = 0.626

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						birth to 7 years old	
	Confounding: Sex, parity, maternal age at delivery, maternal smoking during pregnancy, pre-pregnancy BMI, and annual household income during pregnancy						
Kvalem et al. (2020, 6316210) Medium	Norway, Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, age 10 years: N = 377 Age 16 years: 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 years 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 years All: 1.03 (0.90,1.19); p-value = 0.69 Boys: 0.92 (0.72, 1.19); p-value = 0.55 Girls: 1.15 (0.91, 1.45); p-value = 0.24 SPT 10 years 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 = 0.0.86 16 years All: 1.09 (1.03, 1.15); p-value = 0.001 Boys: 1.07 (0.97, 1.17); p-value = 0.18

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Girls: 0.99 (0.80, 1.23); p-value = 0.93
	Confounding: 10 years: Physical activity at 10 years, mothers' education, BMI at 10 years; 16 years: BMI at 16 years, puberty status at 16 years, mothers' education, physical activity level at 16 years						

Notes: BMI = body mass index; CI = confidence interval; IgE = immunoglobulin E; IQR = interquartile range; MMR = measles, mumps, rubella; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; RR = risk ratio; SD = standard deviation; SPT = skin prick test.
[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							
Goudarzi et al. (2016, 3859523) Medium	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study 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, day-care attendance, environmental tobacco smoke exposure[c]						
Timmermann et al. (2017, 3858497) Medium	Denmark 1997–2000	Cohort	Pregnant women and infants from the CHEF study at ages 5, 7, and 13 years 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	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, 4238372) Medium	China 2012–2015	Cohort	Infants followed up at 6, 12, and 24 months N = 687 children (328 female and 359 male)	Cord blood All: 2.48 (Range = 0.39–65.61) Female: 2.47 (Range = 0.39–18.68) Male: 2.49 (Range = 0.62–65.61)	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) 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, 4238440) Medium	Norway 1992–2002	Cohort, Nested case-control	Children from the ECA study at 0, 2, and 10 years N = 641	Cord blood 5.2 (4.0–6.6)	Atopic dermatitis diagnosed anytime between 0–2 years old, or between 0–10 years 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, 5412076) Medium	Spain 2003–2015	Cohort	Pregnant women and children followed up at ages 1.5, 4, and 7 from the INMA study	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 1,188 at 1.5, N = 1,184 at 4 years, N = 1,066 at 7 years				From ages 1.5 to 7 years: 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. (2019, 5081172) Medium	Taiwan 2001–2005	Cohort	Children at age 2 years N = 839	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)
	Results: Lowest tertile used as reference. **Confounding:** Sex, family income, maternal atopy, breast feeding, and maternal age at childbirth						
Wen et al. (2019, 5387152) Medium	Taiwan 2001–2005	Cohort	General population, children, and adolescents < 18 yrs.; Infants followed from birth up to 5 years 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: CD = Crohn's disease; CIS = clinically isolated serum syndrome; 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.

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, 6833754 Medium	United States	Case-control	Children and adolescents younger than	Serum Cases: 2.02 (IQR = 1.85)	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			21 years with (cases) and without (controls) celiac disease N = 88 (42 girls, 46 boys)	Controls: 1.59 (IQR = 1.64)			Boys: 1.02 (0.24, 4.21)
	Confounding: Genetic susceptibility score, albumin, BMI, age, race (non-Hispanic white vs. other race/ethnicity) and sex[c]						
Steenland et al., 2018, 5079806 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, 7211554) Low	Finland 1999–2005	Cohort	Pregnant women and infants at birth and 3 months 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-month 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 months
Ammitzbøll et al., 2019, 5080379	Denmark 2019	Case-control	Adults with (cases) or without	Serum Cases: 7.14 (5.76–9.93)	Relapsing remitting multiple	Percent change in PFOS comparing MS	−17 (−27, −6); p-value = 0.004 Females: −14 (−28, 3); p-value = 0.093

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low			(controls) RRMS or CIS N = 162 (92 women, 70 men)	Controls: 9.41 (6.41–13.0)	sclerosis (RRMS)	cases vs. healthy controls	Males: −19 (−32, −3); p-value = 0.023
	Confounding: Age, sex, breastfeeding						

Notes: CD = Crohn's disease; CIS = clinically isolated serum syndrome; 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., 2021, 7404102 High for gestation, birth, and childhood exposures (3-year and 8-year) Medium for exposure at 12-year 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)	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				At age 12: 2.4 (1.8–3.2)			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, 5413104 Medium	United States 2003–2012	Cross-sectional	Adolescents aged 12–20 from NHANES N = 2,251 (1,048 female, 1,203 male)	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) 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, 5881345 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, 10176518 Medium	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 years and children aged 8 to 11 years from	Serum Adolescents: 3.3 (2.2–4.9)	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			health surveillance program in Veneto Region Adolescents: N = 6,669 Children: N = 2,693	Children: 2.2 (1.6–3.0)			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) 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, 9960593 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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) 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., 2017, 4238509 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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Age 7 N = 1,086 (535 girls, 551 boys)				
	Confounding: Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child						
Lin et al., 2013, 2850967 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 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, body mass index; 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., 2014, 2851286 Medium	United States 1999–2000, 2003–2008	Cross-sectional	Children ages ≤ 18 years 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Averina et al., 2021, 7410155 Medium	Norway 2010–2011	Cross-sectional	First level high school students ages 15–19 years 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						
Lin et al., 2016, 3981457 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	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, 4238547 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, 4238478 Low	United States 2011–2012	Cross-sectional	Children and adolescents from the World Trade Center Health Registry (WTCHR) N = 308	Serum 3.72 (IQR = 2.82) Comparison: 2.78 (IQR = 2.18)	Augmentation Index (AI) Brachial Artery Distensibility (BAD) Pulse Wave Velocity (PWV)	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)
	Confounding: BMI category, caloric intake, cotinine concentration, physical activity, race, sex						
			Pregnant Women				
Matilla-Santander et al., 2017, 4238432 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 body mass index, previous breastfeeding, parity, gestational week at blood extraction, physical activity, relative Mediterranean Diet Score						
			General Population				
Liao et al., 2020, 6356903 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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 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 anti-hypertensive 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, body mass index, 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, 3859607 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, 4354163 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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					(LVMI) ($g/m^{2.7}$) Relative Wall Thickness (RWT)		
	Confounding: Sex, systolic blood pressure, antihypertensive medication, high density lipoprotein (HDL) and low-density lipoprotein (LDL), cholesterol, blood glucose, waist circumference, triglycerides, body mass index (BMI), education levels, exercise habits, smoking, energy, alcohol intake						
Bao et al., 2017, 3860099 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., 2018, 4238396 Medium	United States 2004–2007	Controlled trial	Overweight and obese adults ages 30–70 in the POUNDS-Lost study	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 621 (384 females, 237 males)				
	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., 2020, 6311641 Medium	United States 1996–2014	Cohort	Adults from the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) N = 957 at baseline, 956 at year 2, and 346 at year 14	Serum Baseline: 26.7 (17.4–40.3) Year 2: 27.6 (19.6–38.9) Year 14: 9.8 (5.9–14.8)	DBP, SBP, pulse pressure (mmHg), and hypertension	DBP, SBP: Regression coefficient per log2-unit increase in PFOS, or by quartiles Hypertension: HR or RR per log2-unit increase in PFOS or by quartiles	SBP: lifestyle arm, baseline to year 2: −2.13 mmHg/year (−3.54, −0.71) 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, 6833625 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, 6988479 Medium	Italy 2017–2019	Cross-sectional	Adults aged 20–39 years from Veneto Region with PFAS	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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			contaminated drinking water DBP and SBP: N = 15,380 (7,428 males, 7,952 females) Hypertension risk: N = 15,786 (7,667 males, 8,119 females)			Hypertension risk: OR per ln-unit increase in PFOS, or by quartiles	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) 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)/diastolic blood pressure (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., 2018, 4238514 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 anti-hypertensive medication. **Confounding:** Age, gender, ethnicity, lifestyle variables (smoking status, alcohol intake and household income), medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents), other components of the metabolic syndrome						
Christensen et al., 2019, 5080398 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	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						
Donat-Vargas et al., 2019, 5080588 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, body mass index, smoking habit, alcohol consumption, physical activity, healthy diet score						
Jeddi et al., 2021, 7404065 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 blood pressure: 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, 4181820 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., 2017, 3858504 Medium	Sweden 2001–2004	Cross-sectional	Adults ages 70+ in Uppsala, Sweden	Plasma 13.23 (9.95–17.77)	CIMT, carotid artery intima-media complex grey	CIMT, CIM-GSM: Regression coefficient 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]
			N = 1,016 (509 females and 507 males)		scale median (CIM-GSM), carotid artery atherosclerotic plaque	Plaque: OR per ln-unit increase in PFOS	
	Confounding: Sex, HDL- and LDL- cholesterol and serum triglycerides, BMI, blood pressure, smoking exercise habits, energy and alcohol intake, diabetes, educational level						
Huang et al., 2018, 5024212 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Congestive heart disease: No association by quartiles, no significant trend; p-trend = 0.9462 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 years and ≥ 50 years. **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, 5381549 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Hutcheson et al., 2020, 6320195 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
	Confounding: Age, BMI, C-reactive proteins, diabetes duration, eGFR, HDL, LDL, history of smoking, race, sex						
Osorio-Yanez et al., 2021, 7542684 Medium	United States 1999	Cohort	Prediabetic adults ages 25+ enrolled in the DPP trial N = 666	Plasma 27.55 (IQR = 19.30)	CAC (Agaston 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, body mass index, race/ethnicity, cigarette smoking, education, treatment assignment, statin use.						
He et al., 2018, 4238388 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Yang et al., 2018, 4238462 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 Hypertension: OR comparing above or below median	DBP, SBP, hypertension: no statistically significant associations
	Outcome: Hypertension evaluated by individual BP components **Comparison:** Logarithm base not specified. **Confounding:** Age						
Chen et al., 2019, 5387400 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, 5080653 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., 2016, 3858533 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: AI = augmentation index; BAD = brachial artery distensibility; BMI = body mass index; 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; GM = geometric mean; HDL = high density lipoprotein cholesterol; HELIX = Human Early-Life Exposome; IQR = Interquartile range; HOME = Heath

Outcomes and Measures of the Environment; LDL = low-density lipoprotein-cholesterol; LVEDD = left ventricular end-diastolic diameter (mm); LVMI = left ventricular mass index (g/m^2); MVD = microvascular disease; NHANES = National Health and Nutrition Examination Survey; PFOA = perfluorooctanoic acid; PFDE = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; MPAH = 2-(N-methyl-PFOSA) acetate; PFNA = perfluorononanoic acid; PFUnDA = perfluoroundecanoic acid; PWV = pulse wave velocity; OR = odds ratio; RWT = relative wall thickness; SBP = systolic blood pressure (mmHg); SD = standard deviation; SE = standard error; TFF1 = Tromsø Fit Futures 1

[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

D.5.2.1 Forest Plots

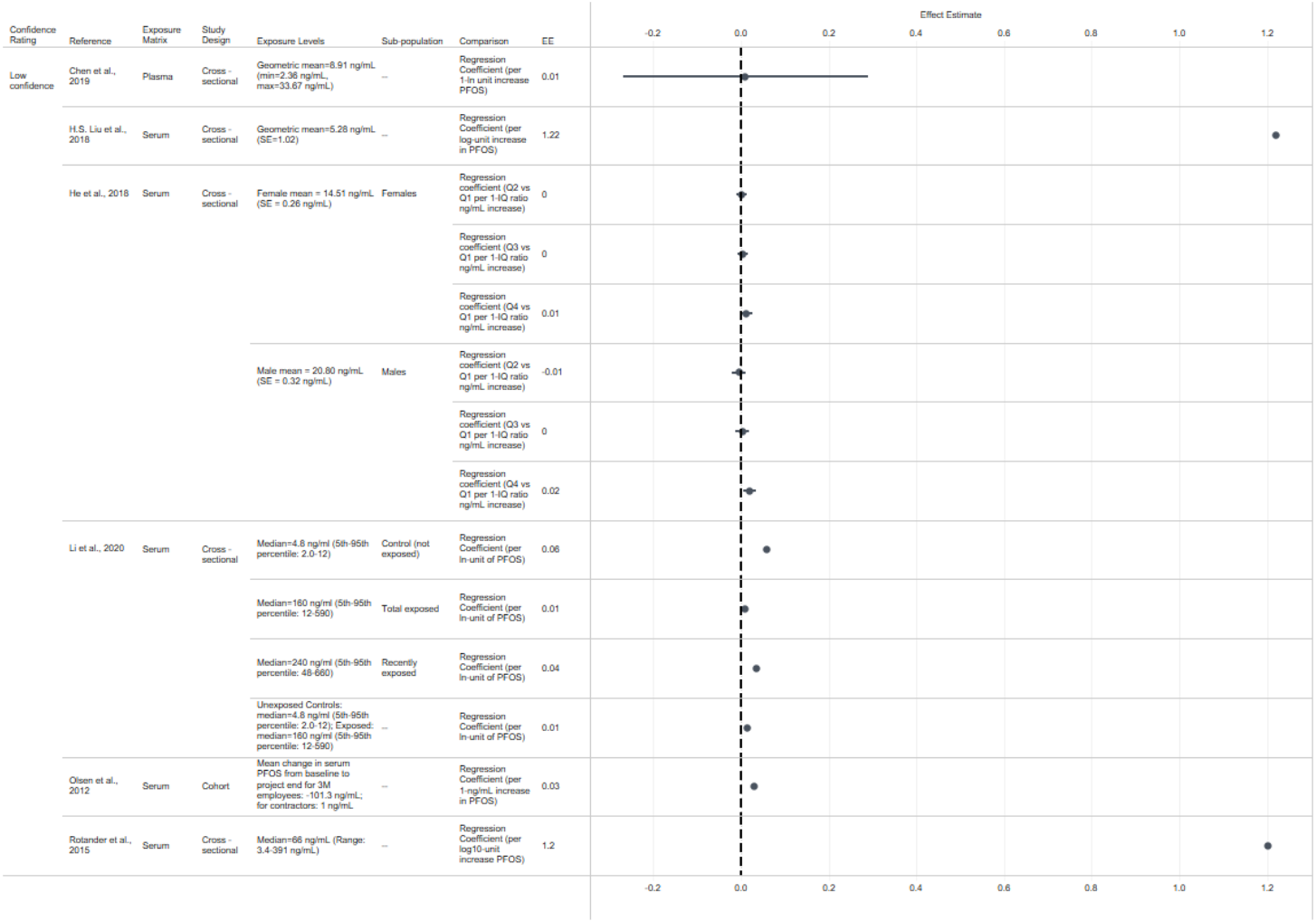


Figure D-2. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

D.5.2.2 Tables

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., 2021, 7404102 High for gestation, birth, and childhood exposures (3-year and 8-year) Medium for exposure at 12-year follow-up	United States 2003–2006	Cohort	Pregnant women and their children followed-up at birth and ages 3, 8, and 12 years 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)
	HOME = Heath 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, 1290820 Medium	United States 1999–2000 and 2003–2004	Cross-sectional	Adolescents ages 12–20 years from NHANES N = 474	Serum Mean (SEM) = 3.11	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)

				(0.05) log10-ng/mL			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, 1291110 Medium	United States 2003–2004	Cross-sectional	Adolescent girls ages 12–19 years 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., 2014, 2850925 Medium	United States 1999–2008	Cross-sectional	Adolescents ages 12–18 years 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 Elevated or depressed: OR per ln-unit increase in PFOS, or 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) 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
	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, 1430763 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 years 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 hours 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, 2850370 Medium	Denmark 1997	Cross-sectional	Children ages 8–10 from Danish component of EYHS N = 400 normal weight, N = 59 overweight	Plasma 41.5 (Range = 6.2–132.5)	Triglycerides (mmol/L)	Percent change per 10-unit increase PFOS	Normal weight: −0.5 (−3.2, 2.4), p-value = 0.75 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., 2015, 3981585 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) 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, 2851005 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, 3981435 Medium	Denmark 1997–2009	Cohort	Members of the EYHS evaluated at ages 9 and 15 (N = 260), 9 and 21 (N = 175), or 15 and 21 (N = 171)	Plasma Median at 9 = 44.5 (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)	Levels (mmol/L) of TG	Percent change in TG at age 15 or 21 per 10 unit increase in PFOS at age 9 or 15	Age 9 to 15: −0.7 (−5.03, 3.77) Age 9 to 21: −1.98 (−8.17, 4.75) 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 years of age). Waist circumference was adjusted for height in order to account for body size.						
Manzano-Salgado et al., 2017, 4238509 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, 5079656 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, body mass index percentiles, fasting time, and exposure to secondhand smoke						
Kang et al., 2018, 4937567 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, second-hand smoking						
Mora et al., 2018, 4239224 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., 2020, 6833719 Medium	Denmark 2010–2012	Cohort	Pregnant women and their children assessed at 3 months and 18 months	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

			N = 260 at 3 months, 83 at 18 months				
	Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI2, education, smoking, sex, and lipid outcome at 3 months						
Spratlen et al., 2020, 5915332 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, 7410155 Medium	Norway 2010–2011	Cross-sectional	First level high school students ages 15–19 years 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, 8442228 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 months, and 9 years Birth: N = 459 (219 female, 240 male) 18 months: N = 334 9 years: 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 months: 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 HDL, age 9 (PFOS age 9)

				9 years: 3.08 (2.42–4.31) Levels at 5 years and by sex at 18 months and 9 years not reported			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 years additionally adjusted for maternal smoking during pregnancy, maternal pre-pregnancy BMI, and parity						
Canova et al., 2021, 10176518 Medium for TC, HDL; Low for LDL, TG	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 years and children aged 8 to 11 years 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)
	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, 9960593 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) 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., 2021, 7026251 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 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., 2014, 2850928 Medium for TC, HDL, and LDL	Norway 2003–2004	Cross-sectional	Women in mid pregnancy (median = 18 weeks of	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	TC Per ln-unit: 8.96 (1.70, 16.22)

Low for Triglycerides			gestation) from MoBa N = 891			PFOS, or by quartiles	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) 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 body mass index, nulliparous or inter-pregnancy 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, 3749113 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, 4238432 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 body mass index, previous breastfeeding, parity, gestational week at blood extraction, physical activity, and relative Mediterranean Diet Score						
Starling et al., 2017, 3858473 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 body mass index, education, gravidity, smoking, and gestational age at blood draw						
Yang et al., 2020, 7021246 Medium	China 2013–2014	Cohort	Pregnant women ages 20–40 years 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) 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, body mass index (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, 7277682 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 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, 1290820 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)

							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, 1291110 Medium	United States 2003–2004	Cross-sectional	Adults ages 20–80 years 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., 2018, 4238514 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 (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Dong et al., 2019, 5080195 Medium	United States 2003–2014	Cross-sectional	Adults age 20–80 from NHANES N = 8814	Serum Mean = 15.6	Levels (mg/dL) of TC, LDL, HDL	Regression coefficient per unit increase PFOS	TC all cycles: 0.4 (0.06, 0.6) 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., 2019, 5080642 Medium	United States 2004–2015	Cross-sectional	Members of NHANES Non-obese N = 1053 females (NF) and 1237 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, 7102734 Medium	United States 2011–2014	Cross-sectional	Adults age 20+ from NHANES N = 1067	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, 6988488 Medium	United States 2007–2014	Cross-sectional	Adults age 20+ from NHANES Non-diabetic non-LLM users: N = 2,872 Diabetic non-LLM users: N = 316 Non-diabetic LLM users: N = 519	Serum Levels not reported	Apolipoprotein B (log10-mg/dL)	Regression coefficient per log10-unit increase in PFOS	Apolipoprotein B 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: N = 293				Diabetic LLM users: 0.02001, p-value = 0.19
	Confounding: Gender, age, age squared, race/ethnicity, poverty income ratio, 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, 1291109 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 = 46494	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) 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, 2919285 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	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

		Non-HDL: N = 670 HDL: N = 384 women, 309 men Triacylglycerols: N = 365 women, 284 men	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 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, 2919150 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
	Confounding: Sex, education, age, BMI, smoking status, intake of alcohol, egg, and animal fat and physical activity						
Fisher et al. 2013, 2919156 Medium	Canada 2007–2009	Cross-sectional	Adults ages 18–74 years 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, 2850962 Medium for TC, HDL Low for TG, LDL	United States Baseline: 2005–2006; Follow-up: 2010	Cohort	Adults ages 20–60 from C8 Short-Term Follow-up Study living in West Virginia and Ohio with PFOA-contaminated drinking water N = 560 (N = 521 for LDL analysis)	Serum Baseline GM (SD) = 18.5 (13.5) Follow-up GM (SD) = 8.2 (7.1)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Percentage decrease (log10 of final and initial ratio change per log10 of ratio change in PFOS)	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) 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., 2019, 5080588 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-year 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, body mass index, smoking habit, alcohol consumption, physical activity and healthy diet score						
Lin et al., 2019, 5187597 Medium	United States 1996–2014	Cohort and cross-sectional	Prediabetic adults age 25+ from the Diabetes	Plasma Median = 27.2	Levels (mg/dL) of TC, LDL, HDL, triglycerides, non-	Regression coefficient per doubling PFOS	<u>Cross-sectional</u> TC: 2.53 (−0.10, 5.16) LDL: 1.38 (−1.02, 3.77)

			Prevention Program (DPP) and Outcomes Study (DPPOS) N = 940 (888 not on metformin)		HDL, and very low density lipids (VLDL); hypercholesterolemia, hypertriglyceridemia	HR or OR for hypercholesterolemia or hypertriglyceridemia per doubling of PFOS	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) 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, 7021512 Medium	Italy 2017–2019	Cross-sectional	Residents of PFAS "Red Area" with contaminated public water supply ages 20–39 N = 15720 (7620 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) 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., 2020, 6988476 Medium	Taiwan 2016–2017	Cross-sectional	Adults aged 55 to 75 that resided in the study area for more than 10 years 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

							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., 2020, 6318644 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, 7762348 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.						
Jeddi et al., 2021, 7404065 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. (2003, 1290020) 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; APFO = ammonium perfluorooctanoate; ApoB = Apolipoprotein B; ApoE = Apolipoprotein E; ApoC-III = Apolipoprotein C-III; CHMS = Canadian Health Measures Survey; DCH = Diet, Cancer and Health; EYHS = European Youth Study; HDL = high density lipids; KorEHS-C = Korea Environmental Health Survey in Children and Adolescents; LDL = low density lipids; HELIX = Human Early-Life Exposome; HOME = Heath Outcomes and Measures of the Environment; HR = hazard ratio; IQR = interquartile range; S-MBCS = Shanghai-Minhang Birth Cohort Study; MoBa = Norwegian Mother and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; SE = standard error; TC = total cholesterol; OR = odds ratio; VLDL = very low-density lipoprotein.

[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, 6356361)	United States 2003–2007	Cohort	Mother-infant pairs from	Cord serum 14.3	Levels of TSH (µIU/L), TT4	Regression coefficient per	Cord serum TSH: 0.09 (–0.06, 0.25)

High for cord serum thyroid hormones; Medium for maternal thyroid hormones			Health Outcome Measures of the Environment (HOME) Study N = 256 for cord serum N = 185 for maternal serum	Maternal serum 5.5	(µg/dL), TT3 (ng/dL), FT4 (ng/dL), and FT3 (pg/mL)	log2-unit increase in PFOS	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, 5080657) Medium	Fernand, Ohio, USA 1991–2008	Cohort	FCC Median age 38 years at enrollment, N = 122 for TSH measurements; 47 male and 75 female N = 144 for TT4 measurements; 63 males and 81 females	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 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 (2019, 6315816) Medium	United States 2007–2012	Cross-sectional	Adults from NHANES aged 20+ Glomerular filtration (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, poverty income ratio, total calories consumed during the last 24h, smoking status, use of drugs						
Jain (2013, 2168068) 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, 3749030) 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 Females 60–80: 9.50	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 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, poverty income ratio, serum cotinine, and race/ethnicity						
Li et al. (2017, 3856460) Low	China 2013–2014	Cross-sectional	Residents of Southern China, ages 1 month to 90 years, 70%	Serum 1.3	Levels of TSH (µIU/mL), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per log-unit IQR	TSH: 0.41 (0.05, 0.76), p-value = 0.024 FT3: −0.14 (−0.24, −0.04), p-value = 0.007

			with thyroid condition N = 202			increase in PFOS	FT4: −0.13 (−0.22, −0.04), p-value = 0.004
	Comparison: Logarithm base not specified. **Confounding:** Age, sex						
Byrne et al. (2018, 5079678) 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 TT4, FT4: No statistically significant associations
	Confounding: Age, sex, smoking status						
Zhang et al. (2018, 5079665) Low	China 2013–2016	Cross-sectional	Women aged 20–40 years, 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, 5918609) 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						
Kim et al. (2020, 6833758) High	South Korea 2012–2017	Cohort	Children, aged 2, 4, 6 years 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, 3981723) 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 Least square means (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, 4241056) 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) 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, 5387078) 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, 5915990) 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, 3860107) Low	Taiwan 2004–2005	Cross-sectional	Newborns from Taiwan Birth Panel Study (TBPS)	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	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)

			N = 118 (64 boys, 54 girls)			increase in PFOS	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) 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, 6833676) 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-hours), dU-cortisone (nmol/24-hours), 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, 5918609) High	Faroe Islands, Denmark 1994–1995	Cross-sectional	Pregnant women and their children Maternal age 28 (SD = 5.6) N = 172 and 153 for measurements in maternal and cord serum, respectively	Maternal blood Geometric mean = 20.86 µg/g	Maternal serum levels of TSH (log-IU/L), T4 (log-pmol/L), FT3 (log-pmol/L), FT4 (log-pmol/L) FT3 resin uptake FT4 index	Regression coefficient per log2-unit increase in PFOS	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) 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, 3350759) Medium	Norway 2007–2009 or until 3 days 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, 4241056) 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, 5412435) Medium	Canada 2019–2012	Cohort	Pregnant women recruited prior	Maternal blood Total PFOS: 4.77	Levels of TSH (log-mIU/mL),	Regression coefficient per unit increase	TSH, linear PFOS Main effect: 0.01 (−0.03, 0.04)

			to 18 weeks of gestation N = 478	Linear PFOS: 2.49 ∑Br-PFOS: 1.08	FT3 (log-pmol/L), FT4 (log-pmol/L) by gestation status and 3 months post-partum	in total, linear, or 1m-PFOS	3 months post-partum: 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, 3981723) 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 Least square means (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; FCC = Fernald Community Cohort; GF = glomerular filtration; GFR = glomerular filtration rate; TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; FT3 = free triiodothyronine; FT4 = free thyroxine; POI = premature ovarian insufficiency TgAb = thyroglobulin antibody; TPOAb = thyroid peroxidase antibody; TT3 = total triiodothyronine; TT4 = total thyroxine; TGN = thyroglobulin.
[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. (2018, 3981371)	Canada, Recruitment 2008–2011	Cohort	Pregnant women and their children,	Maternal blood 4.6	Adiponectin, leptin	Regression coefficient per log10-unit	Adiponectin, leptin: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
High			from the MIREC Study N = 1,175			increase in PFOS	
	Confounding: Maternal age, pre-pregnancy body mass index, sex, and parity[c]						
Buck et al. (2018, 5080288) 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 body mass index, serum cotinine, delivery mode, and infant sex						
Chen et al. (2019, 5080578) 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. (2020, 6833719) High	Denmark, 2010–2012	Cohort	Pregnant women and their infants assessed at birth, 3 months, and 18 months, 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						
Minatoya et al. (2017, 3981691) 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Alderete et al. (2019, 5080614) Medium	United States, 2001–2012	Cohort	Obese Hispanic children (8–14 years), SOLAR Project N = 38	Plasma 12.22	Blood glucose, insulin, 2-hour glucose (mg/dL)), 2-hour insulin, insulin resistance, insulin levels	Regression coefficient per ln- unit increase in PFOS	Glucose (2-hour) 6.2 (−2.3, 14.8) Blood glucose, insulin, 2-hour 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, 3859836) 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 weeks gestation, fruit/vegetable consumption, fish consumption, prenatal vitamin use, maternal serum cotinine concentrations, and child age in months						
Conway et al. (2016, 3859824) 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						
Domazet et al. (2016, 3981435) 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	WC, HOMA-Beta, HOMA-IR, insulin, glucose, skinfold thickness, BMI	Percent change at 15 or 21 years old per 10-unit increase in PFOS at 9 years 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:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Age 9 Males: 44.5 Females: 39.9			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 years of age), and ethnicity, maternal parity, and maternal income in 1997 (9 years of age). Waist circumference was adjusted for height in order to account for body size.						
Domazet et al. (2020, 6833700) Medium	Denmark, 1997	Cross-sectional	Children from EYHS, 9-year-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. (2018, 4238300) 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 months: Positive statistically significant associations.
	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, 3859812) 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),	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Total body fat (%) per high, medium, and low educational status		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, 4937567) Medium	Korea, 2012–2014	Cross-sectional	Children from KorEHS-C Seoul and Gyeonggi, 3–18 years 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, second-hand smoking						
Karlsen et al. (2017, 3858520) Medium	Faroe Islands, recruited 2007–2009 (at birth); follow up at child ages 18 months, 5 years	Cohort	Children, 5 years (BMI) N = 349 Children, 5 years (overweight) N = 371 Children, 18 months (overweight) N = 444	Serum, Maternal serum 5 years: 4.7 18 months: 8.25	BMI z-score, Overweight	Risk Ratio (OW), or Regression coefficient per log10-unit increase in maternal PFOS, or by tertiles (BMI)	BMI z-score 18 months: 0.2 (0.1, 0.4), p-value < 0.05 OW 18 months:1.29 (1.01, 1.64), p-value < 0.05
	Results: Lowest tertile used as reference. **Confounding:** Maternal nationality, age at delivery, pre–pregnancy BMI, smoking during pregnancy, child sex, exclusive breastfeeding duration, child's fish intake at age 5 years						
Kobayashi et al. (2017, 3981430) Medium	Japan, 2002–2005	Cross-sectional	Children from Hokkaido Study on Environment	Maternal serum 5.3	Ponderal index	Regression coefficient per	−1.07 (−1.79, −0.36), p-value = 0.004

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			and Children's Health N = 176			ln-unit increase in PFOS	
	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, 4217244) Medium	Norway and Sweden, Recruitment 1986–1988	Cohort	Pregnant women and their children at 5-year 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 weeks, interpregnancy interval, previous breastfeeding duration and country of residence						
Lopez-Espinosa et al. (2016, 3859832) Medium	United States, 2005–2006	Cohort	Children, ages 6–9 years from the C8 Health Project N = 1123 girls and 1169 boys	Serum Girls: 20.9 Boys: 22.4	Insulin-like growth factor 1 (IGF-1)P(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						
Manzano-Salgado et al. (2017, 4238509) Medium	Spain, Recruitment 2003–2008	Cohort	Mother-child pairs, followed for 8 years, 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Martinsson et al. (2020, 6311645) 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, 3859823) 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 ratio, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	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., 2020, 6391244 Medium	United States, 2013–2014	Cross-sectional	Children aged 3–11 years 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), weight-for-age z-score (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, poverty income ratio, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, sex						
Fleisch et al. (2017, 3858513) Medium for metabolic function	United States, Pregnant women recruited 1999–2002, outcome	Cohort	Pregnant women and their children from Project Viva	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:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low for HOMA-IR	assessed at mid-childhood follow-up		N = 584 Median age at follow-up = 7.7 years				−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. (2018,4354143) High	Denmark, recruitment 2010–2012, outcome assessed 12–20 weeks later	Cohort	Pregnant women, Odense Child Cohort N = 158	Serum 8.37	Blood glucose, insulin, c-peptide, 2-hour glucose, insulin resistance, beta cell function, insulin sensitivity	Percent change per log2-unit increase in PFOS	Blood glucose, insulin, c-peptide, 2-hour glucose, insulin resistance, beta cell function, insulin sensitivity: No statistically significant association
	Confounding: Age, parity, education level, pre-pregnancy BMI						
Mitro et al. (2020, 6833625) High	United States, Recruitment 1999–2002	Cohort	Pregnant women, Project Viva N = 786	Plasma 24.8	WC (cm), BMI (kg/m^2), Adiponectin (ug/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						
Preston et al. (2020, 6833657) High	United States, 1999–2002	Cohort	Pregnant women from Project Viva N = 1,533	Serum 25.7	Gestational diabetes, glucose tolerance, hyperglycemia,	Regression coefficient by quartiles	Glucose blood level, All Q4: 4.3 (0.5, 8.0) < 35 years Q4: 6.5 (2.1, 10.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					glucose blood level		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, 3858473) 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 body mass index (BMI), race/ethnicity, education, smoking during pregnancy, gravidity, and gestational age at blood draw						
Ashley-Martin et al. (2016, 3859831) 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, 3981711) 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)
	Confounding: Pre-pregnancy non-fasting serum lipids, BMI						
Liu et al. (2019, 5881135) Medium	China, 2013–2015	Case-control	Pregnant women without history or family history of diabetes	Serum 3.13	Gestational diabetes (GDM), glucose homeostasis	Regression coefficient per ln-unit increase or by tertiles	GDM: m-PFOS Per ln-unit increase: 1.36 (0.88, 2.11)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 189			sum m-PFOS or L-PFOS	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, 5381534) 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, 5024206) Medium	United States, 2009–2013	Cohort	Pregnant women with singleton pregnancies N = 2,292	Plasma GM = 5.21	GDM	Risk Ratio 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, 6833646) Medium	China, 2012	Cross-sectional	Pregnant women, Shanghai-Minhang Birth Cohort Study N = 705	Plasma 10.7	Glucose (1 hour, fasting)	Regression coefficient per ln-unit increase in PFOS	Glucose (1 hour tolerance test): 0.31 (0.11, 0.50), p-value = 0.003 Glucose after fasting, glucose after 1 hour tolerance test by gestational weeks: No statistically significant association

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	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, 3201206) 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, 3983872) 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. (2018, 5079666) 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 5m-PFOS Cases: 0.36 Controls: 0.36 6m-PFOS Cases: 0.29 Controls: 0.31	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 3m+4m-PFOS T2: 1.81 (0.98, 3.33) T3: 1.88 (1.00, 3.52), p-value < 0.05 5m-PFOS

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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. (2018, 5080352) 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.(2020, 6833677) Medium	China, 2017–2019	Nested case-control	Pregnant women N = 165 cases, 330 controls	Serum Cases: 6.69 Controls: 6.45	GDM	OR per unit increase in PFOS; OR per log10-unit increase in PFOS	GDM Q2: 0.69 (0.34, 2.07) Q3: 0.72 (0.48, 1.90) Q4: 1.07 (0.51, 1.32) p-trend = 0.27 log-PFOS: 0.61 (0.42, 1.65), p-value = 0.21
	Confounding: Maternal age, sampling time, parity, BMI, educational level, and serum lipids						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
General Population							
Cardenas et al. (2017, 4167229) 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 (ug/mL), HbA1c (%), Insulin (fasting) (uU/mL), Glucose (fasting) (uU/mL), HOMA–IR, Insulin (30 min, uU/mL), Proinsulin (fasting, pM), HOMA–B, Insulin (corrected response), Insulinogenic index, Diabetes, HOMA-IR, glucose (30 mins), glucose (2 hours), 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 mins), glucose (2 hours), 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, 5080657) Medium	United States, 1991–2008	Cohort	Adults living in a community with water supply from a PFAS-contaminated aquifer N = 192	Serum 28.4	BMI	Percent change per IQR increase in PFOS	BMI: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Age, year of measurement, sex, education, income, marital status, and BMI						
Cardenas et al. (2019, 5381549) Medium	United States, 1996–2014	Controlled trial	Adults older than 25 without diabetes and with elevated fasting and postload glucose, Diabetes Prevention Program 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. (2016, 3350721) 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, 3859824) 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. (2019, 5083542) Medium	Sweden, 1990–2003, 2001–2012	Case-control	Adults with (cases) and without (controls) type 2 diabetes living in Sweden N = 248	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
	Results: Lowest tertile used as reference; T1 (13, 11–16 ng/mL), T2 (21, 19–23 ng/mL).						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Gender, age, sample year, red and processed meat intake, fish intake, BMI						
Duan et al. (2020, 5918597) Medium	China, 2017	Cross-sectional	Adults, 19 to 87 years 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, body mass index, smoking and alcohol-drinking status, exercising status, education level, and family history of diabetes						
Jain et al. (2019, 5080621) 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, 5079850) Medium	England, mothers recruited 1991–2002, outcome assessed at age 17	Nested case-control studies	Pregnant mothers and their 17-year 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 months						
Liu et al. (2018, 4238396) 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/24h), 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 Per log10-umit increase in PFOS

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							0.8, p-value < 0.05 RMR First 6 months, 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 months, 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. (2018, 4238514) Medium	United States, 2013–2014	Cross-sectional	Adults from NHANES N = 1,871	Serum GM = 5.28	Fasting blood glucose, 2-hour glucose, HbA1c, insulin levels, HOMA-IR, beta cell function,	Regression coefficient per ln-unit increase in PFOS	Fasting blood glucose: 1.96 (SE = 0.79) 2-hour glucose, HbA1c, insulin levels, HOMA-IR, beta cell function, metabolic syndrome, WC:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					metabolic syndrome, WC		No statistically significant associations
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, WC, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Mancini et al. (2018, 5079710) Medium	France, 1990–2012	Cohort	Women aged 40–60, E3N Cohort N = 71294	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, 3860116) 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 mins) (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, 4241053) Medium	United States, recruitment 1989, blood sample collection 1995–2000, outcome	Case-control	Female nurses drawn from the Nurses' Health Study II cohort study, N = 1586	Plasma Cases: 35.7 Controls: 33.1	T2D hemoglobin, insulin, adiponectin	Regression coefficient SD log10-unit increase in PFOS	T2D Per SD increase: 1.15 (0.98, 1.35), p-value = 0.008 OR for T2: 1.63 (1.25, 2.12) OR for T3: 1.62 (1.09, 2.41)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	assessed during biennial follow up through June 2011					OR by tertiles	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. (2019, 5387400) Medium for metabolic syndrome Low for all other outcomes	Croatia 2007–2008	Cross-sectional	Residents of Hvar ages 44–56 years 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, waist	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]
					circumference (cm)		
Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity							

Notes: ALSPAC = Avon Longitudinal Study of Parents and Children; AUC = area under the curve; BMI = body mass index; DM = diabetes mellitus; EYHS = European Youth Heart Study; GDM = gestational diabetes mellitus; GM = geometric mean; GWG = gestational weight gain; HbA1c = Hemoglobin A1c; HOMA = Homeostatic model assessment; HOME = Health Outcomes and Measures of the Environment; IGF = insulin-like growth factor; IQR = interquartile range; IR = insulin resistance; KorEHS-C: Korea Environmental Health Survey in Children and Adolescents; LSM = least square mean; MIREC = Maternal Infant Research on Environmental Chemicals;OR = odds ratio; OW = overweight; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; RR = risk ratio; SD = standard deviation; SOLAR = Study of Latino Adolescents at Risk of Type 2 Diabetes; T1D = type 1 diabetes; WC = waist circumference.

[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, 4442261) 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 Early childhood only: Peabody Picture Vocabulary Test (PPVT-III) 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) Non-Verbal 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Mid-childhood only: Kaufman Brief Intelligence Test Second Edition (KBIT-2) non-verbal and verbal IQ, (WRAML2) design memory and picture memory		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) Q2: −0.1 (−0.8, 0.5) Q3: 0.1 (−0.6, 0.9) Q4: 0 (−0.7, 0.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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, 5381527) High	China, Recruitment: 2012; Follow-up at age 4 years	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) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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, 3789517) High	Faroe Islands, Recruitment: 1997–2000, Follow-up at ages 5 and 7	Cohort	Children at 5 years (N = 508) and 7 years (N = 491)	Serum Maternal: 27.35 (23.19–33.13) 5 years: 16.78 (13.52–21.05) 7 years: 15.26 (12.38–18.99)	Strengths and Difficulties Questionnaire (SDQ) scores: Total score (hyperactivity/inattention, conduct problems, peer relationship problems, emotional symptoms), prosocial behavior, internalizing	Mean difference (autism, internalizing, externalizing, total) or mean ratio (hyperactivity/inattention, conduct, peer relationship, emotional, prosocial) per doubling of PFOS	SDQ total score Prenatal: 0.46 (−0.78, 1.7), p-value = 0.47 5-year serum: 0.51 (−0.5, 1.52), p-value = 0.32 7-year serum: 0.18 (−0.95, 1.31), p-value = 0.76 Hyperactivity/Inattention Prenatal: 1.03 (0.80, 1.31), p-value = 0.84 5-year serum: 1.05 (0.86, 1.29), p-value = 0.64 7-year serum: 0.88 (0.70, 1.11), p-value = 0.27

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					problem, externalizing problems, autism screening (peer-problems minus pro-social)		Conduct Prenatal: 1.03 (0.81, 1.32), p-value = 0.80 5-year serum: 1.00 (0.81, 1.23), p-value = 0.98 7-year 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-year serum: 1.28 (0.91, 1.80), p-value = 0.15 7-year serum: 1.17 (0.82, 1.69), p-value = 0.39 Emotional Prenatal: 1.10 (0.84, 1.44), p-value = 0.49 5-year serum: 1.14 (0.90, 1.45), p-value = 0.26 7-year serum: 1.22 (0.94, 1.58), p-value = 0.13 Prosocial Prenatal: 1.00 (0.91, 1.09), p-value = 0.96 5-year serum: 0.98 (0.91, 1.06), p-value = 0.70 7-year serum: 1.01 (0.92, 1.10), p-value = 0.88 Internalizing Prenatal: 0.35 (−0.35, 1.05), p-value = 0.32

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							5-year serum: 0.44 (−0.15, 1.02), p-value = 0.15 7-year serum: 0.48 (−0.16, 1.13), p-value = 0.14 Externalizing Prenatal: 0.11 (−0.68, 0.89), p-value = 0.79 5-year serum: 0.08 (−0.58, 0.73), p-value = 0.82 7-year 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-year serum: 0.33 (−0.14, 0.8), p-value = 0.17 7-years serum: 0.06 (−0.46, 0.58), p-value = 0.82
	Confounding: Age, sex, maternal age, pre-pregnancy BMI, parity, socio-economic status, alcohol, and smoking during pregnancy						
Braun et al. (2014, 2345999) Medium	United States, Recruitment: 2003–2006; Follow-up at ages 4–5 years	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						
Chen et al. (2013, 2850933) Medium	Taiwan, Recruitment: 2004–2005; Follow-up at age 2 years	Cohort	Pregnant women and their children from the Taiwan	Cord blood Mean = 7.0 (SD = 5.8)	Comprehensive Developmental Inventory (CDI) skill quotients: cognitive, fine-	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Birth Panel Study N = 239		motor, gross-motor, language, self-help, social, whole test		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 months of age, cord blood cotinine levels, postnatal environmental tobacco smoke exposure						
Ghassabian et al. (2018, 5080189) Medium	United States, 2008–2010	Cohort	Children aged 7 years 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) Q4: 0.27 (0.05, 0.49)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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. (2016, 3981536) Medium	Japan, 2002–2005	Cohort	Pregnant women and their infants at 6 and 18 months 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)
	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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Jeddy et al. (2017, 3859807) Medium	Great Britain. Recruitment: 1991–1992; Follow-up at ages 15 and 18 months	Cohort	Mothers and daughters aged 15 and 38 months 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						
Liew et al. (2015, 2851010)	Denmark,	Case-control	Mother-child pairs from	Maternal plasma	ADHD, ASD	RR and OR (stratified by	ADHD: 0.87 (0.74, 1.02) Q4: 0.79 (0.64, 0.98)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium	Recruitment: 1996–2002; Follow-up at average age 10.7 years		Danish National Birth Cohort 215 Cases (39 Females; 176 Males) 545 Controls (33 Females; 180 Males)	Cases: 25.40 (18.73–32.40) Controls: 27.40 (20.40–35.60)		quartile or by sex) per ln-unit increase in PFOS or by quartiles	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, 5079744) Medium	Denmark, Recruitment: 1996–2002; Follow-up at age 5 years	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, 5080602) Medium	Denmark, Recruitment: 1982–1999; Follow-Up: 1993–2009	Case-control	Pregnant women and their children from the Historic Birth Cohort at Statens Serum Institute	Amniotic fluid Cases: 0.61 (Range: 0.61–2.98) Controls: 1.44 (Range: 0.61–4.22)	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]
			37 Cases (7 Females; 29 Males) 50 Controls (15 Females; 35 Males)				
	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, 4239287) Medium	United States, 2007–2009	Case-control	Children and adolescents aged 4.5–9 years 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, 6316905) Medium	Faroe Islands, Recruitment: 1997–2000; Follow-up at age 7 years	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-year serum: 0.00 (−0.08, 0.07) Without Cues Prenatal: −0.04 (−0.19, 0.06) 5-year serum: 0.00 (−0.06, 0.06) SDQ Prenatal: 0.15 (0.08, 0.23) 5-year serum: 0.02 (−0.03, 0.08)
	Confounding: None reported						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Quaak et al. (2016, 3981464) Medium	Netherlands, Recruitment: 2011–2013; Follow-up through age 18 months	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, 6507470) Medium	United States, Recruitment: 2002–2009;	Case-Control	Mother-child pairs from CHARGE with	Maternal serum 5.81 (3.86–9.11)	ASD measured by Autism Diagnostic	OR per increase (ln-transformed or linear scale)	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]
	Follow-up: 2009–2017		children aged 2–5 years N = 453 (239 Cases; 214 Controls; 88 Females; 365 Males)		Interview-Revised (ADI-R)	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, 5918847) 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. (2020, 6364693) Medium	United States, Recruitment: 2001–2001; Follow-up at	Cohort	Pregnant women and their children from the	Cord blood GM = (Range:)	BSID-II scores: Mental and Psychomotor Development	Regression coefficient of mean difference per log-unit	MDI Year 1: −0.61 (−3.17, 1.95) Year 2: 2.36 (−1.23, 5.94) Females: 5.52 (0.64, 10.4)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	age 1, 2, and 3 years		Columbia University Birth Cohort N = 302 (150 Females; 152 Males)		Index (MDI and PDI), Full IQ, Performance IQ, Verbal IQ	increase in maternal PFOS	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, 2922190) 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 coefficient per	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]
						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, 3352166) Medium	United States, Recruitment: 2003–2006; Follow-up at ages 5 and 8 years	Cohort	Children ages 5 and 8 years 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. (2018, 5079675) Medium	United States, Recruitment: 2003–2006; Follow-up at age 3 and 8 years	Cohort	Children from the HOME study N = 204	Serum 3 years: 6.2 (4.5–10.0) 8 years: 3.6 (2.7–4.9)	BRIEF measures of behavioral regulation, metacognition, global executive composite indices	OR per ln-unit increase in PFOS	Behavioral Regulation 3 years: 0.66 (0.29, 1.51) 8 years: 0.40 (0.14, 1.14) Metacognition 3 years: 0.83 (0.42, 1.63) 8 years: 1.53 (0.67, 3.52)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Global Executive Function 3 years: 0.95 (0.45, 2.01) 8 years: 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. (2018, 5079693) Medium	United States, Recruitment: 2003–2006; Follow-up at ages 3 and 8 years	Cohort	Mother-child dyads from the HOME study 204	Serum Prenatal: 12.9 (8.8–17.6) 3 years: 6.2 (4.5–9.9) 8 years: 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 years: −0.4 (−3.2, 2.5) 8 years: −2.5 (−6.0, 1.1) Tau Prenatal: 6.0 (−23.2, 35.2) 3 years: 13.4 (−9.8, 36.5) 8 years: 5.8 (−22.1, 33.7) Visual-spatial scores (VMWM) Learning distance Prenatal: 0.2 (−1.6, 1.7) 3 years: −0.7 (−2.2, 0.7) 8 years: −0.2 (−1.7, 1.3) Learning time Prenatal: −0.1 (−2.8, 2.6) 3 years: −1.1 (−3.5, 1.2) 8 years: −2.1 (−4.9,0.6) Memory retention distance Prenatal: 2.8 (−1.3, 6.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							3 years: 0.3 (−4.7, 5.4) 8 years: 2.1 (−2.9, 7.0) Memory retention time Prenatal: 0.4 (−1.1, 1.9) 3 years: −0.4 (−2.1, 1.3) 8 years: 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, 5080218) Medium	United States, Recruitment: 2003–2006; Follow-up at ages 3 and 8 years	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						
Vuong et al. (2020, 6833684)	United States, Recruitment:	Cohort	Mother-child pairs with	Maternal serum	Wide Range Achievement	Regression coefficient per	7.0 (−2.9, 16.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium	2003–2006; Follow-up at age 8 years		children aged 8 years from the HOME study N = 161	Mean = 13.9 (SD = 7.9)	Test 4 (WRAT-4) reading composite score	log10-unit increase in PFOS	
	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. (2015, 3860120) Medium	Taiwan, Recruitment: 2000–2001; Follow-up at ages 5 years	Cohort	Pregnant women and their children aged 5 and 8 years 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. (2018, 4238294) Medium	United States, Recruitment: 2003–2006; Follow-up at ages 3, 5, and 7 years	Cohort	Pregnant women and their children aged 3, 5, and 7 years from the HOME study N = 167	Serum Maternal: 13.0 (9.1–17.8) 3 years: 6.6 (4.6–10.2) 8 years: 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, sentence Comprehension measured by	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) Word Attack Maternal Serum: 4.1 (−1.2, 9.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Wide Range Achievement Test 4 (WRAT-4)		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, 6711603) Medium	United States, 2003–2016	Cross-sectional	Adults aged 20–69 years 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, poverty-income ratio, smoking status, BMI, noise exposures (occupational, recreational, firearm noise), NHANES cycles						
Gallo et al. (2013, 2272847) Medium	United States, 2005–2006	Cross-sectional	Adults aged 50+ years from the	Serum Range = 0.25–759.2	Memory impairment (self-reported)	OR per doubling of	0.93 (0.90, 0.96) Q2: 0.96 (0.87, 1.07) Q3: 0.86 (0.78, 0.96)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			C8 Health Project N = 21,024			PFOS and by quintiles	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, 5080366) 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, 6833686) 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 dB 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						
Shrestha et al. (2017, 3981382) Medium	United States, 2000–2002	Cross-sectional	Residents aged 55–74 years who lived	Serum 33.7 (23.3–50.8)	Affective state: Beck Depression	Regression coefficient per	Depression: 0.25 (−0.77, 1.26), p-value = 0.63

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			adjacent to Hudson River N = 126		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 Verbal Learning Test total and subscores,	IQR increase in ln-unit PFOS	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]
					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 Intelligence Scale-Revised total scores for		

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					block design and digit symbol coding		
	Confounding: Age, sex, education, serum total PCB						
				Pregnant Women			
Vuong et al. (2020, 6356876) Medium	United States Recruitment: 2003–2006 Follow-up: ~20 weeks gestation and postpartum (4 weeks, 1, 2, 3, 4, 5, and 8 years)	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 years 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; ALSPAC = Avon Longitudinal Study of Parents and Children; ASD = autism spectrum disorder; ASQ-3 = Ages and Stages Questionnaire-3; BMI = body mass index; BRIEF = Behavior Rating Inventory of Executive Function; CDI = Comprehensive Developmental Inventory; CHARGE = Childhood Autism Risk from Genetics and Environment; DaFO88 = Danish Fetal Origins 1988; 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; IQR = interquartile range; LINC = Linking Maternal Nutrition to Child Health; LFHI = low frequency hearing impairment; MoBa = Mother, Father, and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PFOS = perfluorooctane sulfonic acid; RR = risk ratio; SDQ = Strengths and Difficulties Questionnaire; TMICS = Taiwan Maternal and Infant Cohort Study.

[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, 5080657) 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, 2850967) Low	Taiwan, 2006–2008	Cross-sectional	Adolescents and young adults from YOTA study, 12–30 years, 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, 5080465) 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)	CDK (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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Liu et al. (2018, 4238514) Low	United States, 2013–2014	Cross-sectional	Adults from NHANES, 18+ years, N = 1871	Serum GM = 5.28 (SE = 1.02)	Total protein (g/dL)	Regression coefficient per ln-unit increase in PFOS	0.05 (SE = 0.02); p-value < 0.01
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)						
Arrebola et al. (2019, 5080503) 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, body mass index, weight loss during the last 6 months, region of recruitment, smoking habit, alcohol consumption, education, place of residence						
Chen et al. (2019, 5387400) Low	Croatia, 2007–2008	Cross-sectional	Adults, 44–56 years 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 (2019, 5381566) Low	United States, 2005–2014	Cross-sectional	Adults from NHANES, ≥ 20 years N = 8,220	Serum Levels not reported	Levels of albumin in urine (log10-µg/mL), creatinine in	Regression coefficient per log10-unit increase in	Albumin in urine Per log10-unit increase: −0.08 p-value < 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
					urine (log10-mg/dL), albumin-to-creatinine ratio in urine (log10-mg/g), albumin in serum (log10-mg/dL), creatinine in serum (log10-mg/dL)	PFOS, or percent change per 10% increase in PFOS	Negative associations across eGFR stages 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							p-value < 0.05 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), poverty income ration, NHANES survey period						
Jain and Ducatman (2019, 5080378) Low	United States, 2007–2014	Cross-sectional	Adults from NHANES, ≥ 20 years, 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), poverty income ration, NHANES survey period						
Wang et al. (2019, 5080583) 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), pvalue = 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. (2019, 5918630) 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. (2020, 6833670) Low	United States, 2009–2014	Cross-sectional	Adults from NHANES N = 4915 (no CKD = 4103; 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, 2919148) Low	United States, 1999–2000; 2003–2008	Cross-sectional	Children and adolescents from NHANES, 12–18 years, 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, 3859835) Low	United States, 2003–2010	Cross-sectional	Children and adolescents from NHANES, 12–19 years, 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, poverty-income ratio, caregiver education, serum cotinine, prehypertension, insulin resistance, BMI, hypercholesterolemia, race/ethnicity categories						
Qin et al. (2016, 3981721) Low	Taiwan, 2009–2010	Cross-sectional	Children from GBCA Study, 12–15 years, 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)
	Outcome: Hyperuricemia defined as uric acid level ≥ 6 mg/dL.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	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, 4238547) Low	United States 2016 **Confounding:** Age, sex, race	Cross-sectional	Obese children, 8–12 years N = 40	Serum 2.79 (IQR = 2.10)	Creatinine (mg/dL)	Regression coefficient per unit increase in PFOS	0 (−0.02, 0.03)
			Pregnant Women				
Nielsen et al. (2020, 6833687) 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 nonsignificant 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.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Confounding: Number of days between sampling, pregnancy-induced change in BMI							
Occupational Populations							
Rotander et al. (2015, 3859842) Low	Australia, 2013	Cross-sectional	Firefighters with past exposure to AFFF, 17–66 years 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; eGFR = estimated glomerular filtration rate (mL/min per 1.73 m^2); FCC = Fernald Community Cohort; GBCA = Genetic Biomarkers Study for Childhood Asthma; GF = glomerular filtration; GM = geometric mean; LMrev = Lund Malmö Revised; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PONCH = Pregnancy Obesity Nutrition and Child Health study; SD = standard deviation; SE = standard error; YOTA = Young Taiwanese Cohort Study.

[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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)	Outcome	Comparison	Select Results[a]
Etzel et al. (2019, 5043582) Medium	United States, 2003–2010	Cross-sectional	Children and adults from NHANES, ≥ 12 years 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) No other statistically significant associations or trends
	Results: Lowest quintile used as reference group. **Confounding:** Gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty to income ratio, smoking status, 6-month examination time period[c]						
Chen et al. (2019, 5387400) Medium	Croatia 2007–2008	Cross-sectional	Adults, 44–56 years 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 (2020, 6333438) Medium	United States 2003–2016	Cross-sectional	Adults from NHANES, ≥ 20 years 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)	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)	Outcome	Comparison	Select Results[a]
				anemic females: GM = 5.0 (95% CI: 4.4, 5.8)			
	Confounding: Age, BMI, poverty income ratio, serum cotinine, survey year, daily alcohol intake						
Khalil et al. (2018, 4238547) Low	United States, 2016	Cross-sectional	Children with obesity, 8–12 years 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, 5080340) Low	The Netherlands, 2015	Cross-sectional	Dutch men, 40–70 years 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; GM = geometric mean; HIV = human immunodeficiency virus; IQR = interquartile range NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PPT = prothrombin time.

[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, 5043613) Medium	France, Greece, Lithuania, Norway, Spain, United Kingdom 2003–2009	Cohort	Pregnant women and their children, ages 6–12 years, N = 1,033	Maternal and child's serum, plasma, or whole blood Prenatal (maternal) Median = 6.6 (IQR = 5.8) Postnatal (child) Median = 2.1 (IQR = 1.9)	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
	Confounding: Centre 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 body mass index, postnatal passive smoking status, prenatal maternal active, passive smoking status[c]						
Gaylord et al. (2019, 5080201) Medium	New York, US 2014–2016	Cross-sectional	Adolescents and young adults, ages 13–22 years, 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 5Hz, 5–20Hz, 20Hz	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, 4238440)	Norway 1992–2002	Cohort	Infants followed up at 2 years and 10,	Cord blood, Median = 5.2 (4.0, 6.6)	Oslo Severity Score (1–5 vs. 0)	OR per log2-unit increase in PFOS	1.71 (1.16, 2.53), p-value = 0.007

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			N = 641		Oslo Severity Score (6–12 vs. 0)		1.15 (0.71, 1.84), p-value = 0.576
					Reduced lung function at birth		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						
Manzano-Salgado et al. (2019, 5412076) Medium	Spain 2003–2015	Cohort	Pregnant women and their children, followed up at ages 1.5, 4, and 7 years, N = 503 (4 years) N = 992 (7 years)	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, 3869265) 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 1s; FRC = Functional Residual Capacity; FVC = Forced Vital Capacity; PEF = Peak Expiratory Flow rate; RV = residual volume; TLC = total lung capacity; WTCHR = World Trade Center Health Registry.

[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							
Jeddy et al.(2018, 5079850) 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 months[c]						
Cluett et al.(2019, 5412438) 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, bone mineral content (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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	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, 4238547) Low	United States 2016	Cross-sectional	Obese children, ages 8–12 N = 23	Serum 2.79 (IQR = 2.10)	BMD measured as broadband ultrasound attenuation (dB/MHz) and speed of sound (m/s), stiffness index (%)	Regression coefficient per unit increase in PFOS	BMD (broadband ultrasound attenuation) −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, 5080655) 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, 1937226) 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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							2.37 (1.35, 4.16), p-value < 0.01 Q2: 0.65 (0.19, 2.20) Q3: 1.11 (0.29, 4.30) 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, 5079772) 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, 3229485) 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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Q4: −0.034 (−0.059, −0.009), p-value < 0.05 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, 6315798) 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-year weight change						

Notes: aBMD = areal bone mineral density; ALSPAC = Avon Longitudinal Study of Parents and Children; BMD = bone mineral density; BMI = body mass index; GM = geometric mean; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POUNDS-Lost = Prevention of Obesity Using Novel Dietary Strategies Lost clinical trial; Q1 = quartile one; Q4 = quartile four; SD = standard deviation; SE = standard error; SES = socioeconomic status.
[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, 6833710) Medium	Guinea-Bissau 2012–2015	Cohort	Children aged < 2 years 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-month visit	At inclusion: 1.14 (0.66, 1.96) At 9 months: 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, 3858505) Low	Denmark 2010–2015	Cohort	Pregnant women and their children from the Odense Child Cohort, Ages 1–4 years 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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							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						
Hammer et al. (2019, 8776815) 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. (2020, 6315709) 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; PFOS = perfluorooctane sulfonate; RR = risk ratio; RCT = randomized controlled trial.

[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, 5080517) Medium	United States 1999–2002	Cross-sectional	Adolescents from NHANES aged 12–19 years 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 & Waters (2019, 5386081) Medium	United States 2013–2014	Cross-sectional	Children from NHANES aged 3–11 years 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.
[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, 6315698) Medium	China, 2016	Cross-sectional	Adults from the Isomers of C8 Health Project, ages 22–96 years, 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 years: 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 years: 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.
[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, 5080529) 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 body mass index, daily number of cigarettes smoked in first trimester[c]						

Notes: DNBC = Danish National Birth Cohort.
[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[a]	Outcome	Comparison	Select Results[b]
Grice et al. (2007, 4930271) 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, 2919344) Medium	Denmark 1993–2006	Cohort	Adults with no previous cancer diagnosis, Ages 50–65 at enrollment, Prostate cancer, 1,393; Bladder cancer, 1,104;	Serum Mean (5th–95th percentile): Cases, men: 35.1 (17.4–60.9); Controls, men: 35.0 (16.8–62.4);	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
			Pancreatic cancer, 900; Liver cancer, 839	Cases, women: 32.1 (14.0–58.1); Controls, women: 29.3 (14.2–55.6)			Q4: 0.70 (0.46, 1.07) Per unit increase: 0.93 (0.83, 1.03) 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, 2150988) 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						
Ducatman et al. (2015, 3859843) 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	Age 20–49 β = 1, p-value = 0.71; GMR = 0.95 (0.71, 1.28) Age 50–69

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
						GM ratio (GMR) (PSA < 4.0 ng/mL vs. PSA ≥ 4.0 ng/mL)	β = 1, p-value = 0.99; GMR = 1.1 (0.98, 1.23)
	Confounding: Age, smoking status, average alcohol intake, and body mass index[c]						
Ghisari et al. (2017, 3860243) 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
	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						
	Results: Lowest tertile used as the reference group **Confounding:** Age, BMI, cotinine levels, parity, and breastfeeding						
Hurley et al. (2018, 5080646) Medium	California, US 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, 5412451) 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)	Breast cancer	OR per log2-unit increase in PFOS	0.3 (0.1, 0.9), p-value = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
				Controls: 32.1 (14.9–58.2)			
	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, 5381529) 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 **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, 7161466) 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) µg/L	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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
Fry and Power (2017, 4181820) Medium	US 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						
Christensen et al. (2016, 3858533) Low	Wisconsin, US, 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. (2020, 6835434) Low	China 2014–2017	Case-control	Children, < 16, 84	Serum 4.47 (2.48–8.26)	Germ cell tumors	OR per unit increase in PFOS	1.08 (0.96, 1.21)
	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, 6833693) 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 9959632) 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						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
	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, 10176563) 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) 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, 7021502) Low	United States 2005–2012	Cross-sectional	Adults from NHANES, Ages ≥ 20 years, 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Select Results[b]
							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						

Notes: CHDS = The Child Health and Development Studies; DDE = dichlorodiphenyldichloroethylene; DDT = dichloro-diphenyl-trichloroethane; GM = geometric mean; IRR = incidence rate ratio; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; SD = Standard deviation.

[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

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 (2018, 5083631) Results for Decreased Tetanus Antibody Concentrations at Seven Years of Age and PFOS Exposure Measured at Five Years of Age

Budtz-Jørgensen and Grandjean (2018, 5083631) fit multivariate models of PFOS measured at age five years, against $\log_2$-transformed anti-tetanus antibody concentrations measured at the seven-year-old examination controlling for sex, exact age at the seven-year-old examination, and booster type at age five 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 (2018, 5083631) 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, 2018, 5083631}. 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 (2018, 5083631) Table 3), or for the model that did adjust for PFOA ($\log_2$[PFOA]) ($p = 0.71$).

Table E-1 summarizes the results from Budtz-Jørgensen and Grandjean (2018, 5083631) for PFOS at age five years and tetanus antibodies at age seven years. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a BMR of 5% decrease in the antibody concentration in Table 1 of Budtz-Jørgensen and Grandjean (2018, 5083631).6 As Budtz-Jørgensen and Grandjean (2018, 5083631) $\log_2$-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)).

[6] Budtz-Jørgensen and Grandjean (2018, 5083631) computed BMDs and BMDLs using a BMR of 5% decrease in the antibody concentrations. Their formula, BMD = $\log_2(1-BMR)/\beta$, can simply be reversed to solve for $\beta = \log_2(1-BMR)/BMD$. For negative dose-response where more exposure results in lower antibody concentration, the BMDL is based on the lower bound of β, (β_{LB}). Thus, the $\beta_{LB} = \log_2(1-BMR)/BMDL$. The $SE(\beta) = (\beta-\beta_{LB})/1.645$. The p-value is the two-sided probability that $Z <= SE(\beta)/\beta$.

Table E-1. Results specific to the slope from the linear analyses of PFOS measured at age five years and log_2(tetanus antibody concentrations) measured at age seven years from Table 1 in Budtz-Jørgensen and Grandjean (2018, 5083631) 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
PFOS at Age 5	Linear	No	−0.0274	0.0176	p = 0.12	−0.0565
PFOS at Age 5	Linear	Yes	−0.0039	0.0198	p = 0.84	−0.0365

Notes: SE = standard error.

Interpretation of results in Table E-1:

- PFOS is a non-significant predictor in the single-PFAS model (β = −0.0274; p = 0.12).
- Effects of PFOS in the single-PFAS model are attenuated when log_2[PFOA] is included in the model (β = −0.0039; p = 0.84).
- Nevertheless, these data can be used to estimate a BMDL for completeness and to allow comparisons across PFAS.

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, 1239433}. Selecting a BMR to estimate the BMDs and BMDLs involves making judgments about the statistical and biological characteristics of the data set and about the applications for which the resulting BMDs and BMDLs will be used. An extra risk of 10% is recommended as a standard reporting level for quantal data for toxicological data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects as the basis of the POD for a reference value. However, a BMR of 1% has typically been used for quantal human data from epidemiology studies {U.S. EPA, 2012, 1239433}, 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, 2021, 10428576} reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018, 5083631), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018, 5083631) and determined that a different approach should be used to be consistent with EPA guidance {U.S. EPA, 2012, 1239433}, 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 cut-off 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, 2017, 4239492} 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, 9642152) mentions the same concentration. However, the 2018 WHO update {WHO, 2018, 10406857} 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, 1239433}. 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, 1239433} 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.

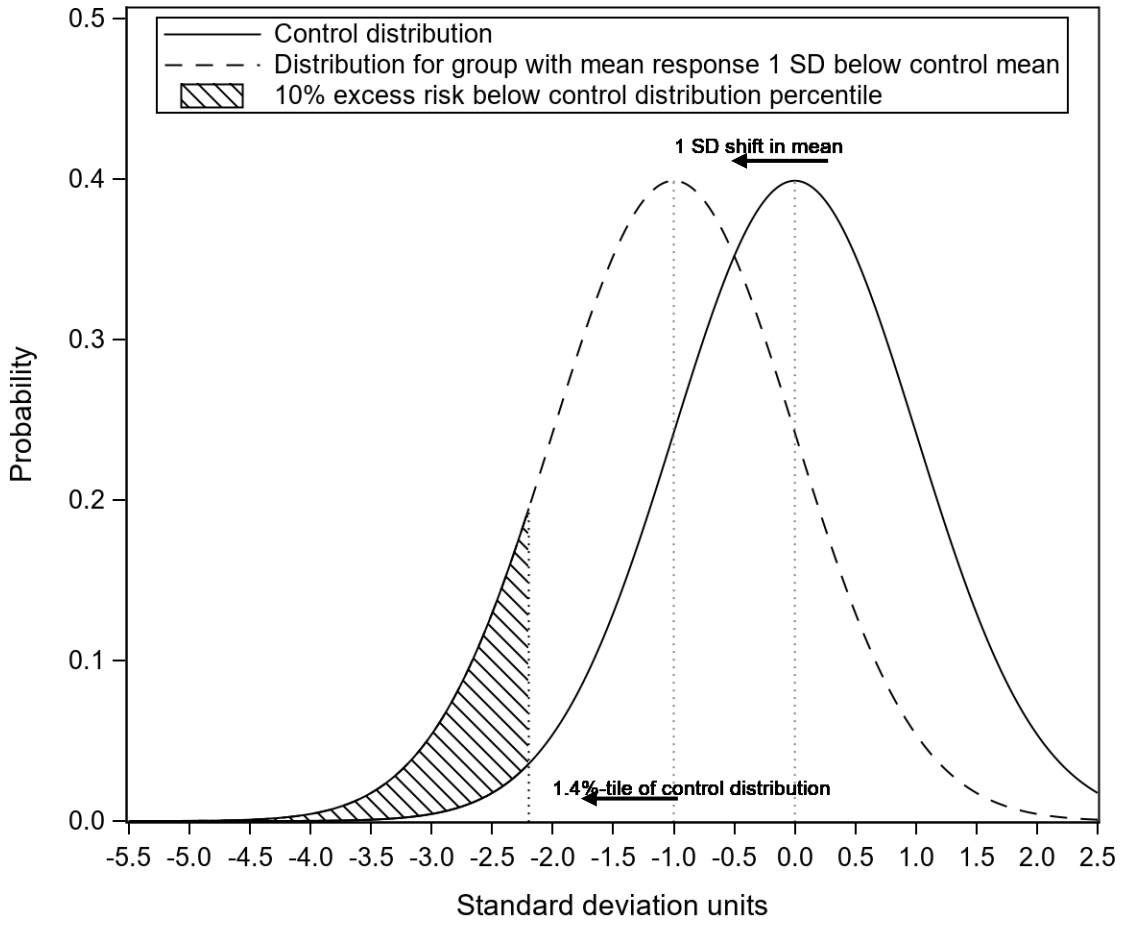


Figure E-1. Difference in population tail probabilities resulting from a one standard deviation shift in the mean from a standard normal distribution, illustrating the theoretical basis for a baseline BMR of 1 SD

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, 1239433}, 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 (2018, 5083631) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study {U.S. EPA, 1991, 732120} 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 U.S. of 13% during 2001–2008 {CDC, 2011, 9998272}. The case-fatality rate can be more than 80% for early lifestage cases {Patel, 1999, 10176842}. Selgrade (2007, 736210) 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, 1239433}, 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). 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, 1248827) 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, 10406286}. 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 seven 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.

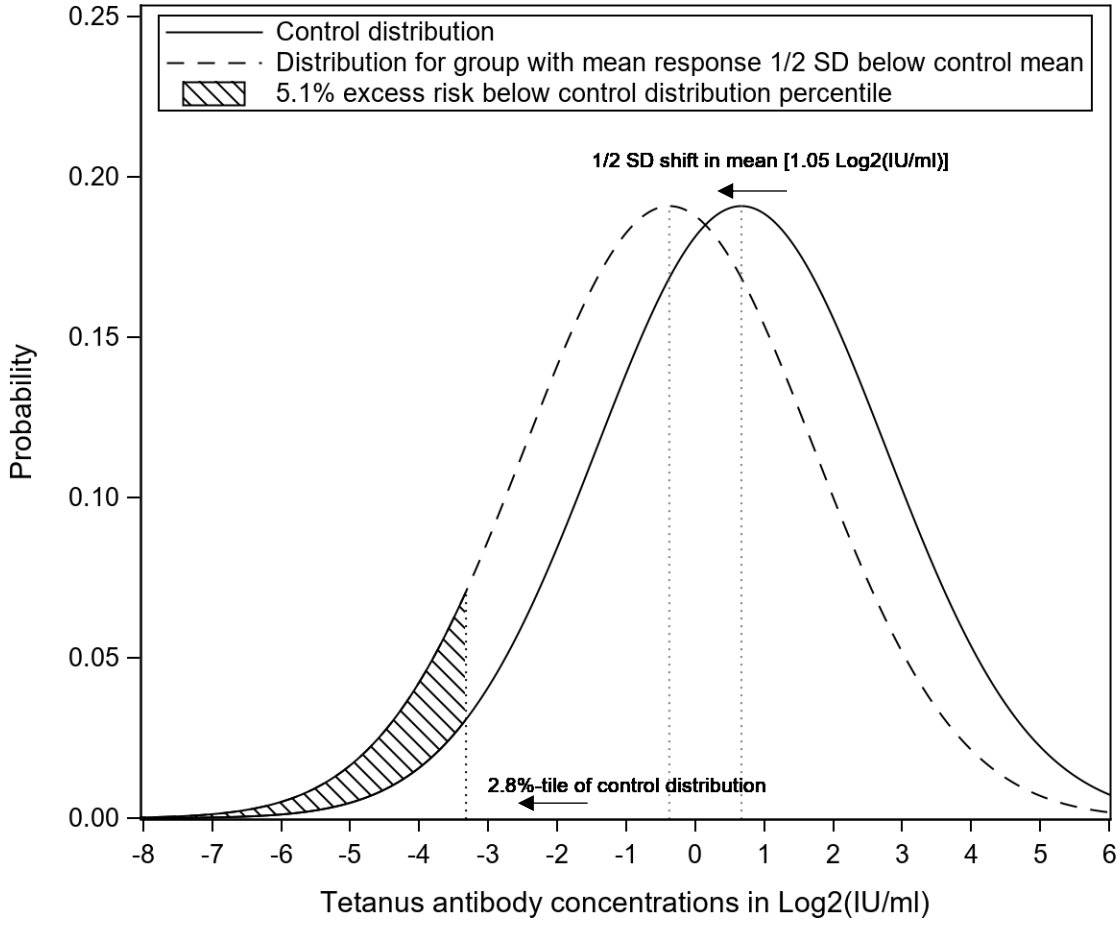


Figure E-2. Difference in population tail probabilities resulting from a ½ standard deviation shift in the mean from an estimation of the distribution of $\log_2$(tetanus antibody concentrations at age seven years)

Table E-2. BMDs and BMDLs for effect of PFOS at age five years on anti-tetanus antibody concentrations at age seven years {Budtz-Jørgensen, 2018, 5083631} 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) $\beta = -0.274$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0565$ per ng/mL	BMD (ng/mL) $\beta = -0.0039$ per ng/mL	BMDL (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:
[a] Denotes the selected POD.

The lowest serum PFOS concentration measured at age five years was 3.3 ng/mL, the 5th percentile was 9.5 ng/mL, and the 10th percentile was 10.7 ng/mL {Grandjean, 2021, 9959716} 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 controls 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. Overall confidence in the BMDLs for Tetanus was judged to be low.

For immunotoxicity related to tetanus associated with PFOS exposure measured at age five 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 (2018, 5083631) Results for Decreased Tetanus Antibody Concentrations at Five Years of Age and PFOS Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018, 5083631) fit multivariate models of PFOS measured perinatally in maternal serum, against log_2-transformed anti-tetanus antibody concentrations measured at the five-year-old examination controlling for sex, and exact age at the five-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 (2018, 5083631) 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 (2018, 5083631). Compared to 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 (2018, 5083631) Table 3), or for the model that did adjust for PFOA (log_2[PFOA]) (p = 0.98).

Table E-3 summarizes the results from Budtz-Jørgensen and Grandjean (2018, 5083631) for tetanus in this exposure window. These regression coefficients (β) and their standard errors (SE) were computed by EPA from the published BMDs and BMDL based on a BMR of 5% change in tetanus antibody concentrations in Table 2 of Budtz-Jørgensen and Grandjean (2018, 5083631).

Table E-3. Results of the linear analyses of PFOS measured perinatally and tetanus antibodies *measured* at age five years from Budtz-Jørgensen and Grandjean (2018, 7276745) 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	−0.0102	0.0095	p = 0.28	−0.0259

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	Yes	0.0021	0.0107	p = 0.85	−0.0156

Notes: SE = standard error.

Interpretation of results in Table E-3:

- PFOS is a non-significant predictor in the single-PFAS model (β = −0.0102; p = 0.28).
- Effects are attenuated when $\log_2$[PFOA] are included in the model (β = 0.0021; p = 0.85).
- Nevertheless, these data can be used to estimate a BMDL for completeness and to allow comparisons across PFAS.

Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft {U.S. EPA, 2021, 10428576} reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018, 5083631), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018, 5083631) and determined that a different approach should be used to be consistent with EPA guidance {U.S. EPA, 2012, 1239433}, 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 cut-off for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, 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 (2018, 5083631) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study {U.S. EPA, 1991, 732120} 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, 1239433}, 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 five years was estimated from two sets of distributional data presented from two different cohorts of five-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018, 5083631). Grandjean et al. (2012, 1248827) reported on 587 five-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017, 4239492) 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 five 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 five 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).[7] To show the impact of the BMR on these results, Table E-4 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-4. BMDs and BMDLs for effect of PFOS measured perinatally and anti-tetanus antibody concentrations at age five years {Budtz-Jørgensen, 2018, 5083631}

BMR	Estimated without control of PFOA		Estimated with control of I	
	BMD (ng/mL) β = −0.0102 per ng/mL	BMDL (ng/mL) β_{LB} = −0.0259 per ng/mL	BMD (ng/mL) β = 0.00207 per ng/mL	β_{LB} =
½ SD	75.9	29.9[a]	–[b]	
1 SD	151.8	59.8	–	

Notes:
[a] Denotes the POD that corresponds to the analyses of PFOS concentrations perinatally and tetanus antibodies at age five 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, 2021, 9959716} 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.

There is *low* confidence in the BMDLs from the PFOS-only model (29.9 ng/mL) and in the multi-PFAS model (49.8 ng/mL). Confidence is diminished by the low quality of the model fit for PFOS in either model compared to 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, 2018, 7276745), and thus this POD is identified with *low* confidence.

For immunotoxicity related to tetanus associated with PFOS exposure measured at age five 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.

[7] 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).

E.1.1.1.3 Timmerman et al. (2021, 9416315)

Timmerman et al. (2021, 9416315) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOS and $\log_{10}$-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, > 1year) 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 −3 (95% CI: −8, 3) (Table 4, Timmerman et al. (2021, 9416315)). 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 (2018, 5083631), 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). 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, 9416315)). 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-5. BMDs and BMDLs for effect of PFOS on anti-tetanus antibody concentrations {Timmerman, 2021, 9416315} 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: 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) (Timmerman, 2021, 9416315), and thus this POD is identified with *low* confidence.

For immunotoxicity related to tetanus associated with PFOS exposure measured at ages five to ten 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-6 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. The comparisons PODs are considered *low* confidence because they are based on a poorly fit PFOS regression parameters.

Table E-6. BMDLs for effect of PFOS on anti-tetanus antibody concentrations using a BMR of ½ SD {Timmerman, 2021, 9416315}

Study	Effect	$BMDL_{½\ SD}$ (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018, 5083631)	PFOS at age five years and anti-tetanus antibody concentrations at age seven years	**18.5**	1.05 $\log_2$(IU/mL)
Budtz-Jørgensen and Grandjean (2018, 5083631)	PFOS perinatally and anti-tetanus antibody concentrations at age seven years	29.9	0.78 $\log_2$(IU/mL)
Timmerman et al. (2021, 9416315)	PFOS and anti-tetanus antibody concentrations at ages seven–10 years	9.66	0.35 $\log_{10}$(IU/mL)

E.1.1.2 Modeling Results for Decreased Diphtheria Antibody Concentrations

E.1.1.2.1 Budtz-Jørgensen and Grandjean (2018, 5083631) Results for Decreased Diphtheria Antibody Concentrations at Seven Years of Age and PFOS Exposure Measured at Five Years of Age

Budtz-Jørgensen and Grandjean (2018, 5083631) fit multivariate models of PFOS measured at age five years, against $\log_2$-transformed anti-diphtheria antibody concentrations measured at the seven-year-old examination controlling for sex, exact age at the seven-year-old examination, and booster type at age five 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 (2018, 5083631) 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, 2018, 5083631}. 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 (2018, 5083631) Table 3), or for the model that did adjust for PFOA ($\log_2$[PFOA]) ($p = 0.34$). Table E-7 summarizes the results from Budtz-Jørgensen and Grandjean (2018, 5083631) for diphtheria in this exposure window. These β and their SE were computed by EPA from the published BMDs and BMDL based on a BMR of 5% decrease in diphtheria antibody concentrations in Table 1 of Budtz-Jørgensen and Grandjean (2018, 5083631).[6]

Table E-7. Results specific to the slope from the linear analyses of PFOS measured at age five years and $\log_2$(diphtheria antibodies) measured at age seven years from Table 1 in

Budtz-Jørgensen and Grandjean (2018, 5083631) 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
PFOS at Age 5	Linear	No	−0.0322	0.0163	p = 0.05	−0.0591
PFOS at Age 5	Linear	Yes	−0.0207	0.0184	p = 0.26	−0.0510

Notes: SE = standard error.

Interpretation of results in Table E-7:

- PFOS is a significant predictor in the single-PFAS model (β = −0.0322; p = 0.05).
- Effects are attenuated when $\log_2$[PFOA] are included in the model (β = −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.

Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft {U.S. EPA, 2021, 10428576} reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018, 5083631), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018, 5083631) and determined that a different approach should be used to be consistent with EPA guidance {U.S. EPA, 2012, 1239433}, 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 cut-off for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, 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 (2018, 5083631) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study {U.S. EPA, 1991, 732120} 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. 1239433}, EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(diphtheria antibody concentrations), and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). A blood concentration for diphtheria antibodies of 0.1 IU/mL is sometimes cited in the diphtheria literature as a 'protective level' (Grandjean et al. (2017, 4239492) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL 'cutoff' level; and Galazka et al (1993, 10228565) 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, 10228563}. 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, 1986, 9978484}. 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, 1989, 9642154; Galazka, 1989, 9642152}."*

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 U.S., but can cause life-threatening airway obstruction, or cardiac failure {Collier, 1975, 9642066}. Among 13 cases reported in the U.S. during 1996–2016, no deaths were mentioned {Liang, 2018, 9978483}. However, diphtheria remains a potentially fatal disease in other parts of the world (Galazka {, 1993, 10228565} 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, 736210) suggests that specific immuno-toxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards—which has the potential to be a more adverse effect that just a single immuno-toxic effect.

Following the technical guidance {U.S. EPA, 2012, 1239433}, EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(diphtheria antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012, 1248827) 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-8 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-8. BMDs and BMDLs for effect of PFOS at age five years on anti-diphtheria antibody concentrations at age seven years {Budtz-Jørgensen, 2018, 5083631} 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) $\beta = -0.0322$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0592$ per ng/mL	BMD (ng/mL) $\beta = -0.0207$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.0510$ 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:
[a] Denotes the selected POD.

The lowest serum PFOS concentration measured at age five years was 3.3 ng/mL, the 5th percentile was 9.5 ng/mL, and the 10th percentile was 10.7 ng/mL {Grandjean, 2021, 9959716} 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 which 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, and overall confidence in the BMDLs for diphtheria was judged to be *medium*.

For immunotoxicity related to diphtheria, associated with PFOS measured at age five 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 (2018, 5083631) Results for Decreased Diphtheria Antibody Concentrations at Five Years of Age and PFOS Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018, 5083631) fit multivariate models of PFOS measured perinatally, against log_2-transformed anti-diphtheria antibody concentrations measured at the five-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 (2018, 5083631) 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 (2018, 5083631). Compared to 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 (2018, 5083631) Table 3), or for the model that did adjust for PFOA ($\log_2$[PFOA]) (p = 0.84). Table E-9 summarizes the results from Budtz-Jørgensen and Grandjean (2018, 5083631) for diphtheria in this exposure window. These β and their SE were computed by EPA from the published BMDs and BMDL based on a BMR of 5% change in diphtheria antibody concentrations in Table 2 of Budtz-Jørgensen and Grandjean (2018, 5083631).[6]

Table E-9. Results of the linear analyses of PFOS measured perinatally and diphtheria antibodies measured at age five years from Budtz-Jørgensen and Grandjean (2018, 7276745) in a single-PFAS model and in a multi-PFAS model

Exposure	Model shape	PFOA adjusted	Slope (β) per ng/mL	SE(β)	Slope (β) fit	Lower bound slope (β_{LB})
Perinatal PFOS	Linear	No	−0.0310	0.0100	p = 0.002	−0.0475
Perinatal PFOS	Linear	Yes	−0.0241	0.0113	p = 0.033	−0.0427

Notes: SE = standard error.

Interpretation of results in Table E-9:

- 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 22% difference in the BMD and 11% difference in the BMDL when PFOS is included in the model.

Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft {U.S. EPA, 2021, 10428576} reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018, 5083631), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018, 5083631) and determined that a different approach should be used to be consistent with EPA guidance {U.S. EPA, 2012, 1239433}, 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 cut-off for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, 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 (2018, 5083631) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study {U.S. EPA, 1991, 732120} 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, 1239433}, EPA derived BMDs and BMDLs associated with a one SD change in the distribution of $\log_2$(tetanus antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age five years was estimated from two sets of distributional data presented from two different birth cohorts of five-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018, 5083631). Grandjean et al. (2012, 1248827) reported on 587 five-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017, 4239492) reported on 349 five-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 five 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 five 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).[8] To show the impact of the BMR on these results, Table E-10 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-10. BMDs and BMDLs for effect of PFOS measured perinatally and anti-diphtheria antibody concentrations at age five years {Budtz-Jørgensen, 2018, 5083631}

BMR	Estimated without control of PFOA		Estimated with control of PFOA	
	BMD (ng/mL) β = −0.031 per ng/mL	BMDL (ng/mL) β_{LB} = −0.0475 per ng/mL	BMD (ng/mL) β = −0.0241 per ng/mL	BMDL (ng/mL) β_{LB} = −0.0427per ng/mL
½ SD	30.6	20.0[a]	39.4	22.3
1 SD	61.3	40.0	78.9	44.5

Notes:

[8] Pooled variance for diphtheria in five-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).

[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, 2021, 9959716} so the estimated BMD for a BMR of ½ SD ($BMDL_{½\ SD}$ = 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 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 which 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. *Medium* confidence in the BMDLs from PFOS linear model (20.0 ng/mL) with control of PFOA since the model fit reasonably well and these BMDLs show low uncertainty about confounding.

For immunotoxicity related to diphtheria, associated with PFOS measured at age five 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, 9416315)

Timmerman et al. (2021, 9416315) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOS against log_{10}-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, 9416315)). 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 (2018, 5083631), 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). 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-11. BMDs and BMDLs for effect of PFOS on anti-diphtheria antibody concentrations {Timmerman, 2021, 9416315} 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: 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) (Timmerman, 2021, 9416315), and thus this POD is identified with *low* confidence.

For immunotoxicity related to tetanus associated with PFOS exposure measured at ages five to ten 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-12 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-12. BMDLs for effect of PFOS on anti-diphtheria antibody concentrations using a BMR of ½ SD {Timmerman, 2021, 9416315}

Study name	Effect	BMDL (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018, 5083631)	PFOS at age five years on anti-diphtheria antibody concentrations at age seven years	**12.5**	0.74 log_2(IU/mL)
Budtz-Jørgensen and Grandjean (2018, 5083631)	PFOS perinatally on anti-diphtheria antibody concentrations at age seven years	20.0	0.95 log_2(IU/mL)
Timmerman et al. (2021, 9416315)	PFOS and anti-diphtheria antibody concentrations at ages 7–10 years	5.6	0.48 log_{10}(IU/mL)

E.1.1.3 Modeling Results for Decreased Rubella Antibody Concentrations

Granum et al (2013, 1937228) investigated the effects of prenatal exposure to perfluorinated compounds on vaccination responses and clinical health outcomes in early childhood in a subcohort of the Norwegian Mother and Child Cohort Study. A total of 56 mother-child pairs, for whom both maternal blood samples at delivery and blood samples from the children at 3 years of age, were evaluated. Antibody titers specific to measles, rubella, tetanus, and influenza were measured. Rubella antibody levels were inversely associated with maternal PFOS (mean = 5.6 ng/mL), but not with any other outcomes.

EPA considered applying a similar approach to those described above for decreased tetanus antibody concentrations in Budtz-Jørgensen and Grandjean (2018, 5083631) and Timmerman et al. (2021, 9416315) to estimate the BMD/BMDL associated with decreased rubella antibody concentrations in Granum et al. (2013, 1937228). Granum et al (2013, 1937228) reported a regression coefficient and 95% confidence interval from multivariate regression analyses of maternal PFOS and anti-Rubella antibody levels (−0.08, 95% CI: −0.14, −0.02). Granum et al (2013, 1937228) also reported summary statistics of rubella antibodies levels at the age of 3 (median = 1.9; 25th, 75th percentiles: 1.5, 2.1). Upon investigation of the extra risk using the distributional data and a cutoff value of 0.1 IU/mL it was determined that this data did not allow for application of a BMR of 1 and ½ SD to provide a reasonably good estimate of 10% and 5% extra risk. The Benchmark Dose Technical Guidance {U.S. EPA, 2012, 1239433} 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 one SD results in about 10% extra risk of being at risk of having an adverse effect. The cut off value of 0.1 IU/mL resulted in 0.003% of the control population at risk of having an adverse effect, a value much smaller than 1.4% which in turn did not result in 10% extra risk.

E.1.2 Modeling Results for Decreased Birthweight

Six *high* confidence studies {Chu, 2020, 6315711; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Wikström, 2020, 6311677; Darrow, 2013, 2850966; Yao, 2021, 9960202} 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, 7643488). 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 $\overline{y} = m\overline{x} + b$ using an average birth weight from an external population as $\overline{y}$, an average exposure as $\overline{x}$ and re-expressed β from the studies as m.

The CDC Wonder site (https://wonder.cdc.gov/natality.html) provides vital statistics for babies born in the United States. There were 3,791,712 all live births in the United States in 2018 according to final natality data. The mean and standard deviation of birth weight were 3261.6 ± 590.7 g (7.19 ± 1.30 lb), with 8.27% of live births falling below the public health definition of low birth weight (i.e., 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 US 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 cut-off 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, 6315711)

Chu et al. (2020, 6315711) 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, 7643488). 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, 6315711), EPA estimated the distribution of exposure by assuming the exposure follows a log-normal 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 2500 g are considered to have low birth weight, and further, low birth weight is associated with a wide range of health conditions throughout life {Hack, 1995, 8632216; Reyes, 2005, 1065677; Tian , 2019, 8632212}. Given this clinical cut-off for adversity and that 8.27% of all live births in the US in 2018 fell below this cut-off, 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[9]. Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cut-off for adversity in the tail of the response distribution. Application of the hybrid approach requires the selection of an extra risk value for BMD estimation. In the case of birth weight, an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from 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 2500 g cut-off value:

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

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

Based on the mean birth weight for all birth in the U.S. in 2018 of 3261.6 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 2500 g. In this case, the mean birth weight would be 3169.2g. Given the median exposure of 2.6 ng/mL from ACE Biomonitoring on Perfluorochemicals as $\overline{x}$, the mean birth weight in the U.S. as $\overline{y}$ and the re-expressed β as m term, the intercept b can be estimated as:

$$b = \overline{y} - m\overline{x} = 3261.6\ g - \left(-11.0\ g(\frac{ng}{mL})^{-1}\right) 2.6\frac{ng}{mL} = 3290.3\ g \qquad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x, using 3290.3 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, 6315711) reported a two-sided 95% confidence interval for the β coefficient, meaning that the lower limit of that confidence interval corresponds to a 97.5% one-sided lower limit. The BMDL is defined as the 95% lower limit of the BMD (i.e., corresponds to a two-sided 90% confidence interval), so the corresponding lower limit on the β coefficient needs to be calculated before calculating the BMDL. First, the standard error of the β coefficient can be calculated as:

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

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{-4.4\ g(\frac{ng}{mL})^{-1} - \left(-17.6\ g(\frac{ng}{mL})^{-1}\right)}{3.92} = 3.37\ g(\frac{ng}{mL})^{-1}$$

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 Sagiv et al. (2018, 4238410)

Sagiv et al. (2018, 4238410) 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 United States cohort. The intercept *b* is 3264.5 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, 4238410) 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 lower limit for the β coefficient from the lower limit on the 95% two-sided confidence interval of −2.6 g per ng/mL. Using the corresponding lower limit (−2.3 g per ng/mL), a BMDL of 41.0 ng/mL is calculated.

E.1.2.3 Starling et al. (2017, 3858473)

Starling et al. (2017, 3858473) 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 United States 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 3275.9 g. The 95% one-sided lower limit 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.1.2.4 Wikström et al. (2020, 6311677)

Wikström et al. (2020, 6311677) 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 lower limit 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.1.2.5 Darrow et al. (2013, 2850966)

Darrow et al. (2013, 2850966) 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 United States 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 3270.5 g. The 95% one-sided lower limit 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, 9960202)

Yao et al. (2021, 9960202) 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. (2018, 5080230), 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 3279.7 g. The 95% one-sided lower limit 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 US in 2018 that fell below the cut-off of 2500 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 cut-off of 2500 g. EPA estimated the study-specific tail probability of live births falling below the public health definition of low birth weight (2500 g) as:

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

$$b = \overline{y} - m\overline{x} = 3261.6 - (\beta_{re-expressed} * 3\frac{ng}{mL})$$

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 ($\bar{x}$), 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-13, are very similar to the main results, presented under the column of "Exact Percentage" in Table E-13, when background exposure was not accounted for while estimating the tail probability.

Table E-13 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 cut-off value. The BMDLs across the studies ranged from 5.0 ng/mL to 57.6 ng/mL.

Table E-13. 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, 6315711)	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)	3290.3	3.37	−16.5	11.0	7.3	9.05%	12.8	8.5
Sagiv et al. (2018, 4238410)	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)	3264.5	0.73	−2.3	85.2	41.0	9.78%	119.8	57.6
Starling et al. (2017, 3858473)	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)	3275.9	8.14	−18.9	19.4	5.7	9.45%	25.0	7.3
Wikström et al. (2019, 6311677)	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)	3283.4	3.94	−14.8	13.7	7.7	9.24%	16.7	9.4
Darrow et al. (2013, 2850966)	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)	3270.5	1.46	−5.8	29.6	17.4	9.60%	40.0	23.3
Yao et al. (2021, 9960202)	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)	3279.7	9.22	−22.1	15.9	5.0	9.34%	19.9	6.3

Notes: 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 US 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 Wikström et al. (2020, 6311677), presented in Table E-14, demonstrate the robustness of EPA's approaches with alternative assumptions on background exposures.

Table E-14. 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, 6311677)	2.6	3283.4	13.7	**7.7**	9.24%	16.7	9.4
	3.0	3286.7	14.1	7.9	9.14%	16.8	9.5
	5.7	3309.2	16.8	9.4	8.53%	17.6	9.9
	23.8	3460.4	34.9	19.6	5.20%	24.1	13.6

Notes:
[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, 6311677). Of the six individual studies, Sagiv et al. (2018, 4238410) and Wikström et al. (2020, 6311677) 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, 6311677) was ultimately selected as it was the lowest POD from these two studies.

E.1.3 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, 2019, 5080195; Lin, 2019, 5187597; Steenland, 2009, 1291109}. These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019, 5080195) investigated an NHANES population (2003–2014), while Steenland et al. (2009, 1291109) investigated effects in a high-exposure community (the C8 Health Project study population). Lin et al (2019, 5187597) collected data from prediabetic adults from the Diabetes Prevention Program (DPP) and DPP Outcomes Study at baseline (1996–1999).

E.1.3.1 Dong et al. (2019, 5080195)

Using data from NHANES (2003–2014) on 8,948 adults, Dong et al. (2019, 5080195) calculated a BMD for PFOS and TC using a hybrid model {Crump, 1995, 2258}. The cut-off 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, 5080195) 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 cut-off point used to define elevated TC or model fit parameters were not provided.

Although the hybrid approach has several advantages {Crump, 1995, 2258}, few details were provided in Dong et al. (2019, 5080195) 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; 5080195) 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, 5080195) reported a regression coefficient β, which we also call *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, 5080195) 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 the following table:

Table E-15. NHANES mean and standard deviation of TC (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 ($\bar{y}$)	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 ($\bar{x}$)	13.73	13.73	15.64	15.64	13.21	13.21	6.13	6.13

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, 10369680}.

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 we use the 95th upper limit for β, 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-16:

Table E-16. BMDs and BMDLs for effect of PFOS on increased cholesterol in Dong et al. (2019, 5080195)

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

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-16 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, 5080195) excluding people taking cholesterol medication, the longest period available, a BMR of 5% and a $BMDL_5$ of 9.3 ng/mL.

E.1.3.2 Steenland et al. (2009, 1291109)

The above hybrid approach was also applied to Steenland et al. (2009, 1291109) using log-transformed values. In Table 4, Steenland et al. (2009, 1291109) reported a linear regression coefficient for change in ln-transformed TC per ln(PFOS): 0.2660 with a standard deviation of 0.00140. The NHANES data used in this approached is summarized in Table E-17 and BMD/BMDL values are presented in Table E-18.

Table E-17. 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

Table E-18. BMDs and BMDLs for effect of PFOS on increased cholesterol in Steenland et al. (2009, 1291109)

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

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, 1291109). 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, 1291109)), summarized in Table E-19. 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-20.

Table E-19. Regression Results for Serum Total Cholesterol by Deciles of serum PFOS from Steenland et al. (2009, 1291109)

Decile	Dose (ng/mL)	N	Regression coefficient[a] (SD)
1	6.37	4629	0.00 (0.192)
2	10.60	4629	0.01 (0.192)
3	13.65	4629	0.01 (0.192)
4	16.19	4629	0.03 (0.192)
5	18.79	4629	0.03 (0.192)

Notes:
[a] Regression coefficient, change in the natural log of total cholesterol

Table E-20. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol in Steenland et al. (2009, 1291109)

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	−10350.92	0.00	−1.16	−1.54	0.76	0.00	25.38	24.66
Exponential 5	–	–	–	–	–	–	–	–	–
Hill	–	–	–	–	–	–	–	–	–
Polynomial Degree 3	0.00	−10588.86	−0.78	−0.78	0.00	45.95	33.33	31.36	26.15
Polynomial Degree 2	0.00	−10588.82	−0.71	–	–	47.85	39.78	–	–
Power	0.00	−10588.89	−0.75	−0.75	0.02	48.56	47.46	32.31	29.22
Linear	0.01	−10589.87	−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

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, 1291109) as shown in Table E-21. 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-22.

Table E-21. Odds ratios for elevated serum TC by quartiles of serum PFOS from Steenland et al. (2009, 1291109)

Quartile	Dose (ng/mL)	N	Incidence	OR	95% CI
1	6.6	11534	1479	1	Ref
2	16.4	11587	1634	1.14	1.05, 1.23
3	23.8	11441	1795	1.28	1.19, 1.39
4	50.55	11400	2158	1.51	1.40, 1.64

Table E-22. Summary of Benchmark Dose Modeling Results for Elevated Total Cholesterol in Steenland et al. (2009, 1291109)

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]	–	3.56x10-6	–	–	–	–	31.08	26.59
Gamma	0.53	39272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.21
Log-Logistic	**0.57**	**39272.40**	**−0.24**	**−0.24**	**−0.05**	**63.18**	**55.91**	**29.93**	**26.39**
Multistage Degree 3	0.01	39282.00	−0.58	−0.58	−1.57	62.48	0.00	40.96	40.29
Multistage Degree 2	0.53	39272.57	−0.28	−0.28	−0.14	63.00	55.88	30.67	27.20
Multistage Degree 1	0.53	39272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.20
Weibull	0.53	39272.57	−0.28	−0.28	−0.14	63.00	55.89	30.67	27.21
Logistic	0.27	39274.11	−0.42	−0.42	−0.62	62.30	56.70	34.49	31.47
Log-Probit	0.35	39274.11	−0.10	−0.10	0.16	66.02	57.02	29.71	14.27
Probit	0.31	39273.81	−0.40	−0.40	−0.55	62.43	56.61	33.93	30.84
Quantal Linear	0.53	39272.57	−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-17 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, 1291109) 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.1.3.3 Lin et al. (2019, 5187597)

Lin et al. (2019, 5187597) collected data from prediabetic adults from the Diabetes Prevention Program (DPP) and DPP Outcomes Study at baseline (1996–1999). This study included 888 pre-diabetic 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, 5187597). 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 percent difference in lipid levels (mg/dL) per quartile of baseline plasma PFOS concentrations (ng/mL), summarized in Table E-23. 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-24. 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-23. Adjusted Mean Differences in Serum Total Cholesterol by Quartiles of Serum PFOS (ng/mL) from Lin et al. (2019, 1291109)

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:
[a] mean ± standard deviation.
[b] Adjusted mean difference in lipid levels (mg/dL) per quartile of baseline plasma PFOS concentration (ng/mL)

Table E-24. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol Lin et al. (2019, 5187597)

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	8863.69	−0.21	−0.21	−0.60	108.34	61.19	88.53	57.34
Exponential 5	–[b]	–	–	–	–	–	–	–	–
Hill	–	–	–	–	–	–	–	–	–
Polynomial Degree 3	0.65	8861.12	−0.35	−0.35	−0.21	261.96	86.09	130.98	66.43
Polynomial Degree 2	0.65	8861.12	−0.34	-	-	262.61	100.07	–	–
Power	0.65	8861.12	−0.34	−0.34	−0.21	262.62	58.47	131.31	66.54
Linear	**0.65**	**8861.12**	**−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.1.3.4 Summary of Modeling Results for Increased Cholesterol

Table E-25 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, 5187597) is considered *low* confidence because it is based on a poorly fit PFOS regression parameter.

Table E-25. BMDLs for effect of PFOS on serum total cholesterol using a BMR of 5%.

Study name	Effect	BMDL (ng/mL)
Dong et al. (2019, 5080195)	Exclude those prescribed cholesterol medication, 1999–2018	**9.34**
Steenland et al. (2009, 1291109)	Exclude those prescribed cholesterol medication	9.52
Lin et al. (2019, 5187597)	Diabetic adults	66.5

E.1.4 Modeling Results for Liver Toxicity

This updated review indicated that PFOS is associated with increases in the liver enzyme ALT (See Main PFOS Document). 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, 1276142) conducted in 47,092 adults from the C8 Study Project (for detailed descriptions of the study and findings see Main PFOS Document and Appendix D). Two additional studies {Lin, 2010, 1291111; Nian, 2019, 5080307} were considered by EPA for POD derivation because they reported significant association in general populations in the U.S and a high exposed population China, respectively. In an NHANES adult population, Lin et al. (2010, 1291111) 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, 5080307) 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.1.4.1 Gallo et al. (2012, 1276142)

Gallo et al. (2012, 1276142) 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 vs. 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 to the

lowest (reference) deciles. The cutoff values used to define elevated ALT levels 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, 2002, 10369681}, and were approximately the 90th percentile of all ALT values in this study.

Elevated ALT

NOAEC/LOAEC method. The results of the logistic regression analysis of elevated ALT across deciles of PFOS are presented in Table E-26. 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-26. The no observed adverse effect concentration (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 to the reference category (the lowest decile of PFOS). The NOAEC based on the elevated ALT data from Gallo et al. (2012, 1276142) is 10.6 ng/mL.

Table E-26. Odds Ratios for Elevated ALT by Decile of PFOS serum concentrations (ng/mL) from Gallo et al. (2012, 1276142). The NOAEC is bolded.

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

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, 1992, 5069}, 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. 10406419}. Through author correspondence EPA obtained the number of participants with and without elevated ALT for each decile of PFOS (Table E-26).

Applying BMDS v3.3rc10 using a BMR of 10% and 5% the data for all ten 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-27, Table E-28, Table E-29, Table E-30. This modeling approach results in BMD and BMDL values higher than the maximum dose included in the modeled data set. The BMD and BMDL values were inside the range of mean exposure values when considering all ten deciles.

Table E-27. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012, 1276142) 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	15296.47	−0.11	−0.11	0.16	28.37	25.58	22.69	20.63
Log-Logistic	0.91	15296.50	−0.11	−0.11	0.17	27.68	22.17	22.50	20.19
Weibull	**0.98**	**15294.50**	**−0.11**	**−0.11**	**0.17**	**27.47**	**23.26**	**22.46**	**20.46**
Logistic	0.52	15296.80	0.67	0.67	0.83	43.97	33.33	25.48	19.53
Log-Probit	0.94	15296.44	−0.10	−0.10	0.14	29.51	22.98	22.98	20.39
Probit	0.51	15296.87	0.69	0.69	0.83	45.41	34.13	25.66	19.47
Quantal Linear	0.45	15297.26	0.80	0.80	0.82	54.66	38.95	26.61	18.96

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.

Table E-28. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012, 1276142) 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	15296.40	−0.09	−0.09	0.12	24.22	18.67	17.44	15.03
Log-Logistic	0.95	15296.41	−0.09	−0.09	0.14	23.67	16.76	17.30	14.58
Weibull	**0.94**	**15296.42**	**−0.09**	**−0.09**	**0.14**	**23.39**	**17.63**	**17.25**	**14.87**
Logistic	0.52	15296.80	0.67	0.67	0.83	41.00	30.25	23.47	17.42
Log-Probit	0.97	15296.36	−0.07	−0.07	0.10	26.47	17.71	17.96	14.79
Probit	0.51	15296.87	0.69	0.69	0.83	42.78	31.38	23.92	17.64
Quantal Linear	0.45	15297.26	0.80	0.80	0.82	54.66	38.95	26.61	18.96

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.

Table E-29. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012, 1276142) 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	15296.46	−0.10	−0.10	0.16	28.47	25.68	22.71	20.60
Log-Logistic	0.92	15296.49	−0.10	−0.10	0.17	27.80	22.17	22.53	20.20
Weibull	**0.98**	**15294.49**	**−0.10**	**−0.10**	**0.17**	**27.60**	**23.80**	**22.49**	**20.44**
Logistic	0.59	15296.40	0.59	0.59	0.79	42.06	32.11	24.42	18.86
Log-Probit	0.94	15296.43	−0.10	−0.10	0.14	29.59	22.97	23.01	20.40
Probit	0.58	15296.47	0.61	0.61	0.79	43.34	32.79	24.53	18.75
Quantal Linear	0.52	15296.83	0.72	0.72	0.79	51.43	36.76	25.04	17.89

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.

Table E-30. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012, 1276142) 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	15296.38	−0.08	−0.08	0.12	23.95	18.49	16.91	14.37
Log-Logistic	0.95	15296.40	−0.08	−0.08	0.13	23.44	16.17	16.78	13.96
Weibull	0.95	15296.40	−0.08	−0.08	0.13	23.14	16.75	16.73	14.27
Logistic	0.59	15296.40	0.59	0.59	0.79	38.74	28.66	22.18	16.50
Log-Probit	**0.98**	**15296.34**	**−0.06**	**−0.06**	**0.09**	**26.43**	**17.13**	**17.48**	14.18
Probit	0.58	15296.47	0.61	0.61	0.79	40.40	29.72	22.58	16.70
Quantal Linear	0.52	15296.83	0.72	0.72	0.79	51.43	36.75	25.04	17.89

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.

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, 1276142), 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, 10369689).

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-31). The unrounded values were used in the calculations:

Table E-31. 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) ($\bar{y}$)	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) ($\bar{x}$)	2.40	1.96	2.37	1.93	1.74	1.26

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-32.

Table E-32. 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

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

$$P = P = P(1 - \Phi$$

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 we use the 95th upper limit for β, 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-33). Note that β95 is different from the upper bound of the 95% confidence interval, since that number is the 97.5th percentile.

Table E-33. BMD and BMDL for effect of PFOS (ng/mL) on increased ALT in Gallo et al. (2012, 1276142)

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	3964.56	2624.95	4884.94	2380.02	2011.20	948.37
BMDL	1213.59	799.02	1426.49	733.99	618.43	307.81
BMR=10%, P(0) Lognormal						
BMD	11660.73	6185.61	11609.86	5444.59	5311.44	2772.69
BMDL	2873.18	1584.68	2848.49	1421.63	1343.43	725.26

For increased ALT associated with PFOS exposure, the POD is based on the data Gallo et al. (2012, 1276142), a BMR of 5% and a $BMDL_5$ of 56.79 ng/mL.

E.1.4.2 Nian et al. (2019, 5080307)

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, 5080307). 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, 5080307). 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-34.

For increased ALT associated with PFOS exposure, the POD is based on the data Nian et al. (2019, 5080307), a BMR of 5% and a $BMDL_5$ of 15.1 ng/mL.

Table E-34. BMD and BMDL for effect of PFOS (ng/mL) on increased ALT in Nian et al. (2019, 5080307), 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

E.1.4.3 Summary of Modeling Results for Liver Toxicity

Table E-35 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-35. BMDLs for effect of PFOS on serum ALT using a BMR of 5%.

Study name	BMDL (ng/mL)
Gallo et al. (2012, 1276142)	56.79
Nian et al. (2019, 5080307)	**15.12**

E.2 Toxicology Studies

E.2.1 Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075)

EPA conducted dose response modeling of the Butenhoff et al. (2012, 1276144)/Thomford (2002, 502907) 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 long-standing 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, 1239433}. The dose and response data used for the modeling are listed in Table E-36. The AUC normalized per day (AUC_{avg}) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. BMD analysis was conducting 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-36. Dose-Response Modeling Data for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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-37 and Figure E-1 and Figure E-2. 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-37. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

	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.

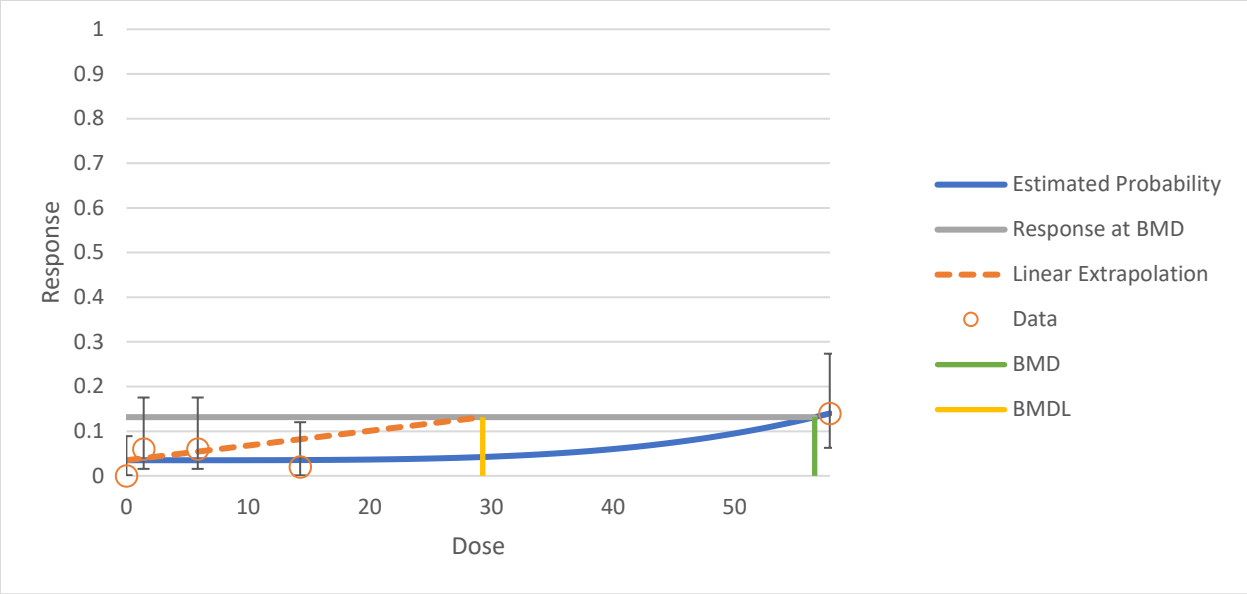


Figure E-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

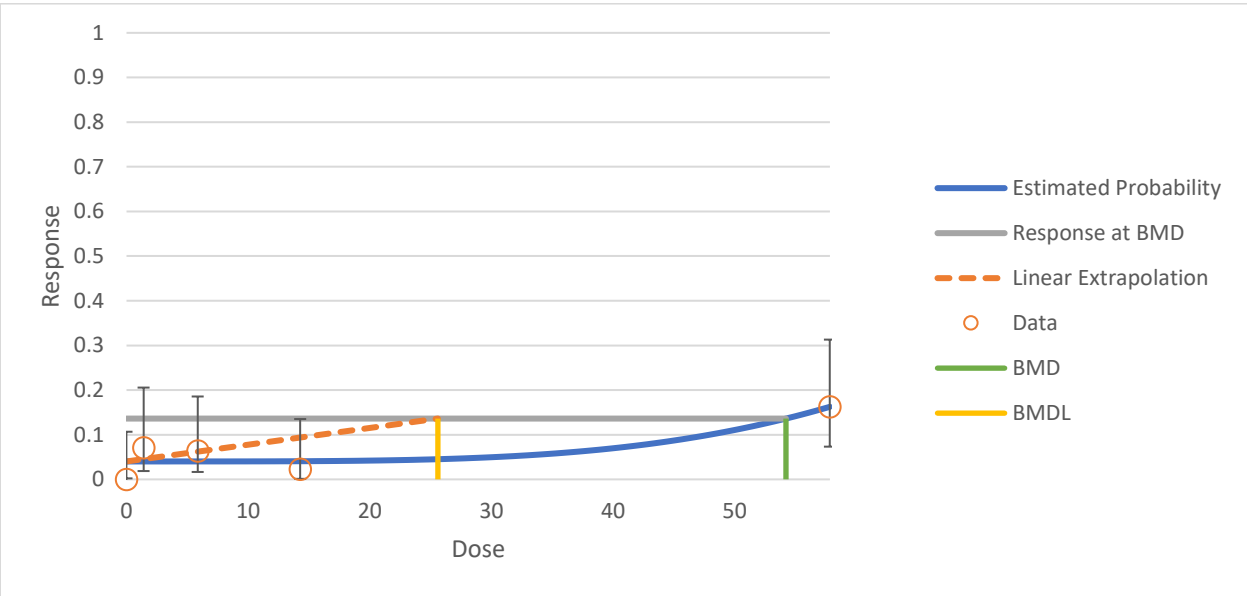


Figure E-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor

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, 1239433}. The dose and response data used for the modeling are listed in Table E-38. The AUC_{avg} was selected for this model because the AUC

accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. BMD analysis was conducting 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-38. Dose-Response Modeling Data for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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-39 and Figure E-3 and Figure E-4. 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, 26.1mg/L.

Table E-39. Summary of Benchmark Dose Modeling Results for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

	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	0.526	114.5	–0.434	-0.633	67.6	29.7	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
	Multistage Degree 3	0.526	114.5	–0.434	-0.633	67.6	29.7	
	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**	

	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			
								to the Multistage Degree 1 model.
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.

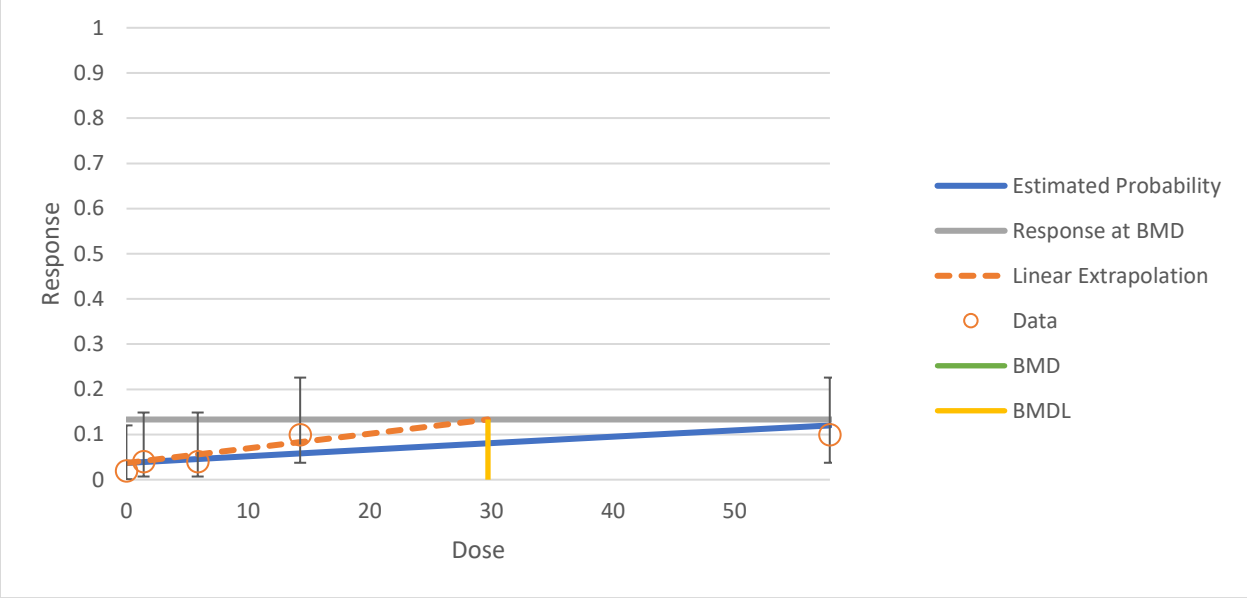


Figure E-3. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

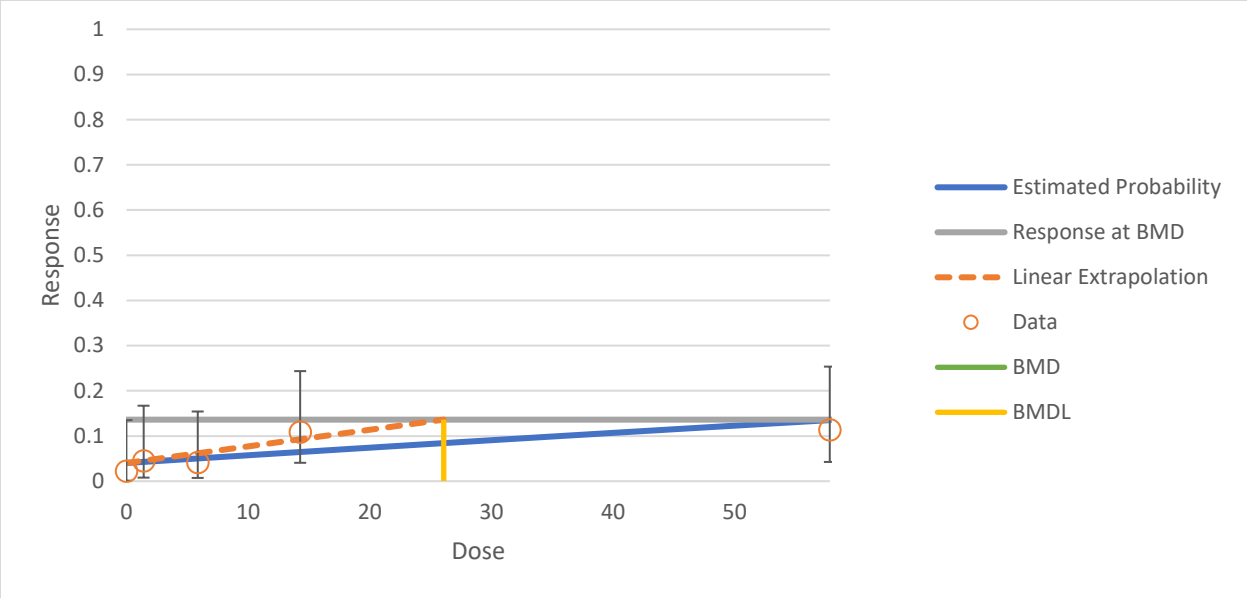


Figure E-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Incidence of Islet Cell Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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, 1239433}. The dose and response data used for the modeling are listed in Table E-40. The AUC_{avg} was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. BMD analysis was conducting 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-40. Dose-Response Modeling Data for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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-41 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 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 25.1mg/L and for the number of animals alive at the time of first tumor is 21.7mg/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, 21.7mg/L. The combined islet cell adenomas and carcinomas in males were not considered further because the response of the islet cell adenomas and carcinomas in the high dose group was not statistically different from the control group, though the trend of response across dose groups was statistically significant.

Table E-41. Summary of Benchmark Dose Modeling Results for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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.

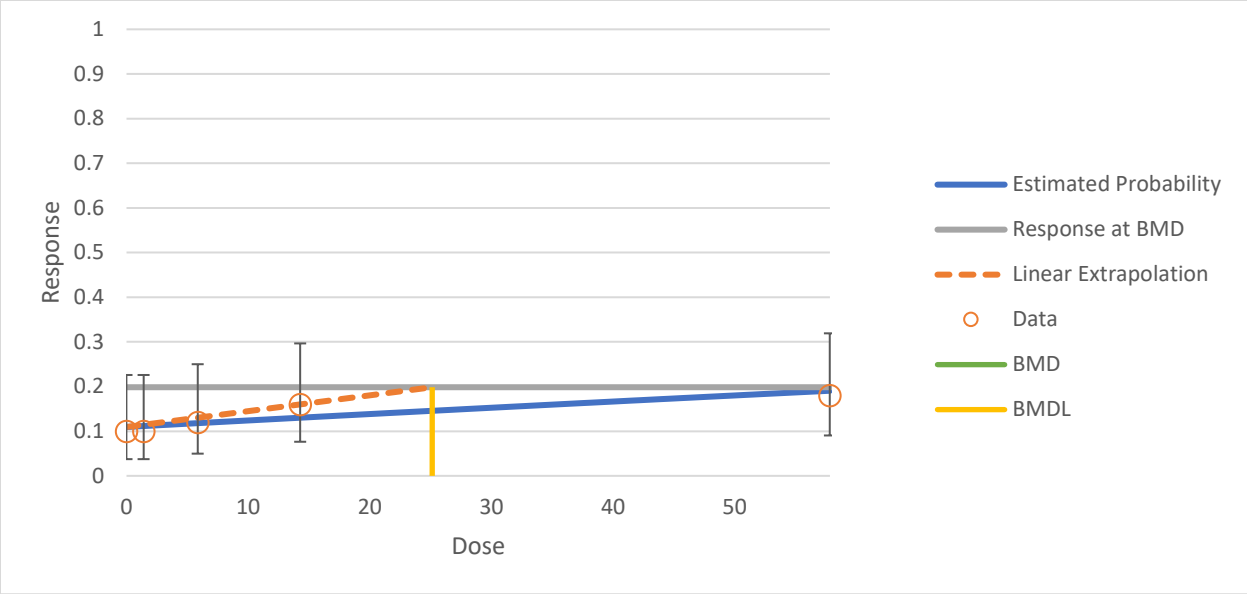


Figure E-5. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

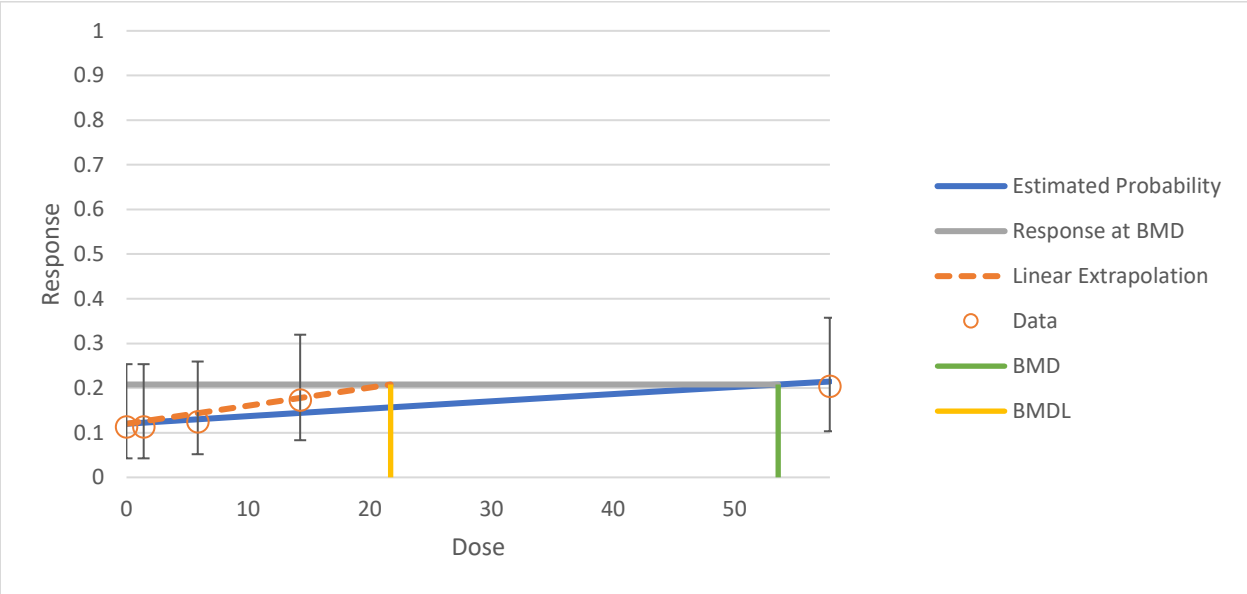


Figure E-6. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Combined Incidence of Islet Cell Adenomas and Carcinomas in Male Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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, 1239433}. The doses and response data used for the

modeling are listed in Table E-42. The AUC_{avg} was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. BMD analysis was conducting 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-42. Dose-Response Modeling Data for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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-43and 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 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-43. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

	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[b]	0.601	69.2	0.00105	-0.668	68.3	37.4	EPA selected the Multistage Degree 1 model. All multistage models had adequate fit (p-values greater than 0.1), the BMDLs were
	Multistage Degree 3[b]	0.598	69.3	0.00722	-0.665	69.0	37.4	
	Multistage Degree 2	0.586	69.3	0.02918	-0.655	70.5	37.3	

	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 1	**0.761**	**67.3**	**0.08232**	**-0.608**	**73.0**	**37.2**	sufficiently close (less than threefold difference), the Multistage Degree 1 model had the lowest AIC.
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.

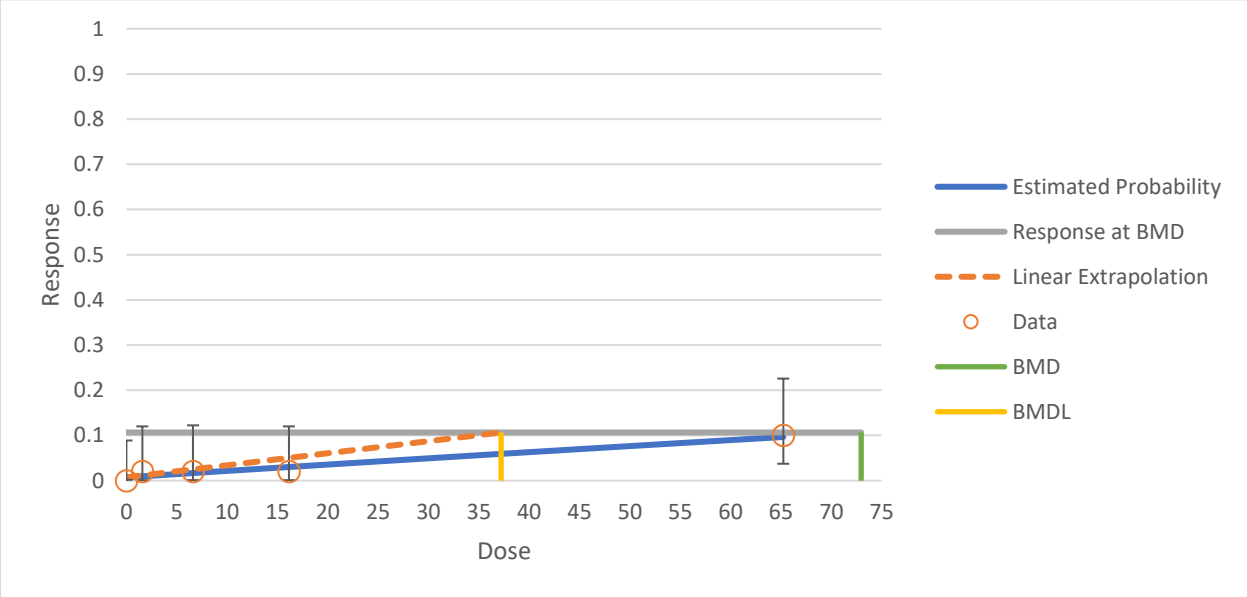


Figure E-7. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Female Rats Following Exposure to

PFOS, for Number of Animals Per Group at Start of Study {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

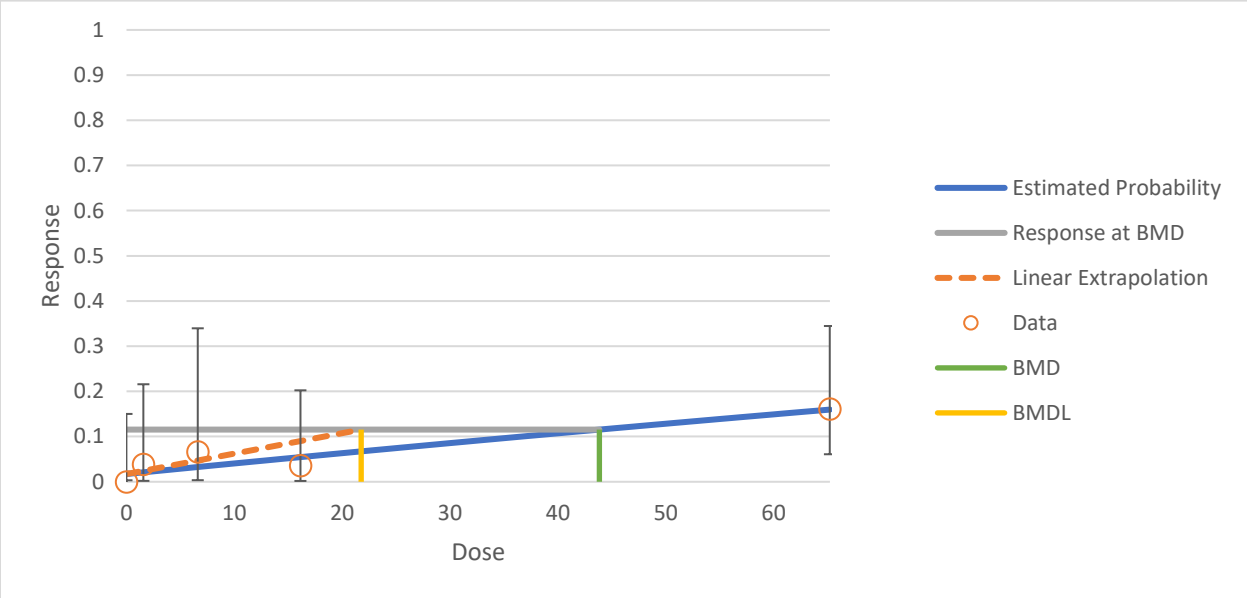


Figure E-8. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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, 1239433}. The dose and response data used for the modeling are listed in Table E-44. The AUC_{avg} was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. BMD analysis was conducting 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 and Carcinomas in Female Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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-45 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 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-45. Summary of Benchmark Dose Modeling Results for Data for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

	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 (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 3	0.597	73.4	0.0081	-0.667	61.2	33.2	
	Multistage Degree 2	0.581	73.5	0.0331	-0.663	60.6	33.0	
	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.

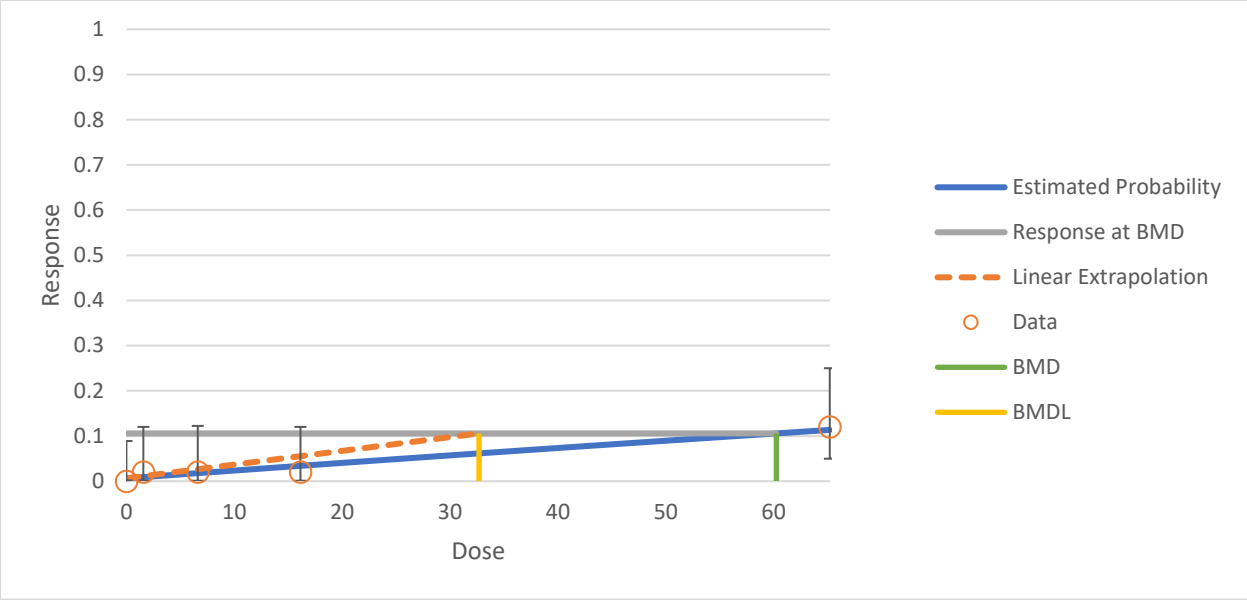


Figure E-9. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Start of Study {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

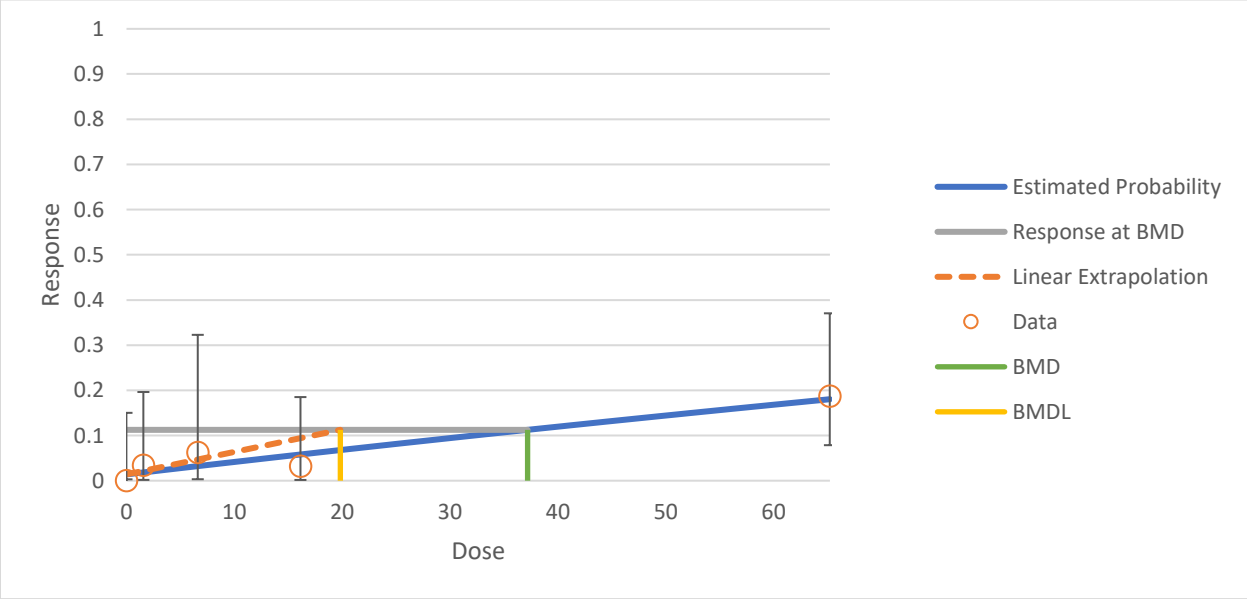


Figure E-10. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocellular Adenomas and Carcinomas in Female Rats Following Exposure to PFOS, for Number of Animals Per Group at Time of First Tumor {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

E.2.1.6 Individual Cell Necrosis in the Liver

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, 1239433}. The doses and response data used for the modeling are listed in Table E-46. The average concentration over the final week of study $C_{last7,avg}$, was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of individual cell necrosis in the liver.

Table E-46. 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, 2012, 1276144/Thomford, 2002, 5029075}

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-47 and Figure E-11. The Log-Logistic model was selected based on adequate p-values (greater than 0.1) and had the lowest AIC mong 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-47. 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, 2012, 1276144/Thomford, 2002, 5029075}

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.

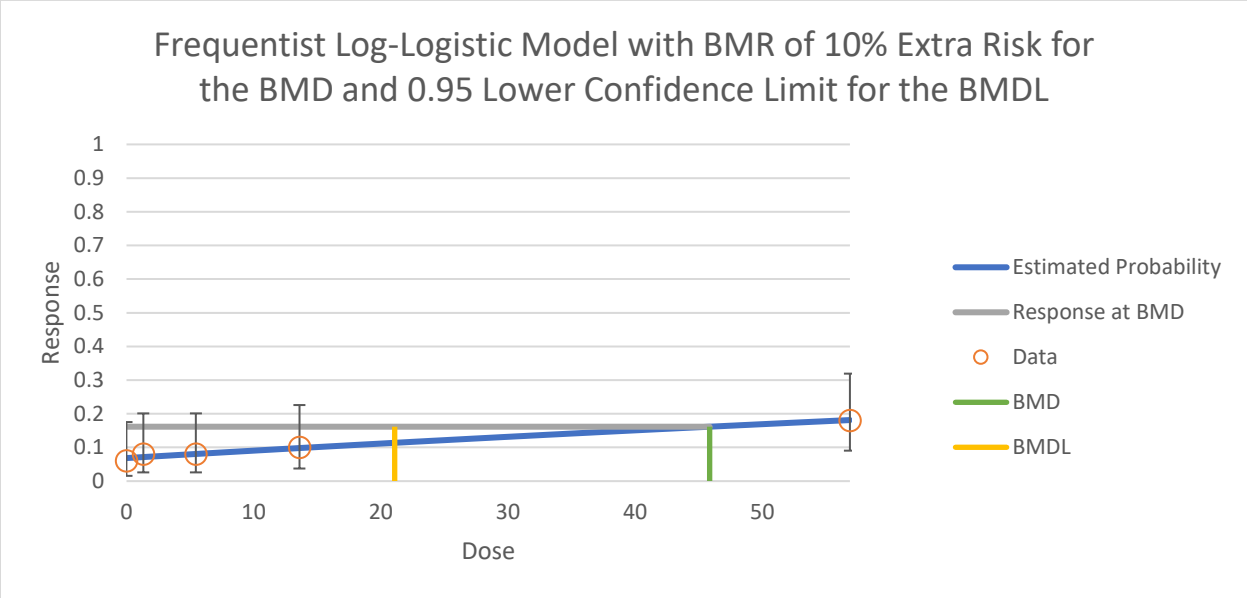


Figure E-11. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Individual Cell Necrosis in the Liver in Female Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

BMD = benchmark dose; BMDL = benchmark dose lower limit.

Increased incidence of individual cell necrosis in the liver was observed in male 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, 1239433}. The doses and response data used for the modeling are listed in Table E-48. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of individual cell necrosis in the liver.

Table E-48. Dose-Response Modeling Data for Individual Cell Necrosis in the Liver in Male Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	50	3
0.024	1.1	50	2
0.098	4.5	50	6
0.242	11.0	50	4
0.984	44.8	50	10

BMD modeling results for individual cell necrosis in the liver are summarized in Table E-49 and Figure E-12. The best fitting model was the Log-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 Gamma model had the lowest AIC. The $BMDL_{10}$ from the selected Log-Logistic model is 14.2 mg/L.

Table E-49. Summary of Benchmark Dose Modeling Results for Individual Cell Necrosis in the Liver in Male Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

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	0.369	162.02	0.003	−0.201	25.3	1.9	EPA selected the Log-Logistic. 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	0.283	162.59	−0.004	−0.397	40.4	14.9	
Log-Logistic	**0.563**	**160.04**	**−0.007**	**−0.004**	**28.4**	**14.2**	
Multistage Degree 4	0.560	160.05	−0.016	−0.028	29.2	15.6	
Multistage Degree 3	0.560	160.05	−0.016	−0.028	29.2	15.6	
Multistage Degree 2	0.560	160.05	−0.016	−0.028	29.2	15.6	
Multistage Degree 1	0.560	160.05	−0.016	−0.028	29.2	15.6	
Weibull	0.560	160.05	−0.016	−0.028	29.2	15.6	
Logistic	0.533	160.19	−0.030	−0.184	34.7	24.3	
Log-Probit	0.386	161.98	−0.667	0.180	23.8	6.5	
Probit	0.536	160.17	−0.031	−0.165	34.0	23.0	

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.

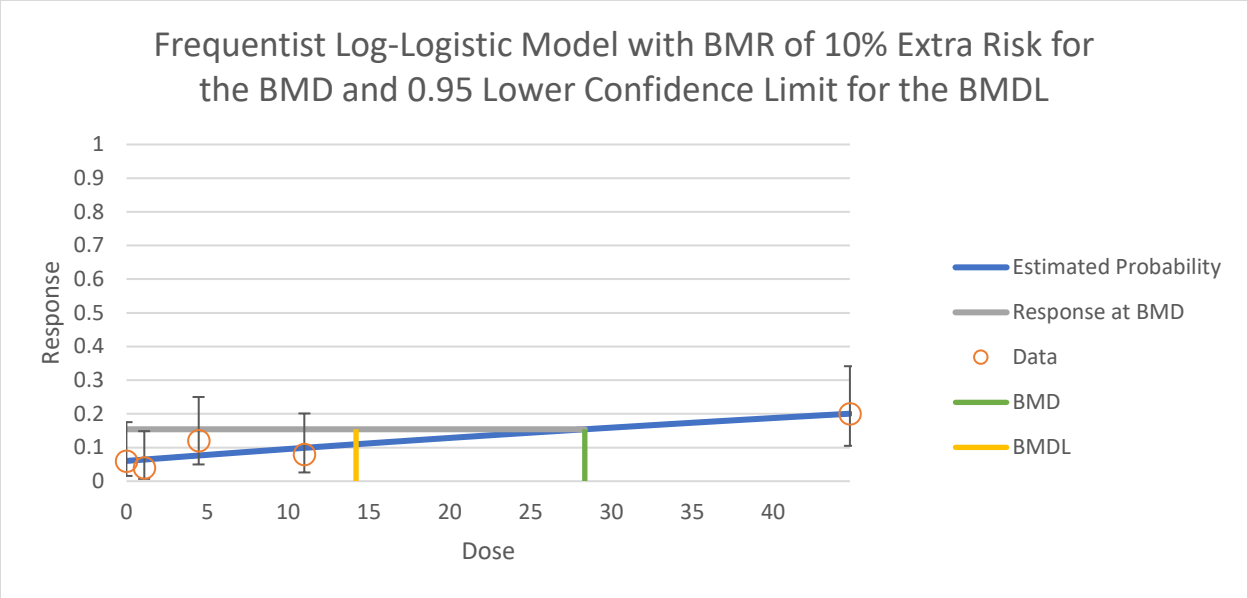


Figure E-12. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Individual Cell Necrosis in the Liver in Male Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS {Butenhoff, 2012, 1276144/Thomford, 2002, 5029075}

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.2 Lee et al. (2015, 2851075)

EPA conducted dose response modeling of the Lee et al. (2015, 2851075) study using the BMDS 3.2 program. This study addresses fetal body weight in F_1 male and female CD-1 mice and the number of dead fetuses in P_0 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 change in the mean equal to 0.5 standard deviations from the control mean and 5% change were chosen. The doses and response data used for the modeling are listed in Table E-50. The $C_{avg,pup,gest}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effect resulting in decreased fetal body weight.

Table E-50. Dose-Response Modeling Data for Fetal Body Weight in F_1 Male and Female CD-1 Mice Following Exposure to PFOS {Lee, 2015, 2851075}

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

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g)[a]
8	14.0	10	1.1 ± 0.2

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

BMD modeling results for fetal body weight are summarized in and Table E-51. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table E-51. Summary of Benchmark Dose Modeling Results for Fetal Body Weight in F_1 Male and Female CD-1 Mice Following Exposure to PFOS (constant variance) {Lee, 2015, 2851075}

Model	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 $BMD_{0.5SD}$	Dose Group near BMD_5	Control Dose Group					
Exponential 2	0.002	−19.3	−0.7	−0.7	2.2	2.0	1.5	1.8	1.4	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.002	−19.3	−0.7	−0.7	2.2	2.0	1.5	1.8	1.4	
Exponential 4	0.360	−29.4	0.4	−0.7	0.4	0.4	0.2	0.5	0.3	
Exponential 5	0.360	−29.4	0.4	−0.7	0.4	0.4	0.2	0.5	0.3	
Hill	0.685	−30.1	0.1	0.1	0.1	0.3	0.2	0.3	0.2	
Polynomial Degree 3	0.001	−17.5	−2.4	−0.6	2.4	2.5	1.9	2.2	1.8	
Polynomial Degree 2	0.001	−17.5	−2.4	−0.6	2.4	2.5	1.9	2.2	1.8	
Power	0.001	−17.5	−2.4	−0.6	2.4	2.5	1.9	2.2	1.8	
Linear	0.001	−17.5	−2.4	−0.6	2.4	2.5	1.9	2.2	1.8	

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.

E.2.2.2 Number of Dead Fetuses

Increased mean response of fetal body weight was observed in P_0 female CD-1 mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen. The doses and response data used for the modeling are listed in Table E-52. The average concentration normalized per day during gestation ($C_{avg,dam,gest}$) and maximum maternal concentration during gestation ($C_{max,dam}$) were both considered and shown below because fetal death could be a result of exposure during a sensitive window of development where a C_{max} metric is a more appropriate dose metric or an accumulation of exposure where an average concentration metric is more appropriate. The $C_{avg,dam,gest}$ was selected for this model.

Table E-52. Dose-Response Modeling Data for Number of Dead Fetuses in P_0 Female CD-1 Mice Following Exposure to PFOS {Lee, 2015, 2851075}

Administered Dose (mg/kg/day)	Internal Dose		Number per Group	Mean Response (incidence)
	$AUC_{avg,dam,gest}$ (mg/L)	$C_{max,dam}$ (mg/L)		
0	0	0	10	0.6 ± 0.3
0.5	2.1	9.2	10	1.6 ± 0.5
2	8.5	37.0	10	4.8 ± 0.5
8	34.1	147.8	10	7.6 ± 1.1

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

The BMD modeling results for fetal body weight for $C_{avg,dam,gest}$ and $C_{max,dam}$ are summarized in Table E-53 and Table E-54, respectively. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint using the $C_{avg,dam,gest}$ internal dose metric.

Table E-53. Summary of Benchmark Dose Modeling Results for Number of Dead Fetuses for $C_{avg,dam,gest}$ in P_0 Female CD-1 Mice Following Exposure to PFOS (nonconstant variance) {Lee, 2015, 2851075}

Model	Goodness of Fit		Scaled Residual		$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Exponential 2	< 0.0001	148.1	4.2	−3.1	8.2	6.5	No models had adequate fit (p-values were less than 0.1).
Exponential 3	< 0.0001	148.1	4.2	−3.1	8.2	6.5	
Exponential 4	0.045	76.8	0.5	0.5	0.2	0.2	
Exponential 5	–[a]	74.8	$-4.1 \times e^{-3}$	$-4.1 \times e^{-3}$	0.4	0.2	
Hill	–[a]	74.8	$-4.1 \times e^{-3}$	$-4.1 \times e^{-3}$	0.5	0.3	
Polynomial Degree 3	< 0.0001	120.7	−1.1	−1.1	0.4	0.3	
Polynomial Degree 2	< 0.0001	120.7	−1.1	−1.1	0.4	0.3	
Power	< 0.0001	120.7	−1.1	−1.1	0.4	0.3	

Model	Goodness of Fit		Scaled Residual		$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Linear	< 0.0001	120.7	−1.1	−1.1	0.4	0.3	

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.
[a] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

Table E-54. Summary of Benchmark Dose Modeling Results for Number of Dead Fetuses for $C_{max,dam}$ in P_0 Female CD-1 Mice Following Exposure to PFOS (nonconstant variance) {Lee, 2015, 2851075}

Model	Goodness of Fit		Scaled Residual		$BMD_{0.5SD}$ (mg/L)	$BMDL_{0.5SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Exponential 2	< 0.0001	148.1	4.2	−3.1	35.3	28.1	No models had adequate fit (p-values were less than 0.1).
Exponential 3	< 0.0001	148.1	4.2	−3.1	35.3	28.1	
Exponential 4	0.045	76.8	0.5	0.5	1.0	0.7	
Exponential 5	–[a]	74.8	$-4.1 \times e^{-3}$	$-4.1 \times e^{-3}$	1.9	1.0	
Hill	–[a]	74.8	$-4.1 \times e^{-3}$	$-4.1 \times e^{-3}$	2.3	1.3	
Polynomial Degree 3	< 0.0001	120.7	−1.1	−1.1	1.9	1.2	
Polynomial Degree 2	< 0.0001	120.7	−1.1	−1.1	1.9	1.2	
Power	< 0.0001	120.7	−1.1	−1.1	1.9	1.2	
Linear	< 0.0001	120.7	−1.1	−1.1	1.9	1.2	

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.
[a] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

E.2.3 Luebker et al. (2005, 757857)

EPA conducted dose response modeling of the Luebker et al. (2005, 757857) study using the BMDS 3.2 program. This study addresses pup body weight relative to the litter at 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-55. The $C_{avg,pup,gest}$ was selected for this model

rather than alternate metrics such as C_{max} because the average concentration normalized per day during gestation is expected to better correlate with an accumulation of effect resulting in decreased pup body weight.

Table E-55. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (LD5) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS {Luebker, 2005, 757857}

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	15.4	17	8.6 ± 1.9
0.8	30.8	17	8.5 ± 2.8
1	38.5	17	8.1 ± 2.5
1.2	46.1	17	7.5 ± 2.7
1.6	61.5	17	7.2 ± 2.7
2	76.9	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. The BMD modeling results for pup body weight relative to the litter at LD 5 are summarized in Table E-56 and Figure E-13. The Polynomial Degree 6 model was selected as it had the lowest AIC among the viable models. The $BMDL_5$ from the selected Polynomial Degree 6 model is 10.1 mg/L.

Table E-56. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter (LD5) in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance) {Luebker, 2005, 757857}

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	0.069	602.1	–0.73	−0.7	0.38	18.9	11.7	10.8	7.2	EPA selected the Polynomial Degree 6 model. All models had adequate fit (p-values greater than 0.1), and the Polynomial Degree 6 model was selected as it had the lowest AIC among the viable models.
Exponential 3	0.199	599.9	–0.69	−0.3	1.45	43.8	20.4	30.7	12.7	
Exponential 4	0.069	602.1	–0.73	−0.7	0.38	18.9	11.7	10.8	7.2	
Exponential 5	0.199	599.9	–0.69	−0.3	1.45	43.8	20.4	30.7	12.7	
Hill	0.142	601.3	–0.36	−0.2	1.33	41.4	19.1	28.2	16.4	
Polynomial Degree 6	**0.808**	**594.2**	0.01	**−0.7**	**0.93**	33.1	17.9	**16.6**	**10.1**	
Polynomial Degree 5	0.736	594.6	–0.03	−0.7	0.96	34.1	18.1	17.4	10.2	
Polynomial Degree 4	0.640	595.3	–0.29	−0.7	0.99	35.0	18.3	18.7	10.4	
Polynomial Degree 3	0.381	598.1	–0.28	−0.8	1.02	35.7	18.4	20.6	10.6	
Polynomial Degree 2	0.266	599.1	–0.23	−0.9	1.06	35.6	17.9	22.6	10.6	
Power	0.245	599.3	–0.36	−0.2	1.34	41.8	18.8	28.5	11.3	
Linear	0.133	600.3	–0.81	−0.8	0.34	19.7	13.3	11.3	8.2	

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

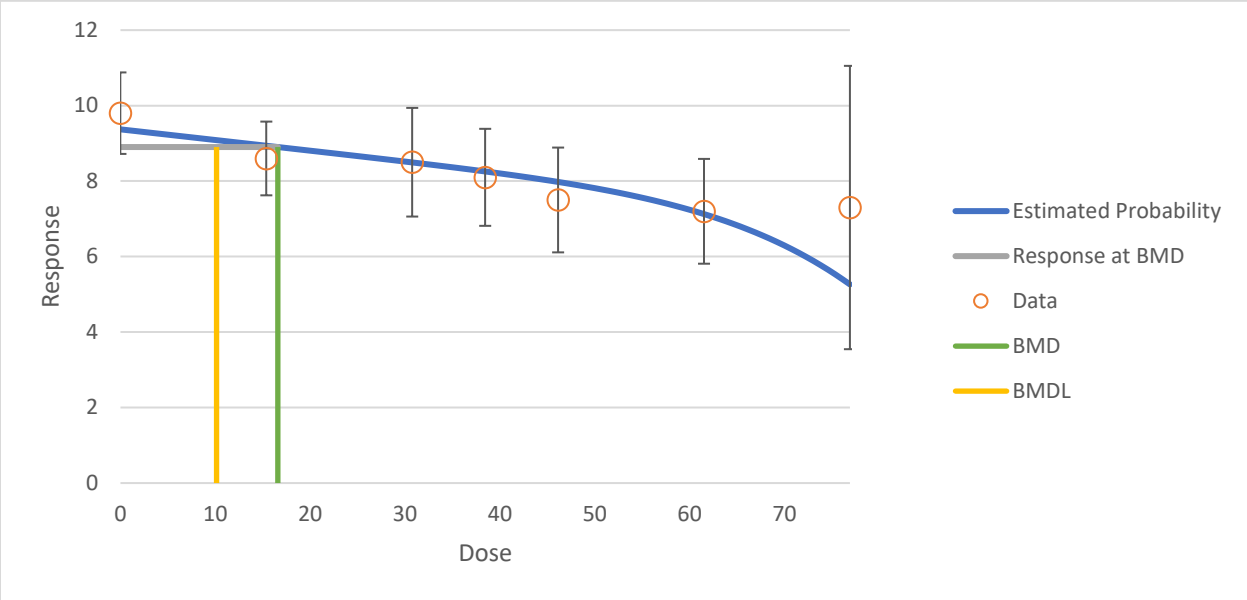


Figure E-13. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 6 Model for Pup Body Weight Relative to the Litter at LD5 in F_1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS {Luebker, 2005, 757857}

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.4 NTP (2019, 5400978)

EPA conducted dose response modeling of the NTP (2019, 5400978) 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, 1239433}. The doses and response data used for the modeling are listed in Table E-57. The $C_{last7,\ avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of extramedullary hematopoiesis in the spleen.

Table E-57. Dose-Response Modeling Data for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	10	1
0.312	10.2	10	1

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
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-58 and Figure E-14. 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-58. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

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.

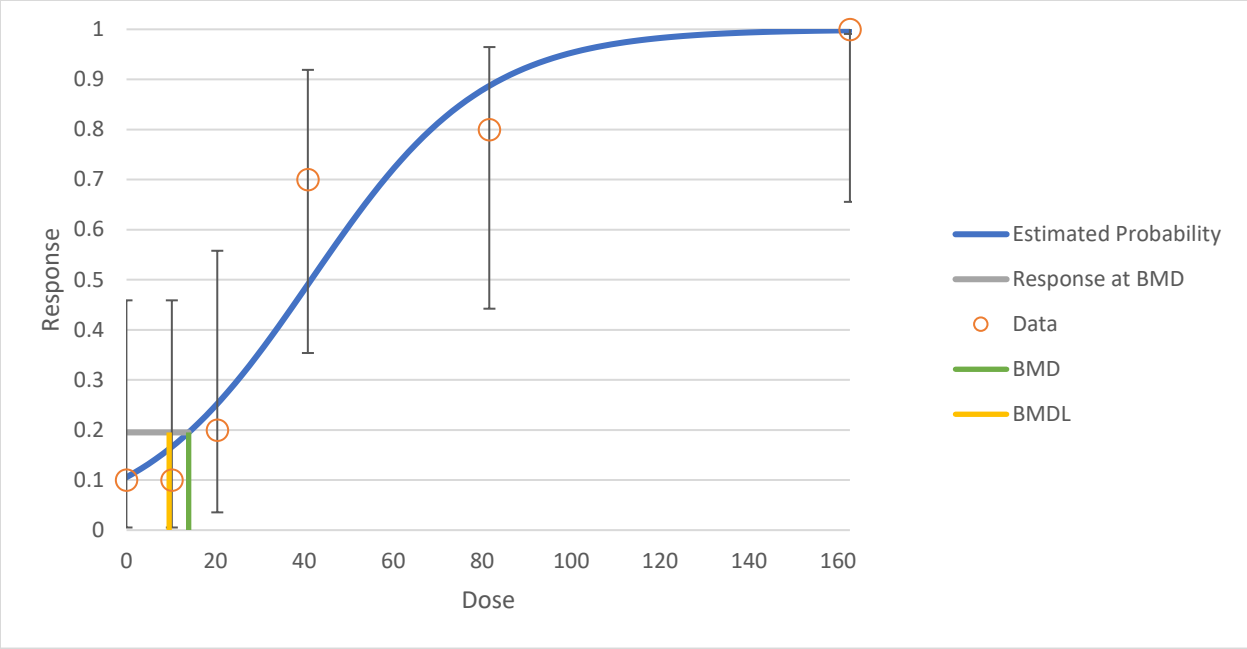


Figure E-14. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Logistic Model for Extramedullary Hematopoiesis in the Spleen in Male Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

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, 1239433}. The doses and response data used for the modeling are listed in Table E-59. The $C_{last7, avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of extramedullary hematopoiesis in the spleen.

Table E-59. Dose-Response Modeling Data for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

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-60 and Figure E-15. 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-60. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

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.

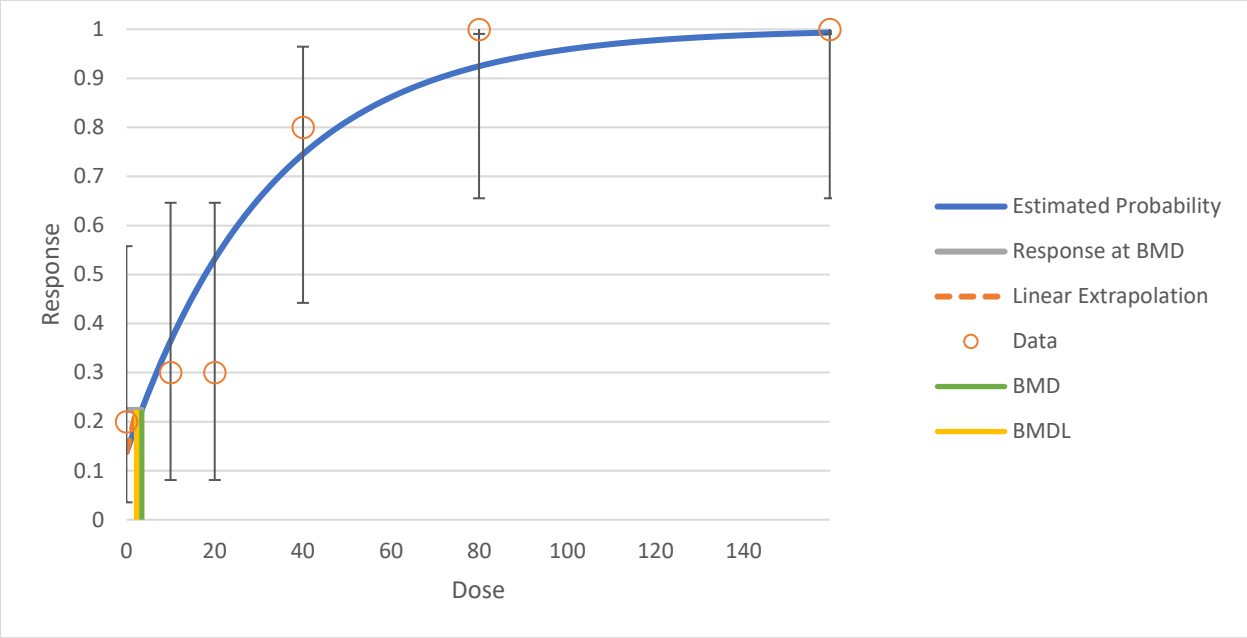


Figure E-15. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS {NTP, 2019, 5400978}

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.5 Zhong et al. (2016, 3748828)

EPA conducted dose response modeling of the Zhong et al. (2016, 3748828) 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.

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. 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, 1239433}. The doses and response data used for the modeling are listed in Table E-61. The $C_{avg,pup,gest,lact}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of decreased plaque forming cell response of splenic cells from across the gestation and lactation lifestages.

Table E-61. Dose-Response Modeling Data for PFC Response of Splenic Cells in F_1 male C57BL/6 mice Following Exposure to PFOS {Zhong, 2016, 3748828}

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]

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (# cells per 10^6 spleen cells)[a]
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-62 and Figure E-16. 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-62. Summary of Benchmark Dose Modeling Results for Plaque Forming Cell Response of Splenic Cells in F_1 Male C57BL/6 Mice Following Exposure to PFOS (constant variance) {Zhong, 2016, 3748828}

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.

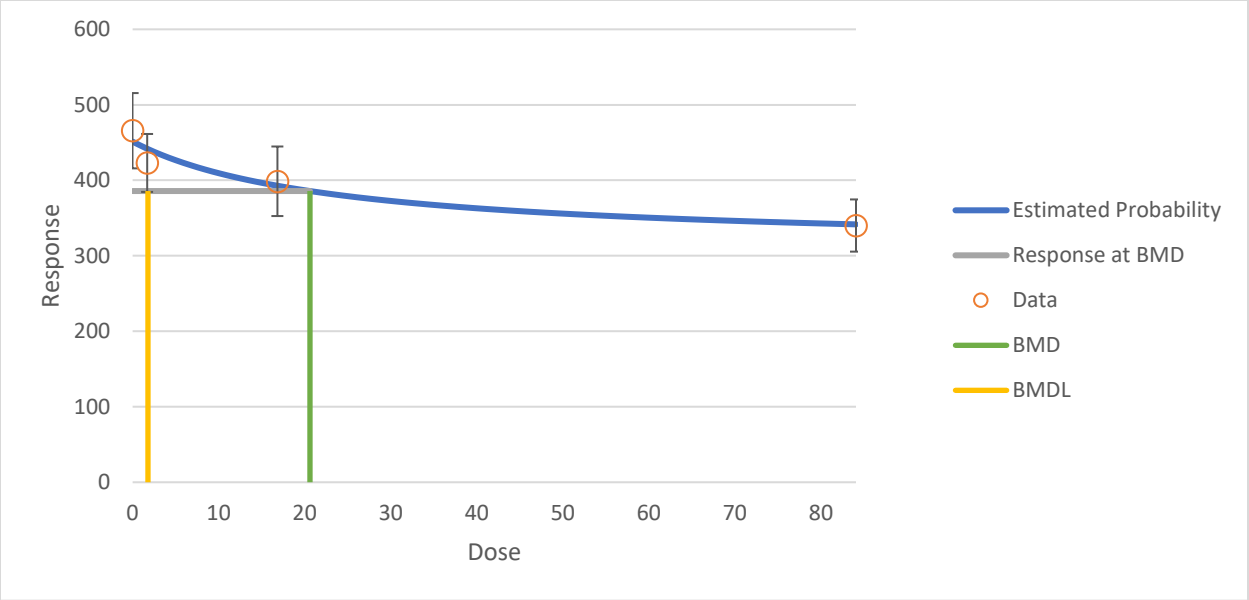


Figure E-16. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for PFC Response of Splenic Cells in F_1 Male C57BL/6 Mice Following Exposure to PFOS {Zhong, 2016, 3748828}

BMD = benchmark dose; BMDL = benchmark dose lower limit.

Appendix F. Pharmacokinetic Modeling

For the animal pharmacokinetic model, model predictions from Wambaugh et al. (2013, 2850932) 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, 2850932) (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 CIs. The application of the model outputs in the derivation of a human RfD can be found in the PFOS Main Document.

F.1 Comparison of Fits to Training Datasets Used in Wambaugh et al. (2013, 2850932)

The following figures show comparisons of the model predicted serum concentrations to the data used for model training. Fits also presented in supplemental material of Wambaugh et al. (2013, 2850932).

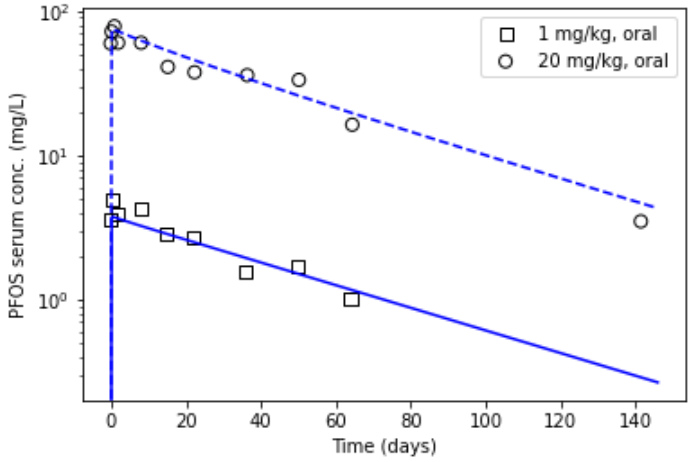


Figure F-1. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Female CD1 Mice

1 mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

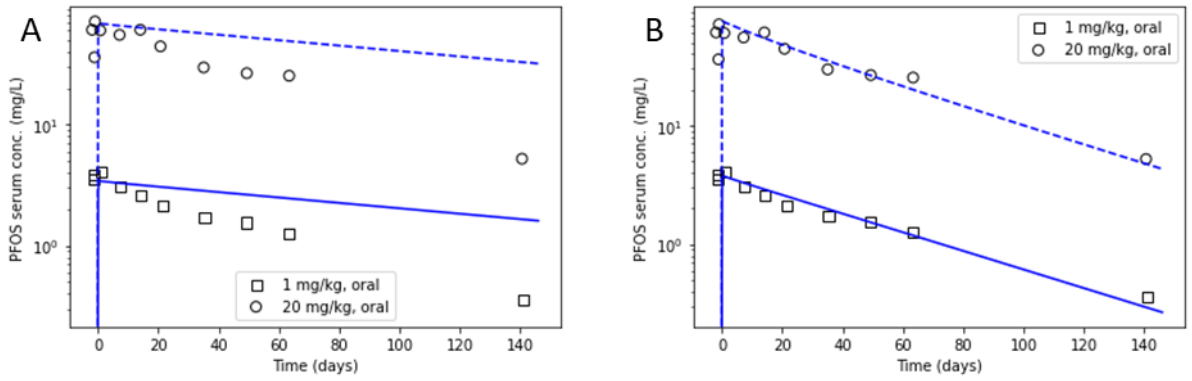


Figure F-2. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Male CD1 Mice

A) Fits to observed male data using male-specific model. B) Fits to observed male data using female-specific model parameters. 1 mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

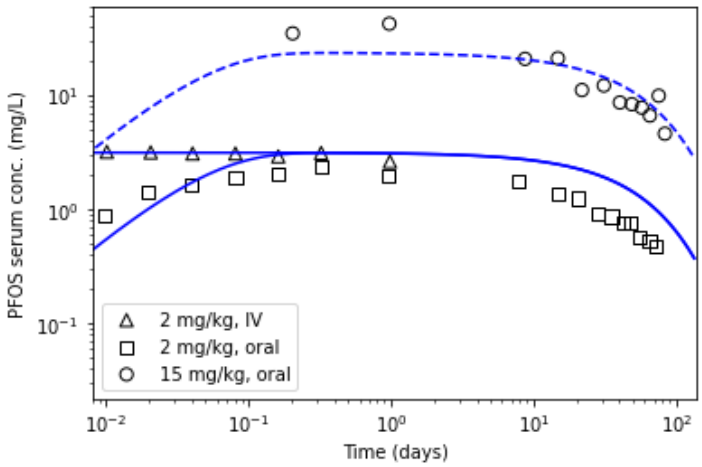


Figure F-3. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single IV Dose of 2 mg/kg or a Single Oral Dose of 2 or 15 mg/kg PFOS to Male Sprague-Dawley Rats

2 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.

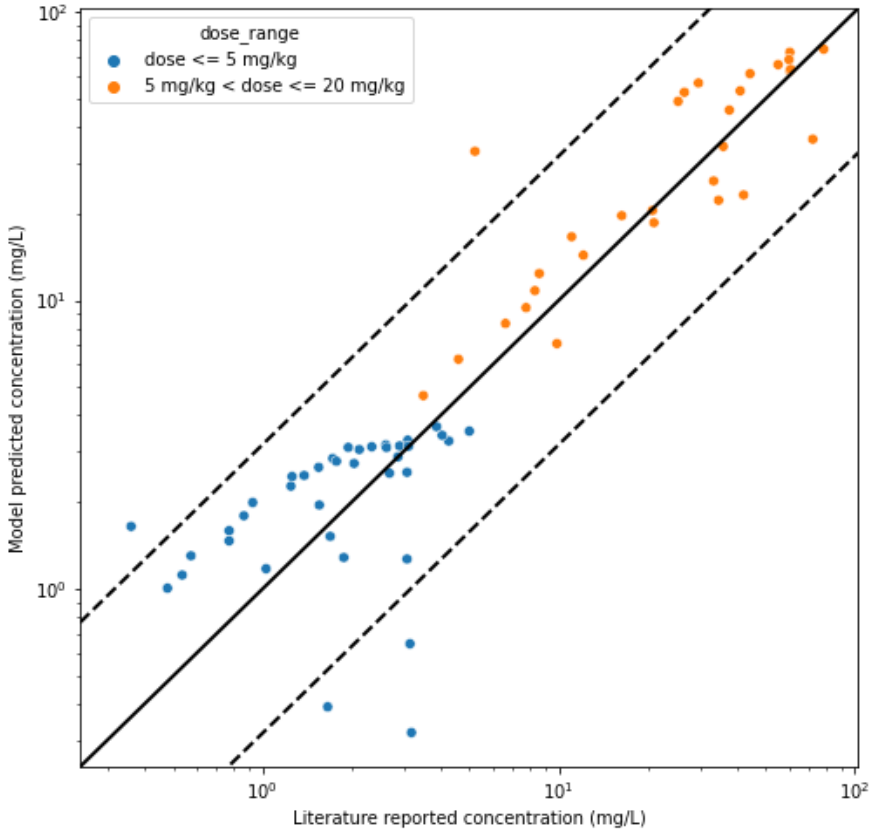
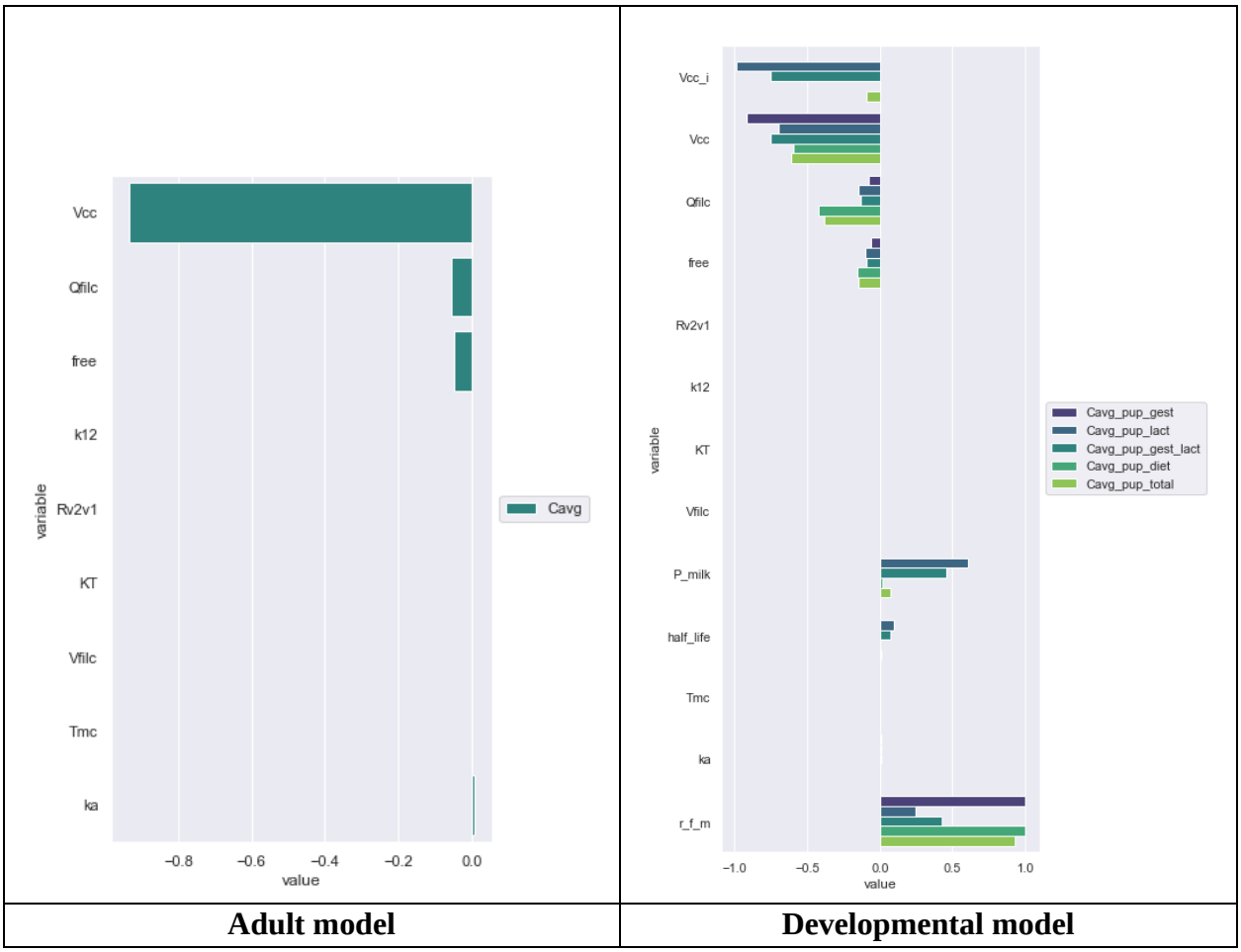


Figure F-4. Model Prediction Summary for PFOS Training Data

Model predictions on the training data result in a mean squared log error (MSLE) of 0.174. Dashed lines represent +/- one-half $\log_{10}$.

We conducted a local, one-at-a-time sensitivity analysis 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, 2850932) parameterization.

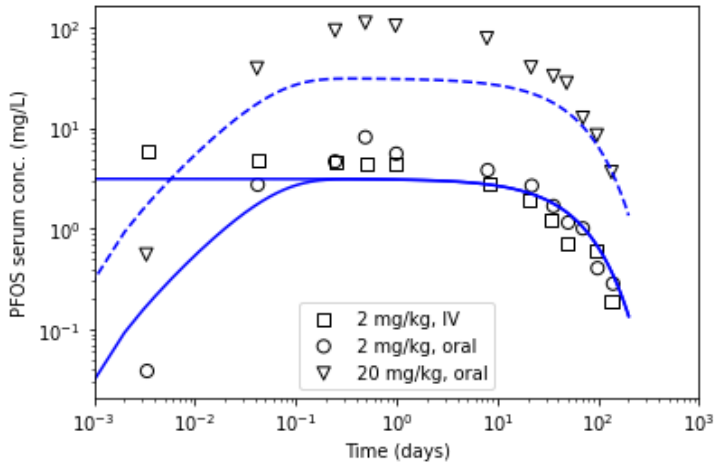


Figure F-6. mentally Observed Serum Concentrations {Huang, 2019, 7410147} and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Male Sprague-Dawley Rats

2 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.

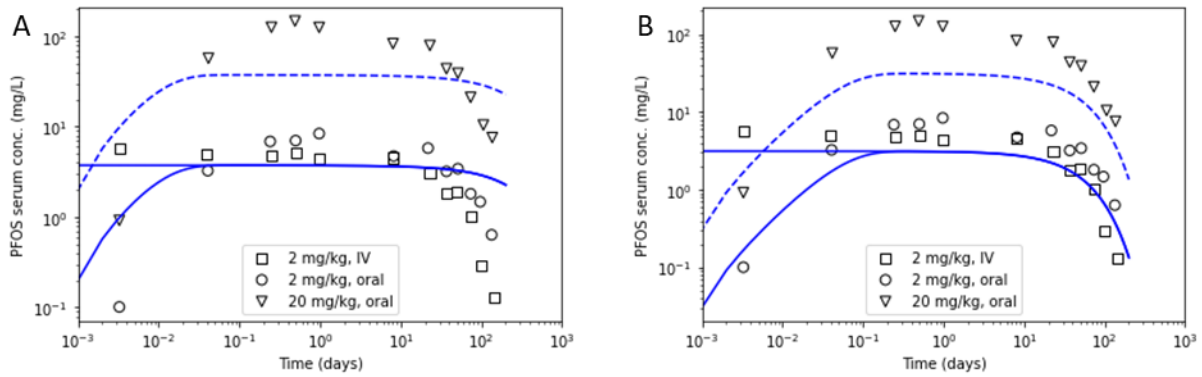


Figure F-7. Experimentally Observed Serum Concentrations {Huang, 2019, 7410147} and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Female Sprague-Dawley Rats

A) Fits to observed female data using female-specific model parameters. B) Fits to observed female data using male-specific model parameters.
2 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.

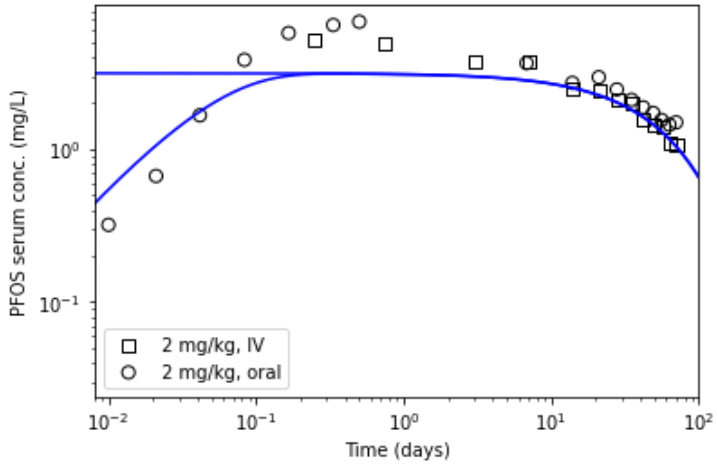


Figure F-8. Experimentally Observed Serum Concentrations {Kim, 2016, 3749289} and Median Prediction for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 mg/kg PFOS to Male Sprague-Dawley Rats

2 mg/kg intravenous (IV) dose represented by the squares; 2 mg/kg oral dose represented by the circles.

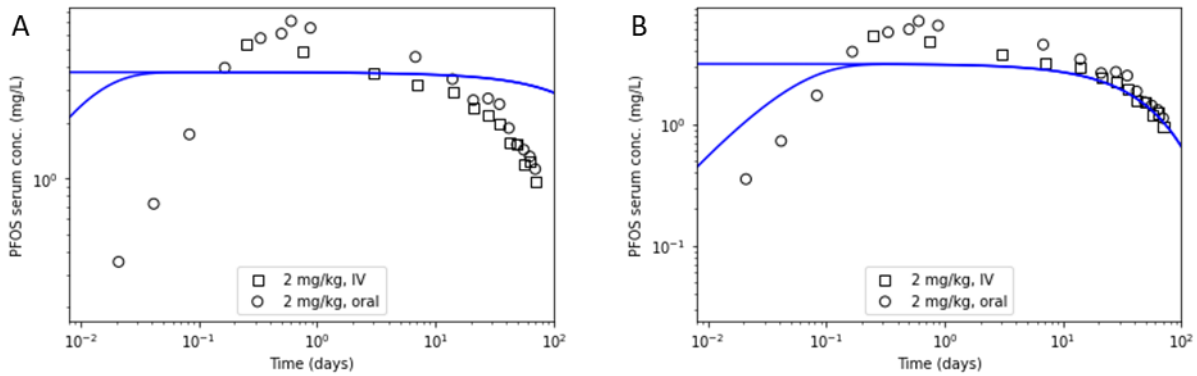


Figure F-9.Experimentally Observed Serum Concentrations {Kim, 2016, 3749289} and Median Prediction for a Single IV Dose of 2 mg/kg an Oral Dose of 2 mg/kg PFOS to Female Sprague-Dawley Rats

A) Fits to observed female data using female-specific model parameters. B) Fits to observed female data using male-specific model parameters.
2 mg/kg intravenous (IV) dose represented by the squares; 2 mg/kg oral dose represented by the circles.

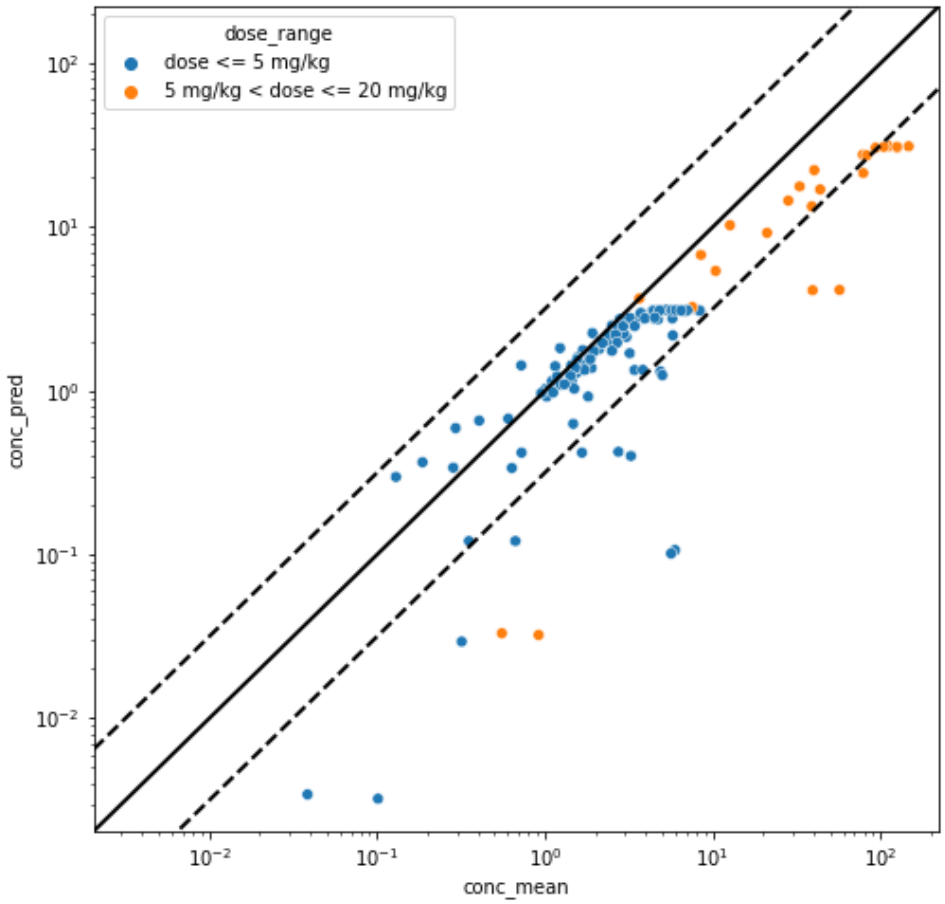


Figure F-10. Model Prediction Summary for PFOS Test Data

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 main document (see PFOS Main Document), the human model was implemented in R/MCSim from the original AcslX model {Verner, 2016, 3299692}. 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.

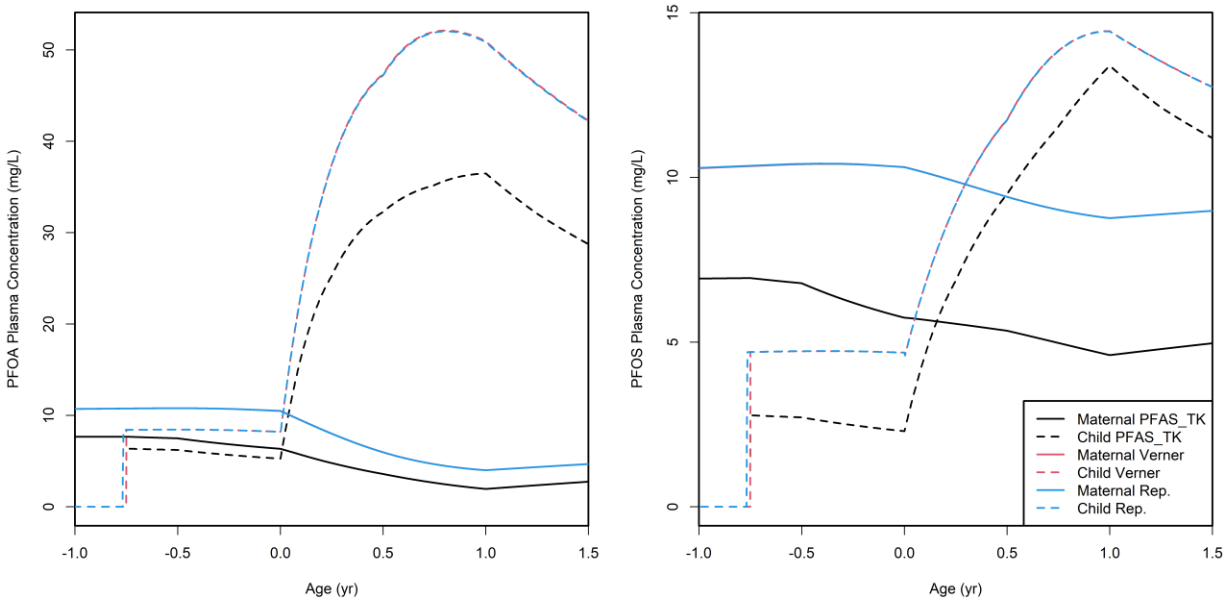


Figure F-11. Model Comparison

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, 2011, 786546}. 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, 1290877) 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. (2015, 3299692), we chose to model this scenario 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, 2013, 1937228}, 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. (2015, 3859839) 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.

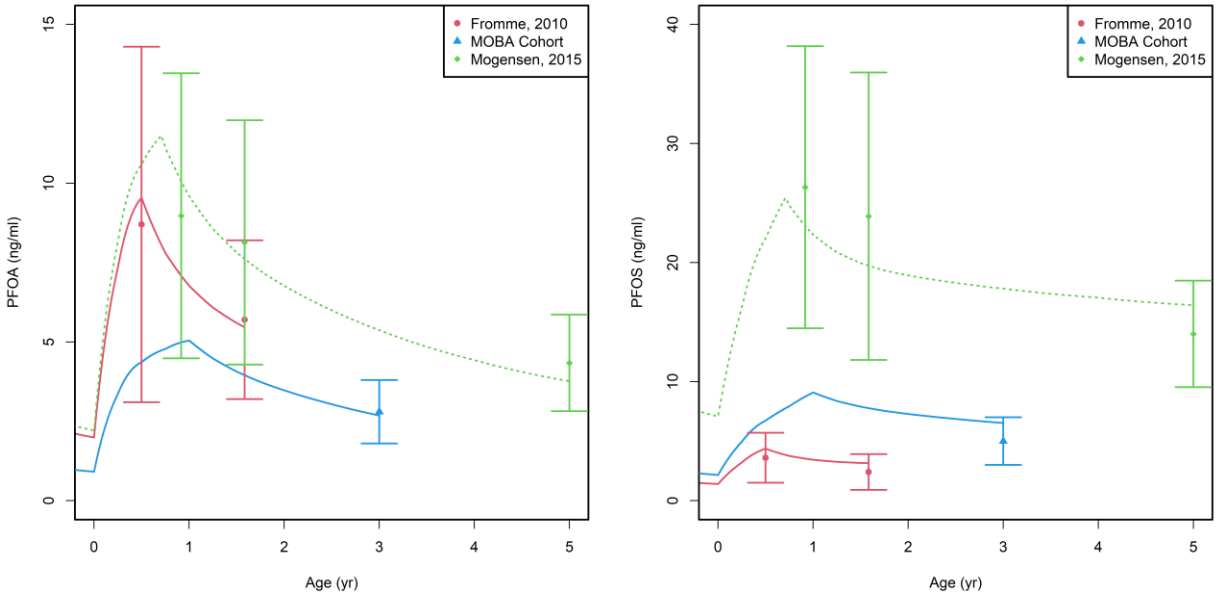


Figure F-12. Predicted Child Serum Levels Compared to Reported Values

These values were calculated using the updated parameters with constant Vd and exposure relative to body weight.

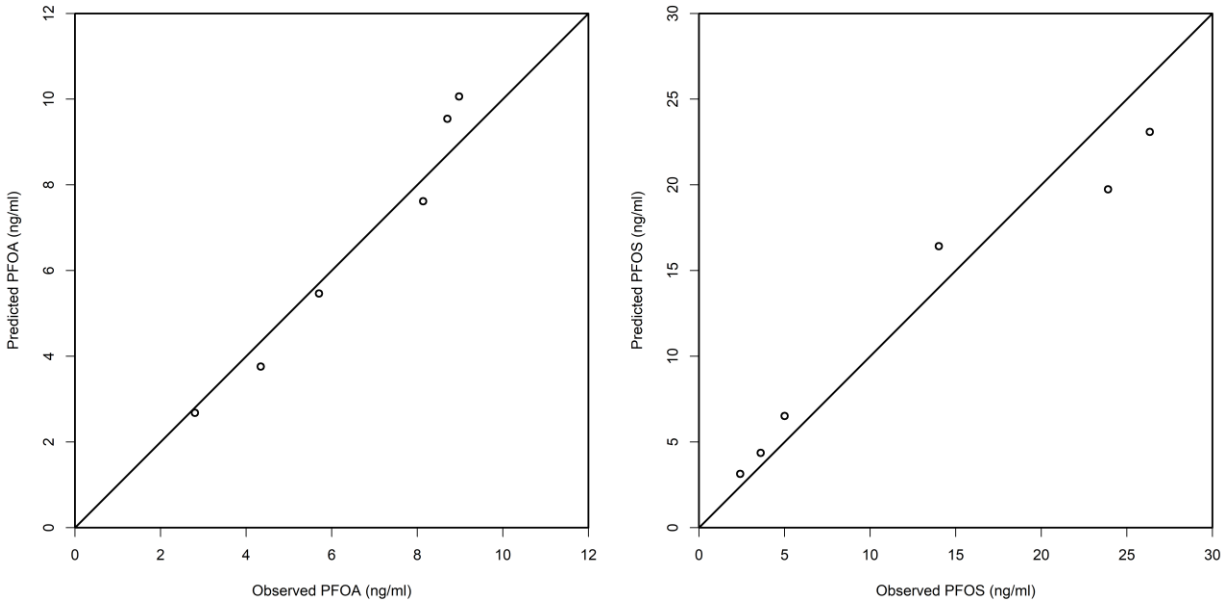


Figure F-13. Comparison of Predicted and Observed Child Serum Levels

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, we see a similar effect from V_d and half-life as in the maternal serum, because cord blood levels are based on maternal levels in the model, but we also see a high sensitivity 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, we see 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 comparted to 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.

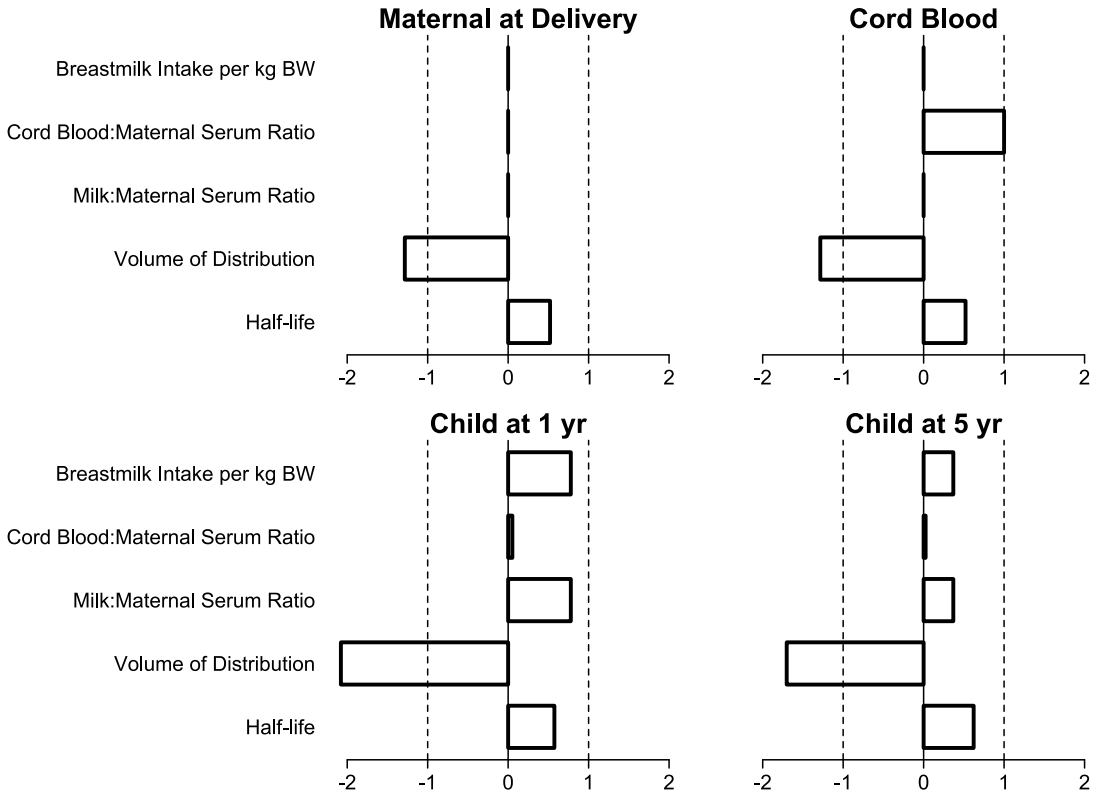


Figure F-14. Sensitivity Coefficients

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, 2019, 5080506} 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, we integrated the V_d scaling in the MDH model into our model (Figure F-14). The main effect is to reduce the peak serum levels in children that occurs due to exposure through breastmilk. Based on mean relative error (for PFOA and PFOS combined), we determined that the model with constant V_d had better performance.

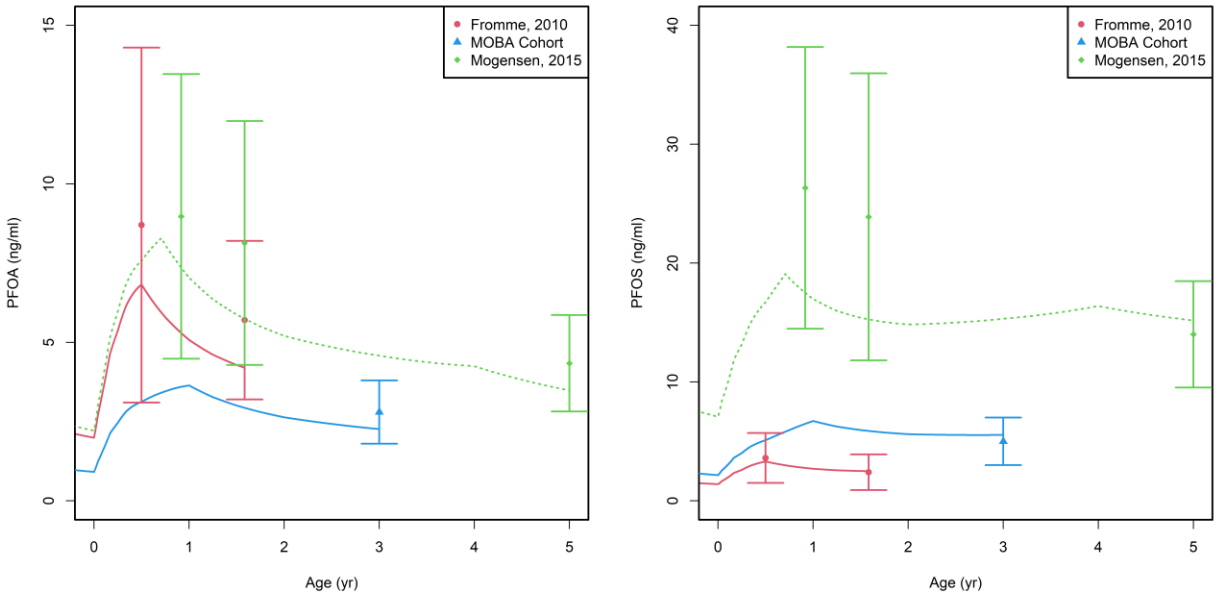


Figure F-15. Predicted Child Serum Levels Compared to Reported Values with Increased Volume of Distribution in Children as was Implemented in the Minnesota Department of Health Model

We 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). As discussed in the main document (see Main PFOS Document), this approach was not used for dosimetric extrapolation due primarily to the poor fit to the PFOS dataset. 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.

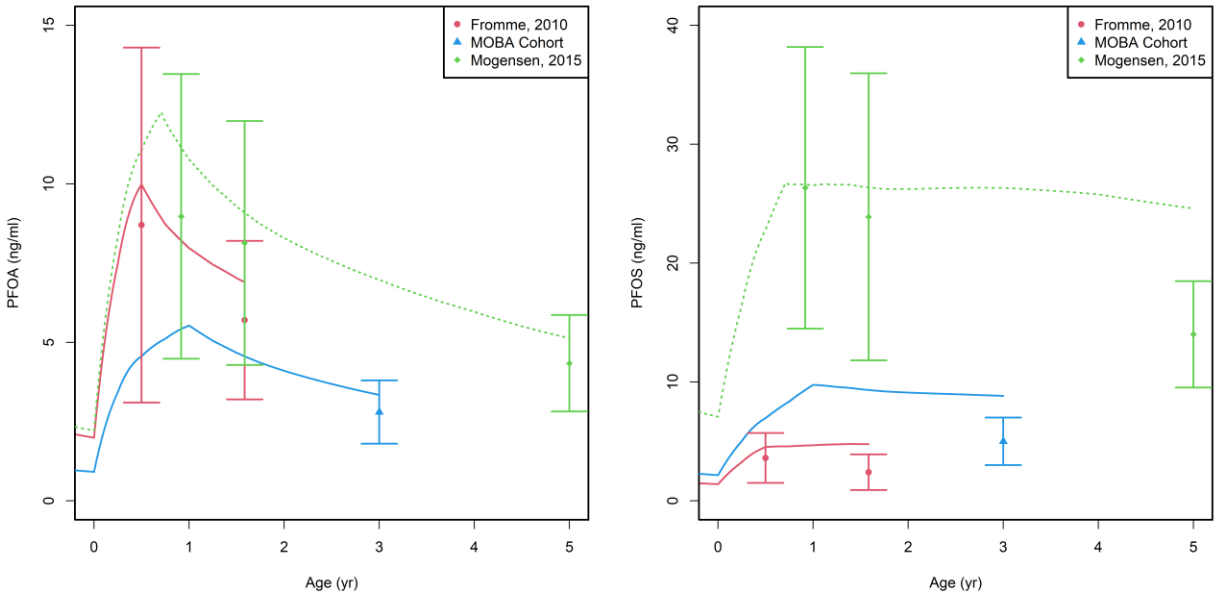


Figure F-16. Predicted Child Serum Levels Compared to Reported Values with Constant Volume of Distribution and Variable Exposure Based on Drinking Water Intake

Appendix G. Relative Source Contribution

G.1 Background

EPA applies a RSC when calculating the MCLG to account for the fraction of an individual's total exposure allocated to drinking water. EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (i.e., PFOS) 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 an exposure for an individual in the general U.S. population estimated to equal 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. The purpose of the RSC is to ensure that the level of a contaminant (e.g., MCLG value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD {U.S. EPA, 2000, 19428}. 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%. In the case of PFOS, other potential sources include diet, ambient and indoor air, incidental soil and dust ingestion, consumer products, and others.

The RSC is derived by applying the Exposure Decision Tree approach published in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* {U.S. EPA, 2000, 19428}. The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure. To determine the RSC to be used in the MCLG calculation, EPA considers whether there are significant known or potential uses/sources other than drinking water, the adequacy of data or strength of evidence available for each relevant exposure source and pathway, and whether information on each source is available to quantitatively characterize exposure. The RSC is developed to reflect the exposure to the general population or a sensitive population within the general population.

In cases in which there is a lack of sufficient environmental data and/or exposure data, 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, 19428}. Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG. For disproportionately affected subpopulations, such as the occupationally exposed or site-impacted (e.g., by a particular source or industry) where there may be higher than average PFAS concentrations in drinking water, it may be appropriate to apply an RSC greater than 20% if there is sufficient information to quantitatively characterize sources other than drinking water. This is a less conservative approach from a public health perspective and would result in a higher MCLG for those disproportionately affected populations.

G.2 Literature Review

In 2019, EPA's Office of Research and Development (ORD) conducted a broad 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, 2021, 7277659}. In this study, literature was identified that reported exposure measures from household media paired with occupant PFAS concentrations in blood serum. Second, systematic evidence mapping was conducted for literature reporting measured occurrence of PFAS chemicals in exposure media {Holder, 2021 *in prep.*, 9419128}. 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 and coworkers (2022, 10273296) 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 science 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 {Wu, 2014, 2533322; Makey, 2017, 3860102; Bryne, 2017, 4165183; Kim, 2019, 5080673; Balk, 2019, 5918617; Poothong, 2019, 5080584}. 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, 2022, 2022, 10476098}. 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 (2022., 7277659) described above focused on indoor pathways and therefore excluded non-indoor pathways such as drinking or surface water or soil. Ninety-seven articles fell into this excluded group (i.e., PFOS was measured in sera or a non-indoor environmental medium). Because the combination of PFOS measured in sera and drinking water is potentially informative for deriving the RSC, these 97 papers were reviewed for this effort, though are not described in this appendix.

G.2.2 Evidence Mapping

Holder et al. (2021 *in prep.*, 9419128) investigated evidence for important pathways of exposure to PFAS chemicals by reviewing literature reporting measured occurrence of PFAS chemicals in exposure media. The review focused on eight PFAS chemicals (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 and published between 2003–2020. ICF's *litstreamTM* 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. 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 related to 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.

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, 2016, 3603365}. 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 manufacturing of PFOS in the United States was discontinued voluntarily by its primary manufacturer in 2002 and was completely phased out of U.S. production in 2016. However, some limited uses of PFOS-related chemicals (i.e., PFOS replacements such as PFBS) remain for which alternatives are not currently available. Exposure

to PFOS can occur through food, including fish and shellfish, house dust, air, and contact with consumer products {U.S. EPA, 2016, 3603365}.

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, 2009, 1291085; Trudel, 2008, 214241; Vestergren, 2009, 1290815}. 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 {Domingo, 2017, 3981385; East, 2021, 9416543}. 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, 2008, 214241}. 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, 6984182; Domingo, 2017, 3981385}.

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, 9419076}. 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, 2019, 9638790; FDA, 2019, 9638792}. 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, 9419064}. PFOS was below the lower limit of quantification (LLOQ; 4 ng/L) in all 30 samples analyzed in a study of domestic and imported carbonated water and non-carbonated bottled water {FDA, 2016, 9419013}. 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, 9419076}. 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, 2010, 729962}.

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, 6984182}. 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, 2020, 6311690}. 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, 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. The authors reported a significant positive correlation between the observed and modeled serum concentrations for PFOS ($r = 0.29$, $p < 0.05$). The correlation existed despite the model underestimating serum concentrations of PFOS by a factor of 4, which was attributed to the long half-life and decreased exposure over recent years. While the authors did not separately quantify intake from food and drinking water, an earlier article from the same research group {Papadopoulou, 2017, 3859798} 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, 2850114) 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 ($p < 0.05$).

G.3.1.1 Food Contact Materials

Since the 1960s, the FDA has authorized several broad classes of PFAS for use in food contact substances due to their non-stick and grease, oil, and water-resistant properties. The authorization of the use of a food contact substance requires that available data and information demonstrate that there is a reasonable certainty of no harm for that use.

- 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, 9419078}

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, 2018, 9419077}. 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.

FDA (2020, 9419079) recently prohibited a few PFAS chemicals in food packaging. They announced in January 2021 that three manufacturers would begin a 3-year phase-out of their sales of some products containing 6:2 FTOH for use as food contact substances in the U.S. marketplace. After the phase-out period, they estimated that it could take up to 18 months to exhaust existing stocks of paper and paperboard products containing these food contact substances from the market. A fourth manufacturer informed FDA that they have stopped sales of their short-chain PFAS products to the U.S. market. Maine, Washington, New York, and Vermont passed restrictions on PFAS in packaging, as have cities like San Francisco and Berkeley.

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, 2018, 9419077}. 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 and Heckman LLP, 2020, 9419081}. Schaider at al. (2017, 3981864) 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, 9419077), 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, 2019, 5080553}. 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, 3981827) reported no measurable concentrations of any PFSA, including PFOS, in any of the samples. In a second study, Zabaleta et al. (2020, 6505866) 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.3.1.2 Fish and Shellfish

PFOS has been shown to bioaccumulate and biomagnify with increasing trophic level in a variety of freshwater ecosystems {Kannan, 2005, 1290874; Martin, 2004, 1291044; Penland, 2020, 6512132; Xu, 2014, 5079760} and saltwater ecosystems {de Vos, 2008, 2919394; Houde, 2006, 1290875; Loi, 2011, 1274155; Powley, 2008, 1332751; Tomy, 2004, 1332758} 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 {Haukas, 2007, 2158020; Kannan, 2005, 1290874; Kelly, 2009, 1276129; Martin, 2004, 1291044; Tomy, 2004, 1332758}. 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, 2014, 2850900; Kannan, 2005, 1290874; Martin, 2004, 1291044}.

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 {Giesy, 2001, 1290854; Houde, 2006, 1290875}.

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, 10369692)	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, 10369694)	Largemouth bass Smallmouth bass Black crappie White crappie Walleye/sauger Yellow perch White bass Northern pike Lake 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.

Reference	Most Commonly Sampled Species	Site Description	Results
	Brown trout Rainbow trout Brook trout		
U.S. EPA (2011, 10369695)	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 (2016, 10369696)	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, 2919419) 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, 2587663) 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, 6512132) 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, 6512132) measured was 482.9 ng/g ww, from the eggs of a redhorse fish sample.

Houde et al. (2006, 1290875) 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, 5387058) 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, 6833737) 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, 10601281), 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, 9638787}.

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.3.2 Consumer Product Uses

An early investigation of consumer exposure to PFOS by Trudel et al. (2008, 214241) 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, 2324799) 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, 9416541) 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, 2850246) 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 {Gremmel, 2016, 3858525; van der Veen, 2020, 6316195}. PFOS was detected in one-third of the jackets tested by Gremmel et al. (2016, 3858525) 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, 2020, 6316195}.

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 {Shoeib, 2011, 1082300; Gebbink, 2015, 2850068; NJDWQI, 2018, 5026035; Poothong, 2020, 6311690}. 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 {Shoeib, 2011, 1082300; Kim, 2019, 5080673; Wu, 2014, 2533322; Poothong, 2020, 6311690; Makey, 2017, 3860102; Byrne, 2017, 4165183; Fraser, 2013, 2325338}.

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, 2019, 5080673}. One study of Alaska Natives noted that PFOS was the predominant compound in dust samples {Byrne, 2017, 4165183}.

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, 2011, 2325317}. 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, 2007, 1289790}. 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, 2005, 1289802}. 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, 9419086}. 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, 9638786}. 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, 9638786}.

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, 9638786} and Japan {Sasaki, 2003, 5081390}. 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, 2003, 5081390}.

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, 2004, 1291044}. 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, 2007, 1049488}. The highest concentrations were reported in Manchester, United Kingdom. Similarly, high concentrations, 150 pg/m^3 for were reported Paris, France {ECCC, 2018, 9638786}.

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, 2007, 1289794}. 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, 2010, 1291056}. 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 with 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 used the Exposure Decision Tree methodology to derive the RSC for this MCLG (Figure G-1) {U.S. EPA, 2000, 19428}. Findings from studies on populations in the United States, with supporting evidence from Canada and Western Europe, suggest that diet, particularly fish, is the major contributor to total PFOS exposure among adults, typically with dust as an important additional exposure medium, especially for sensitive populations. Additional exposure sources are consumer products and air (Box 2; Figure G-1). However, adequate data are not available to describe central tendency and high-end exposures for all relevant exposure sources and pathways (Box 3; Figure G-1). There is sufficient data on the physical/chemical properties, fate and transport, and generalized information characterizing the likelihood of exposure to PFOS via relevant sources (Box 4; Figure G-1). There are significant known or potential sources other than drinking water (Box 6; Figure G-1), although there is not enough information available for each pathway, particularly dust, air, consumer products, and food contact materials, to characterize

exposure (Box 8A; Figure G-1). Therefore, an RSC of 20% (0.20) should be used (Box 8B; Figure G-1).

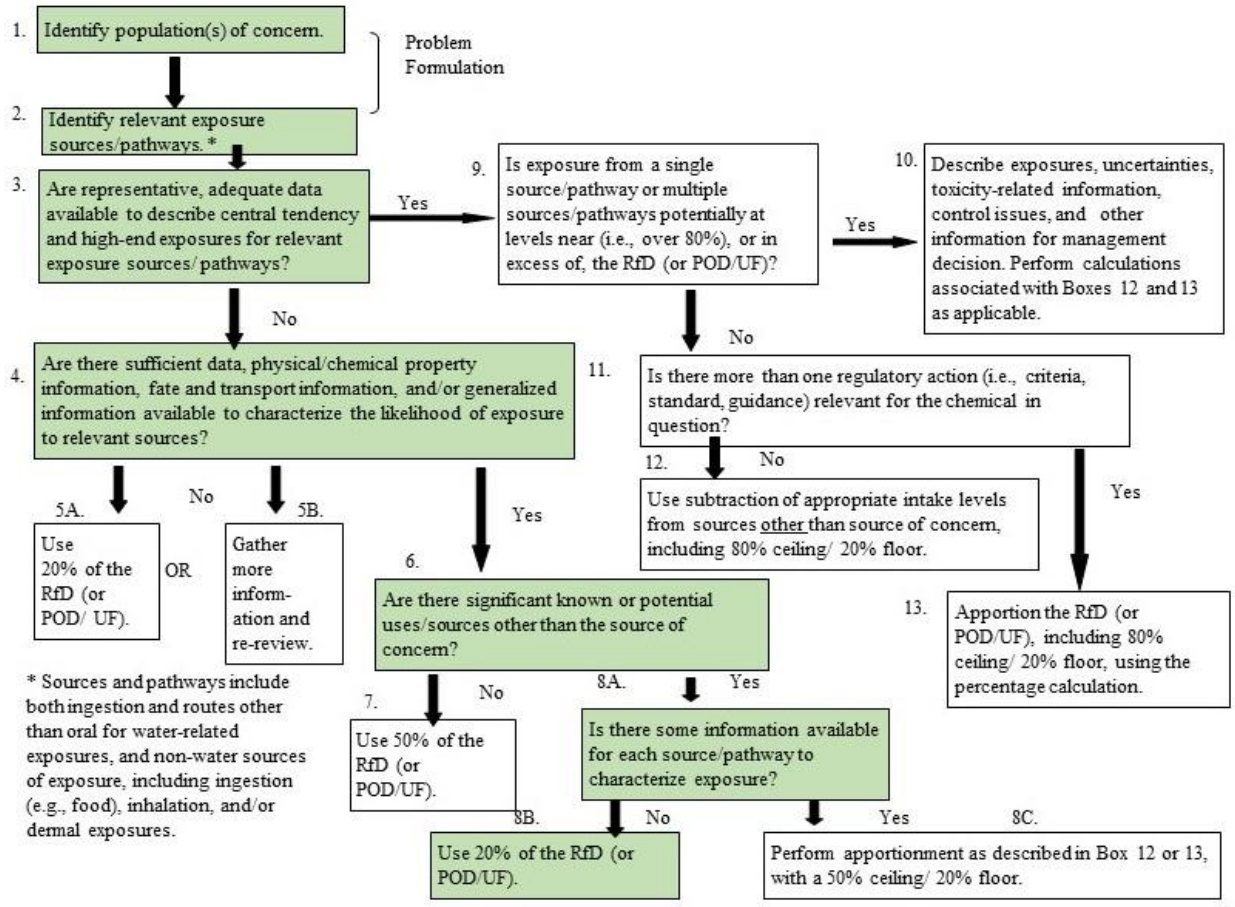


Figure G-1. Application of the Exposure Decision Tree {U.S. EPA, 2000, 19428} for PFOS

Green highlighted boxes indicate selections made at each branch of the Decision Tree.
POD = point of departure; RfD = reference dose; UF = uncertainty factor.

In summary, based on the physical properties, detected levels, and available exposure information for PFOS, food, and air are potentially significant sources. Following the Exposure Decision Tree in EPA's 2000 Methodology {U.S. EPA 2000, 19428}, significant potential sources other than drinking water ingestion exist; however, information is not available to quantitatively characterize exposure from these different sources. Therefore, EPA recommends an RSC of 20% (0.20) for PFOS.