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EXTERNAL PEER REVIEW DRAFT
Proposed Approaches to the Derivation of a
Draft Maximum Contaminant Level Goal for
Perfluorooctane Sulfonic Acid (PFOS)
(CASRN 1763-23-1) in Drinking Water

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(PFOS)
(CASRN 1763-23-1) in Drinking Water

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Acronyms and Abbreviations

17-OHP	17-hydroxyprogesterone	AUC _{avg_dam_gest}	AUC during gestation
5-mC	5' methylated cytosine	AUC _{avg_dam_lact}	AUC during lactation
Ab	Antibody	AUC _{avg_dam_gest_lact}	AUC combined gestation and lactation
ABC	ATP-binding cassette		
ABCG2	ATP-binding cassette transporter G2	AZI	azithromycin-dihydrate
aBMD	areal bone mineral density	BBB	blood brain barrier
ACD	anterior chamber depth	BCERP	Breast Cancer and Environment Research Program
ACTH	adrenocorticotrophic hormone	BCRP	Breast Cancer Resistance Protein
ADGap	anopenile distance	BDI	Beck Depression Inventory
ADHD	attention deficit hyperactivity disorder	BMD	benchmark dose
ADME	absorption, distribution, metabolism, and excretion	BMD ₁₀	dose corresponding to a 10% change in response
AFFF	aqueous film forming foam	BMDL	benchmark dose lower limits
AGD	anogenital distance	BMDL ₁₀	dose level corresponding to the 95% lower confidence limit of a 10% change
AGDas	anoscrotal distance		
AIC	Akaike information criterion	BMDS	Benchmark Dose Software
ALP	alkaline phosphatase	BMI	body mass index
ALSPAC	Avon Longitudinal Study of Parents and Children	BMR	benchmark response
ALT	alanine aminotransferase	BRIEF	Behavior Rating Inventory of Executive Function
AMH	anti-müllerian hormone		
ApoC-III	apolipoprotein C-III	BUN	blood urea nitrogen
ASBT	apical sodium-dependent bile salt transporter	BWT	birthweight
ASD	autism spectrum disorder	BW	body weight
ASQ	Ages and Stages Questionnaire	C _{7,avg}	average concentration over the final week of study
AST	aspartate aminotransferase	CAD	coronary artery disease
ATP III	Adult Treatment Panel III	CalEPA	California EPA
ATSDR	Agency for Toxic Substances and Disease Registry	CAPA	Caucasian Asian Pediatric Adult
AUC	area under the curve	CASRN	Chemical Abstracts Service Registry Number

C_{avg}	average blood concentration	CRP	C-reactive protein
$C_{avg_pup_gest}$	C_{avg} during gestation	C-section	cesarian section
$C_{avg_pup_lact}$	C_{avg} during lactation	CSF	cancer slope factor
$C_{avg_pup_gest_lact}$	C_{avg} combined gestation and lactation	CSM	cholestyramine
CBCL 1.5–5	Child Behavior Checklist 1.5–5	CTX	type I collagen
CCL	Contaminant Candidate List	CVD	cardiovascular disease
CDC	Centers for Disease Control and Prevention	CYP	cytochrome P450 aromatase
CDI	comprehensive developmental inventory	DaFO88	Danish Fetal Origins 1988
CHARGE	Childhood Autism Risk from Genetics and Environment	DBP	diastolic blood pressure
CHD	coronary heart disease	DFI	DNA fragmentation index
CHECK	Children’s Health and Environmental Chemicals in Korea	DHEA	dehydroepiandrosterone
CHEF	Children's Health and the Environment in the Faroes	DHEAS	dehydroepiandrosterone sulfate
CHF	congestive heart failure	DM	diabetes mellitus
CHIAM7.4	chromatographic index	DNA	deoxyribonucleic acid
CHO	Chinese hamster ovary	DNBC	Danish National Birth Cohort
CI	confidence interval	DPP	Diabetes Prevention Program
CIMT	carotid artery intima-media thickness	DPPOS	Diabetes Prevention Program and Outcomes Study
CKD	chronic kidney disease	DWI	drinking water intake
C_{last-7}	blood concentration over the last 7 days	E2	estradiol
CKD-EP	Chronic Kidney Disease Epidemiology Collaboration study	E3	estriol
CL	clearance	ED-RIA	equilibrium dialysis-radioimmunoassay
Cl_{total}	total daily clearance	EFSA	European Food Safety Authority
C_{max}	maximum blood concentration	eGFR	estimated glomerular filtration rate
CNS	central nervous system	ENT1	equilibrative nucleoside transporter
CRH	corticotropin releasing hormone	EPA	U.S. Environmental Protection Agency
		ER	estrogen receptor
		ER+	estrogen receptor positive
		EYHS	European Youth Heart Study
		F ₁	first generation

F ₂	second generation	HFPO	hexafluoropropylene oxide
FAI	free androgen index	Hib	<i>Haemophilus influenzae</i> type b
FCC	Fernald Community Cohort	HOMA-B	homeostasis model assessment of beta-cell function
FDA	U.S. Food and Drug Administration	HIV	human immunodeficiency virus
FEF _{25 – 75%}	forced expiratory flow at 25–75%	HOMA	Homeostatic Model Assessment
FEV ₁	forced expiratory volume in one second	HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
FR α	folate receptor alpha	HOME	Health Outcomes and Measures of the Environment
FSH	follicle stimulating hormone	HPLC	high performance liquid chromatography
FT3	free triiodothyronine	HPLC-MS/MS	HPLC coupled to mass spectrophotometry
FTI	free thyroxine index	HR	Hazard Ratio
FVC	forced vital capacity	HRL	health risk limit
GABA	gamma-aminobutyric acid	HSA	human serum albumin
GBCA	Genetic and Biomarkers study for Childhood Asthma	HUMIS	Norwegian Human Milk Study
GD	gestational day	IARC	International Agency for Research on Cancer
GF	glomerular filtration	IBD	inflammatory bowel disease
GFR	glomerular filtration rate	ID	intellectual disability
GGT	γ -glutamyltransferase	IDL	intermediate density lipoprotein
GI	gastrointestinal	IgE	immunoglobulin E
GLP	good laboratory practice	IGF-1	insulin-like growth factor 1
HAI	hemagglutinin inhibition	IgM	immunoglobulin M
HAWC	Health Assessment Workspace Collaborative	IHD	ischemic heart disease
Hb	hemoglobin	IL-2	interleukin 2
HbA1c	hemoglobin A1c	IL-4	interleukin 4
HDL	high density lipoprotein cholesterol	IMS	imaging mass spectrometry
HED	human equivalent dose		
HERO	Health and Environmental Research Online		
HESD	Health Effects Support Document		
HFMD	hand, foot, and mouth disease		

INUENDO	Biopersistent Organochlorines in Diet and Human Fertility	MDI	mental developmental index
IQ	intelligence quotient	MeFOSAA	2-(N-Methyl- perfluorooctane sulfonamido) acetic acid
IQR	interquartile range		
IRIS	Integrated Risk Information System	Me-PFOSA-AcOH	2-(N-Methyl- perfluorooctane sulfonamido) acetic acid
IUFD	intrauterine fetal death		
IV	intravenous	MIREC	Maternal-Infant Research on Environmental Chemicals
K _{mem/w}	membrane/water partition coefficients		
K _{oc}	organic carbon-water partitioning coefficient	MMR	measles, mumps, and rubella
LBW	low birthweight	MOA	mode of action
LD	lactational day	MoBa	Mother, Father, and Child Cohort Study
LDL	low density lipoprotein cholesterol	MPAH	n-methyl-PFOSA
L-FABP	liver fatty acid binding protein	mPLP	mouse prolactin-like protein
LH	luteinizing hormone	MRL	Minimum Reporting Level
LIFE	Longitudinal Investigation of Fertility and the Environment	mRNA	messenger RNA
		MRP	multidrug resistance- associated protein
LINC	Linking Maternal Nutrition to Child Health	MS	multiple sclerosis
LLOQ	lower limit of quantification	NAG	n-acteyl-b- glucosaminidase
LME	latent effects mixed model	NCCA	National Coastal Condition Assessment
LOAEL	lowest-observed-adverse- effect level	NCI	National Cancer Institute
LOD	limit of detection	NHANES	National Health and Nutrition Examination Survey
LOQ	limit of quantification		
LPS	lipopolysaccharide	NHS	Nurses' Health Study
MALDI	Matrix-Assisted Laser Desorption/Ionization	NIEHS	National Institute for Environmental Health Sciences
MCDI	MacArthur Communicative Development Inventories for Infants	NK	natural killer
		NOAA	National Oceanic and Atmospheric Administration
MCLG	Maximum Contaminant Level Goal	NOAEL	no-observed-adverse- effect level

NPDWR	National Primary Drinking Water Regulation	PFC PFCA	plaque forming cell perfluorinated carboxylic acids
NRSA	National Rivers and Streams Assessment	PFDA PFDoDA	perfluorodecanoic acid perfluorododecanoic acid
NT	not tested	PFHpA	perfluoroheptanoic acid
NTCP	sodium/taurocholate cotransporting polypeptide	PFHxA PFHxS	perfluorohexanoic acid perfluorohexanesulfonate
NTP	National Toxicology Program	PFNA PFOA	perfluorononanoic acid perfluorooctanoic acid
OAT	organic anion transporter	PFOS	perfluorooctane sulfonic acid
OATP	organic anion transporting polypeptides	PFSA	perfluorosulfonic acid
OCC	Odense Child Cohort	PFUnDA	perfluoroundecanoic acid
OECD	Organisation for Economic and Co-operation and Development	PHQ-9	Patient Health Questionnaire
OR	odds ratio	P _{ion}	anionic permeability
ORD	Office of Research and Development	PK	pharmacokinetic
OSS	Oslo severity score	PND	postnatal day
OST	Office of Science and Technology	PNW	postnatal week
P ₀	parental generation	POD	point of departure
PBPK	physiologically based pharmacokinetic	POD _{HED}	point of departure human equivalent dose
PC	partition coefficient	POI	premature ovarian insufficiency
PCOS	polycystic ovary syndrome	PONCH	Pregnancy Obesity Nutrition and Child Health study
PDI	psychomotor developmental index	POPUP	Persistent Organic Pollutants in Uppsala Primiparas
PECO	Population, Exposure, Comparator, and Outcome	POUNDS-Lost	Prevention of Obesity Using Novel Dietary Strategies Lost
PEF	peak expiratory flow rate	PPAR	peroxisome proliferator activated receptor
PFAA	perfluoroalkyl acids	ppb	parts per billion
PFAS	perfluoroalkyl and polyfluoroalkyl substances	ppm	parts per million
PFBA	perfluorobutanoic acid	ppt	parts per trillion
PFBS	perfluorobutane sulfonate	PR	progesterone receptor
		PR+	progesterone receptor positive

PSA	prostate specific antigen	TSCATS	Toxic Substance Control
PTB	preterm birth		Act Test Submissions
PWS	public water systems	TSH	thyroid stimulating
QA	Quality Assurance		hormone
RfD	reference dose	TT3	total triiodothyronine
RIA	radioimmunoassay	TT4	total thyroxine
R _{PM}	ratio of PFOS in placenta relative to maternal serum	TTE	transplacental transfer efficiencies
RSC	relative source contribution	UCMR 3	Third Unregulated Contaminant Monitoring Rule
RSV	respiratory syncytial virus	UF	uncertainty factors
rT3	reverse T3	UF _A	interspecies uncertainty factor
RTECS	Registry of Toxic Effects of Chemical Substances	UF _D	database uncertainty factor
SAB	Science Advisory Board	UF _H	intraspecies uncertainty factor
SBP	systolic blood pressure	UF _L	extrapolation uncertainty factor
SD	standard deviation	UF _S	uncertainty factor for extrapolation from a subchronic to a chronic exposure duration
SDQ	Strengths and Difficulties Questionnaire	UF _{TOT}	total uncertainty factors
SERT	serotonin transporter	V _d	volume of distribution
SES	socioeconomic status	VI	visual impairment
SGA	small for gestational age	VLDL	very low-density lipoprotein cholesterol
SHBG	sex hormone binding globulin	VMWM	Virtual Morris Water Maze
SMBCS	Shanghai-Minhang Birth Cohort Study	WBC	white blood cell
SRBC	sheep red blood cell	WBHGB	whole blood hemoglobin
SWAN	Study of Women's Health Across the Nation	WHO	World Health Organization
SWDA	Safe Water Drinking Act	WTC	World Trade Center
T3	triiodothyronine	WTCHR	World Trade Center Health Registry
T4	thyroxine		
TA	thyroid antibody	ww	wet weight
TC	total cholesterol		
TDS	Total Diet Study		
TgAb	thyroglobulin antibody		
TiAb	title-abstract		
T _{max}	Time to C _{max}		
TNP	trinitrophenyl		
TPO	thyroid peroxidase		
TPOAb	thyroid peroxidase antibody		

1.0 Background

The U.S. Environmental Protection Agency (EPA) has initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) for per- and polyfluoroalkyl substances (PFAS) under the Safe Drinking Water Act (SDWA). The agency is seeking comment from the EPA Science Advisory Board (SAB) on key scientific issues related to the development of the NPDWR. As part of this proposed rulemaking, EPA has prepared this white paper, *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water*, that derives oral toxicity values and a relative source contribution for perfluorooctane sulfonic acid (PFOS). This paper is being submitted for scientific review by EPA SAB along with three other documents:

- *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic acid (PFOA) (CASRN 335-67-1) in Drinking Water*
- *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)*
- *Analysis of Avoided Cardiovascular Disease Risk from Reduced PFOA and PFOS exposure: Methodology and Preliminary Results*

This draft document, as well as the PFOA draft document, develop a number of values, including toxicity values, that could be use in a human health risk assessment. While the PFOA and PFOS approaches documents do not constitute risk assessments, the values were derived using human health risk assessment guidance, guidelines, and current methods. Fit-for-purpose systematic review methods, also consistent with current EPA methods, were used to develop the toxicity values within the timeline to rule proposal and in order to follow a transparent and scientifically robust process to identify, evaluate, and synthesize the best available science.

EPA is seeking review of this document along with the other three documents that together will ultimately inform development of the NPDWR for PFOS and PFOA. EPA will revise the draft documents based on SAB comments and recommendations. The health effects information from each of these documents will be incorporated into any analyses that will be used to establish MCLGs and NPDWRs.

PFAS are a large group of anthropogenic chemicals that include PFOA, PFOS, and thousands of other chemicals. The universe of environmentally relevant PFAS, including parent chemicals, metabolites, and degradants, is greater than 9,000 compounds. 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}. Comparatively, the number of PFAS currently used 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}. The chemical structures of PFAS make them repel water and oil, remain chemically and thermally stable, and

exhibit surfactant properties; these properties make PFAS useful for commercial and industrial applications and purposes, and are also the properties that make some PFAS 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 PFAS co-occur in exposure media (e.g., air, water, ice, sediment), and in tissues and blood of aquatic and terrestrial organisms, and humans.

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}. These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl and polyfluoroalkyl substances. PFOA and PFOS belong to the perfluoroalkyl acids (PFAA) of the non-polymer perfluoroalkyl substances category of PFAS and are among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review see Podder, 2021, 9640865).

1.1 Evaluation of PFOS Under SDWA

1.1.1 History of PFOS Under SDWA

SDWA, as amended in 1996, requires EPA to publish a list of unregulated contaminants every 5 years that are not subject to any current proposed or promulgated NPDWRs, are known or anticipated to occur in public water systems (PWSs), and might require regulation under SDWA. This list is known as the Contaminant Candidate List (CCL). PFOS is included on the third CCL (CCL 3) {U.S. EPA, 2009, 1508321} and on the fourth CCL (CCL 4) {U.S. EPA, 2016, 6307617}.

After they were listed on the CCL 3 in 2009, EPA initiated the *Health Effects Support Document (HESD) for Perfluorooctane Sulfonic Acid (PFOS)* and one for another PFAS, PFOA, to assist federal, state, tribal and local officials, and managers of drinking water systems in protecting public health when these chemicals are present in drinking water {U.S. EPA, 2016, 3603365; U.S. EPA, 2016, 3982043}. The two HESDs were peer-reviewed in 2014 and were revised based on consideration of peer reviewer and public comments and inclusion of additional studies published through December 2015. The 2016 HESD for PFOS {U.S. EPA, 2016, 3603365} provides a reference dose (RfD) and cancer assessment that serve as the basis for the non-regulatory Health Advisory {U.S. EPA, 2016, 3982043}.

SDWA requires EPA to make regulatory determinations for at least five CCL contaminants every 5 years. EPA initiates the process for developing a NPDWR when the agency makes a determination to regulate based on three criteria:

- The contaminant may have an adverse effect on the health of persons.
- The contaminant is known to occur or there is substantial likelihood the contaminant will occur in PWSs with a frequency and at levels of public health concern.
- In the sole judgment of the Administrator, regulating the contaminant presents a meaningful opportunity for health risk reductions.

To make these determinations, the agency considers a range of information, including data to analyze occurrence of these compounds in finished drinking water and data on health effects.

In the *Final Regulatory Determinations for Contaminants on the Fourth Drinking Water Contaminant Candidate List* (2021, 9640861), the agency made a determination to regulate PFOA and PFOS with a NPDWR. The agency found that PFOA and PFOS may have adverse health effects; that PFOA and PFOS occur in PWSs with a frequency and at levels of public health concern; and that, in the sole judgment of the Administrator, regulation of PFOA and PFOS presents a meaningful opportunity for health risk reduction for persons served by PWSs {U.S. EPA, 2021, 7487276}.

1.2 Purpose of this Document

The primary purpose of this document is to derive an updated chronic oral draft RfD, cancer slope factor (CSF) if relevant data are available (as needed), and a draft relative source contribution (RSC) for PFOS for SAB review. These toxicity values and RSC values build upon the information provided in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and Health Advisory {U.S. EPA, 2016, 3982042}, respectively. EPA will incorporate SAB feedback as appropriate to finalize the values derived in this assessment and subsequently derive an MCLG for the NPDWRs for PFOS and PFOA.

Secondary purposes of this document are to:

- Provide a description of the literature searches conducted and fit-for-purpose systematic review methods used to identify health effects information (epidemiological, toxicological studies and physiologically based pharmacokinetic (PBPK) models), published since the 2016 HESDs for PFOA and PFOS that could potentially influence future PFOA or PFOS drinking water regulatory actions.
- Describe screening against the Populations, Exposures, Comparator, and Outcome (PECO) criteria and tracking studies containing supplemental material that are potentially relevant to an assessment during the literature screening process.
- Briefly summarize studies identified from the literature search that meet PECO criteria and create a literature inventory to identify those that are most appropriate to derive an oral point of departure (POD) for chronic toxicity value derivation on the basis of several study design considerations.
- Describe study evaluations of epidemiological and animal toxicological studies considered plausibly useful for POD derivation on the basis of study design.
- Describe data fully extracted from studies that could be used for POD derivation on the basis of study design and study evaluation results.
- Describe the dose-response analysis conducted on the studies identified for POD derivation. Derive a draft RfD and/or CSF, as needed, for PFOS.
- Identify additional analyses needed for finalizing a draft MCLG and key data gaps.

1.2.1 MCLG Approach

1.2.1.1 Inputs of MCLG Calculation for Noncancer Effects for PFOA and PFOS

For chemicals exhibiting a threshold for toxic effects, EPA establishes the MCLG based on an oral RfD. The chronic RfD is an estimate (with uncertainty spanning perhaps an order of

magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime.

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. For dose response data in an animal model, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD when data for a particular endpoint were not amenable to BMD modeling.

The general steps for deriving an RfD for PFOS are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints related to oral exposure to PFOS. This step involves selecting the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study quality, and characterized for adverse health outcomes, the risk assessor selects health endpoints/outcomes judged to be relevant to human health and among the most sensitive, defined as effects observed in the lower dose range. Considerations that might influence selection of endpoints include data with dose response, percent change from controls, adversity of effect, and consistency across studies.

Step 1a (for dose response data in an animal model): Convert Administered Dose to an Internal Dose. A toxicokinetic model is used to make predictions of the internal dose in lab animals used in toxicity studies or in humans based on the administered dose used in the study (Section 4.1.3.1). A number of dose-metrics across life stages were selected for simulation in a mouse, rat, monkey, or human. Concentrations of PFOS in blood were considered for all the internal dose-metrics.

Step 2: Conduct BMD Modeling. Using EPA's *Benchmark Dose Technical Guidance Document* {U.S. EPA, 2012, 1239433}, a benchmark response (BMR) is selected and BMD modeling is applied to the endpoints selected as most relevant. The BMR is a predetermined change (percent or standard deviation) in the response rate of an adverse effect. It serves as the basis for obtaining the benchmark dose lower limit (BMDL), which is the 95% lower bound of the BMD. A set of BMD models are fit to the dose-response data that describe the dataset of the identified adverse effect. From the set of models, either a best fitting model with the corresponding BMD and BMDL is derived or, if no adequate models are found, the NOAEL or LOAEL identified in step 1 is used as the POD.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (either a BMDL, NOAEL, or LOAEL) is then converted to a HED following the method described in Section 4.1.3.2. Briefly, a toxicokinetic model for human dosimetry is used to simulate the HED from the animal PODs from Step 2. It is also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling. For the human and animal endpoints of

interests, serum concentration was identified, based on the available data, as a suitable internal dosimetry target.

Step 4: Provide rationale for selecting uncertainty factors (UFs). UFs are selected in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans (if applicable), the duration of exposure in the critical study compared to the lifetime of the species studied, and the completeness of the toxicology database.

Step 5: Calculate the chronic RfD. The RfD is calculated by dividing POD_{HED} by the selected UF.

$$RfD = \left(\frac{POD_{HED}}{UF_{TOT}} \right)$$

where:

- POD_{HED} = calculated from the BMDL or NOAEL/LOAEL using the human pharmacokinetic (PK) model presented in Section 4.1.3.2.
- UF_{TOT} = Total UF established in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, duration of exposure in the critical study compared to the lifetime of the species studied, and completeness of the toxicology database.

Once the RfD is determined, the MCLG is derived by considering other known or potential sources of exposure, using the RSC. The RSC is used in the calculation of the MCLG and is based on actual exposure data, or, if data are not available, a value of 20% is assumed for effects based on lifetime exposure. This allows 80% of the total exposure to come from sources other than drinking water, such as exposure from food, inhalation, or dermal contact. In assessments completed after the EPA RSC decision tree was published in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* {U.S. EPA, 2000, 19428}, a maximum RSC value of 80% rather than 100% is used to allow for potential unidentified sources even when exposure data from some sources are available. In the event that one of the identified toxicological assessments includes an updated RSC based on new literature, the updated RSC will be considered for use in deriving the MCLG on a case-by-case basis. The drinking water intake (DWI) used to calculate MCLGs is intended to protect the target population for which the critical effect was identified.

$$MCLG = \left(\frac{Oral\ RfD}{DWI} \right) * RSC$$

Where:

- Oral RfD = Oral reference value (mg/kg/day)
- DWI = Drinking water intake (L/kg/day)
- RSC = Relative source contribution (%)

As stated previously, the purpose of this draft document is to derive a draft RfD and/or CSF and a draft RSC for PFOS for SAB review. EPA will incorporate SAB feedback on health effects information into analyses that are used to establish MCLGs and NPDWRs for PFOS.

1.2.1.2 Inputs of MCLG Calculation for Cancer Endpoints

Under the 2005 guidelines, a descriptive weight of evidence expert judgment is made, based on all available animal, human, and mechanistic data, as to the likelihood that an agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed {U.S. EPA, 2005, 9638795}. If a chemical is *carcinogenic to humans* or *likely to be carcinogenic to humans* by the oral route, the MCLG is typically set at zero because it is assumed, in the absence of other data indicating otherwise, that there is no known threshold for carcinogenicity. For the descriptors of *Suggestive evidence of carcinogenic potential*, *Inadequate information to assess the carcinogenic potential*, and *Not likely to be carcinogenic to humans*, the RfD approach is used. A cancer narrative is also included to provide a more complete description of the weight of evidence and conditions of carcinogenicity. The suggested cancer descriptors available in the 2005 guidelines are:

- *Carcinogenic to humans*
- *Likely to be carcinogenic to humans*
- *Suggestive evidence of carcinogenic potential*
- *Inadequate information to assess carcinogenic potential*
- *Not likely to be carcinogenic to humans*

Compound descriptors are possible if a chemical has different carcinogenic responses with different dose or MOA¹. MOA information enters into both the qualitative and quantitative portions of the assessment. The MOA determines such issues as the human relevance of the observed tumors. MOA must be considered separately for every target organ.

The 2005 guidelines recommend a two-step process for the quantitation step. First, a model is used to fit a dose-response curve based on the doses and associated tumors from the cancer bioassay. The model is used to identify a POD. For cancer assessment, the POD is used for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD₁₀ (the dose corresponding to a 10% increase in tumors), and the BMDL₁₀ (the 95% lower confidence limit on the BMD₁₀) are also reported and are often used as the POD. PK models have been developed to calculate the HED for animal oral exposures.

In the second step of the low-dose extrapolation, the POD is extrapolated to the low-dose region of interest for environmental exposures. The approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). 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 ($\Delta\text{response}/\Delta\text{dose}$) 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

¹ MOA is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. It is contrasted with “mechanism of action,” which implies a more detailed understanding and description of events.

data indicating potential mutagenicity) determines if age-dependent adjustment factors are applied in the quantification of risk to account for additional sensitivity of children.

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 U.S. EPA’s established practice of developing such values, taking into consideration the factors summarized in the characterization of the POD.” In these cases, an RfD-like value is calculated based on the key event² for carcinogenesis or the tumor response.

1.3 Chemical Identity

Physical and chemical properties and other reference information for PFOS are provided in Table 1. Please see the 2016 PFOS Health Advisory {U.S. EPA, 2016, 3982043} for additional details on the chemical and physical properties (Section 2.1) and environmental fate (Section 2.3).

Table 1. Chemical and Physical Properties of PFOS

Property	PFOS, Acidic Form ^a ; Experimental Average (Experimental Range) ^b	Source (# of References Supporting Reported Value)
Chemical Abstracts Service Registry Number (CASRN) ^c	1763-23-1	EPA CompTox Chemicals Dashboard
Chemical Abstracts Index Name	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid	
Synonyms	Perfluorooctane sulfonic acid; heptadecafluoro-1-octane sulfonic acid; PFOS acid	EPA CompTox Chemicals Dashboard
Chemical Formula	C ₈ HF ₁₇ O ₃ S	EPA CompTox Chemicals Dashboard
Molecular Weight	500.13 g/mol	EPA CompTox Chemicals Dashboard
Color/Physical State	White powder (potassium salt)	{OECD, 2002, 1337017}
Boiling Point	169 °C (133–249 °C)	EPA CompTox Chemicals Dashboard (4)
Melting Point	84.1 °C (15.2–185 °C) (predicted)	EPA CompTox Chemicals Dashboard (predicted)
Vapor Pressure	2.48×e ⁻⁶ mm Hg	EPA CompTox Chemicals Dashboard (1)
Henry’s Law Constant	1.80×e ⁻¹¹ atm·m ³ /mol (predicted)	EPA CompTox Chemicals Dashboard (predicted)
K _{oc}	2.57 L/kg of organic carbon	{Higgins, 2006, 5084923}
K _{ow}	5.61 (4.30–7.03)	EPA CompTox Chemicals Dashboard (4)
Solubility in Water	0.00114 mol/L (6.25×e ⁻⁹ –2.27 mol/L)	EPA CompTox Chemicals Dashboard (2)

K_{oc} = organic carbon-water partitioning coefficient; K_{ow} = octanol-water partition co-efficient.

² The key event is defined as an empirically observed precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element.

^a PFOS is commonly produced as a potassium salt (CASRN 2795-39-3). Properties specific to the salt are not included.

^b Unless otherwise noted.

^c The CASRN given is for linear PFOS, but the toxicity studies are based on a mixture of linear and branched; thus the RfD applies to the total linear and branched.

1.4 Occurrence Summary

Data from the third Unregulated Contaminant Monitoring Rule (UCMR 3) are currently the best available nationally representative finished water occurrence information for PFOA and PFOS {U.S. EPA, 2017, 9419085; U.S. EPA, 2021, 7487276}. UCMR 3 monitoring occurred recently (between 2013 and 2015). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed for PFOA and PFOS. The minimum reporting level (MRL)³ for PFOA was 0.02 mg/L and the MRL for PFOS was 0.04 mg/L. A total of 1.37% of samples had reported detections (greater than or equal to the MRL) of at least one of the two compounds.

To examine the occurrence of PFOS and PFOA in aggregate, EPA summed the concentrations detected in the same sample to calculate a total PFOS/PFOA concentration. EPA noted in the 2016 Health Advisories for PFOA and PFOS that the RfDs for both PFOA and PFOS are based on similar developmental health effects and are numerically identical {U.S. EPA, 2016, 3603365; U.S. EPA, 2016, 3603279}. When these two chemicals co-occur at the same time and location in drinking water sources, EPA recommended consideration of the sum of the concentrations {U.S. EPA, 2016, 3603365; U.S. EPA, 2016, 3603279} and did so in the analysis for *Regulatory Determination for Contaminants on the Fourth Drinking Water Contaminant Candidate List* {U.S. EPA, 2021, 7487276; U.S. EPA, 2021, 9640861}. The maximum summed concentration of PFOA and PFOS was 7.22 mg/L⁴ and the median summed value was 0.05 mg/L. Summed PFOA and PFOS concentrations exceeded one-half the health risk limit (HRL)⁵ (0.035 mg/L) at a minimum of 2.4% of PWSs (115 PWSs) and exceeded the HRL (0.070 mg/L) at a minimum of 1.3% of PWSs (63 PWSs). Since UCMR 3 monitoring occurred, certain sites where elevated levels of PFOA and PFOS were detected may have installed treatment for PFOA and PFOS, may have chosen to blend water from multiple sources, or may have otherwise remediated known sources of contamination. The identified 63 PWSs serve a total population of approximately 5.6 million people and are located in 25 states, tribes, or U.S. territories {U.S. EPA, 2017, 9419085}.

Data from more recent state monitoring demonstrate occurrence in multiple geographic locations consistent with UCMR 3 monitoring {U.S. EPA, 2021, 7487276}. At the time of publication of the final regulatory determinations for PFOA and PFOS, the finished water data available from fifteen states collected since UCMR3 showed that there were at least 29 PWSs where the summed concentrations of PFOA and PFOS exceeded the EPA HRL {U.S. EPA, 2021, 7487276}. EPA notes that some of these data are from targeted sampling efforts and thus may not be representative of levels found in all PWSs within the state or represent occurrence in other states. The state data demonstrate occurrence in multiple geographic locations and support

³ 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 (USEPA, 2021).

⁴ Sum of PFOA + PFOS results rounded to 2 decimal places in those cases where a laboratory reported more digits.

⁵ An HRL is a health-based concentration against which the agency evaluates occurrence data when making decisions about regulatory determinations.

EPA’s finding that PFOA and PFOS occur with a frequency and at levels of public health concern in drinking water systems across the United States. See U.S. EPA, 2021, 7487276 for further discussion.

2.0 Methods for PFOA/PFOS Health Effects Systematic Review

2.1 Problem Formulation for the Systematic Review, Specific Aims, and Population, Exposure, Comparator, and Outcome Criteria

This section summarizes the assessment methods for the PFOA and PFOS Health Effects Systematic Review. Systematic review methods used were largely consistent with the recent draft EPA IRIS Handbook {U.S. EPA, 2020, 7006986} and is consistent with current human health risk assessment practices {U.S. EPA, 2002, 88824}. EPA’s draft IRIS Systematic Review Handbook {U.S. EPA, 2020, 7006986} has incorporated feedback from the National Academy of Sciences at workshops held in 2018 and 2019.

2.1.1 *Incorporation of Data from the 2016 Health Effects Support Documents*

For this assessment, EPA built upon the data included and analyses conducted in the 2016 HESD for PFOS {U.S. EPA, 2016, 3603365}. Outlined below are the processes followed for literature identification and inclusion into that assessment.

Relevant literature and data were identified through the following methods:

- Bimonthly literature searches conducted by the EPA library staff (2009–2015),
- Searches conducted by the New Jersey Department of Environmental Protection library staff (2012–2015),
- Recommendation by EPA internal and external peer reviewers, and
- Submission through public comments on the draft assessments.

Literature and other data sources were selected for retrieval, review, and inclusion in the HESD using the following criteria:

- The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
- Elements of the study design merit its inclusion in the draft document based on its contribution to the MOA or the quantification approach.
- The study elucidates the MOA for any toxicity endpoint or toxicokinetic property associated with PFOS exposure.
- The effects observed differ from those in other studies with comparable protocols.
- The relevance of the study to drinking water exposures and to the U.S. population also were considered.

An evaluation of available literature and other data sources was performed by EPA to determine data acceptability. The following study quality considerations from EPA’s *A Review of the*

Reference Dose and Reference Concentration Processes {U.S. EPA, 2002, 88824} were used to select studies for inclusion in the HESD:

- Clearly defines and states hypothesis.
- Adequately describes the study protocol, methods, and statistical analyses.
- Evaluates appropriate endpoints. Toxicity depends on the amount, duration, timing, and pattern of exposure, and could range from frank effects (e.g., mortality) to more subtle biochemical, physiological, pathological, or functional changes in multiple organs and tissues.
- Applies appropriate statistical procedures to determine an effect.
- Establishes dose-response relationship (i.e., NOAEL and/or LOAEL) or data amenable to modeling of the dose response to identify a POD for a change in the effect considered to be adverse (out of the range of normal biological viability). The NOAEL is the highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effects between the appropriate control group and the exposed population. The LOAEL is the lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the appropriate control group and the exposed population.

This information is provided as context because while many of the epidemiological and animal studies from the 2016 document are qualitatively incorporated into this assessment, they have not undergone the systematic review detailed below. Specifically, only the animal studies supporting the candidate RfDs derived in the 2016 HESD were incorporated into the systematic review methods outlined below. All other studies referenced from the 2016 HESD adhered to the criteria described above, but study confidence between studies included in the 2016 HESD and this assessment cannot be compared. Therefore, only the animal studies supporting the candidate RfDs derived in the 2016 HESD were considered quantitatively in this assessment.

2.1.2 *PECO Criteria for the Updated PFOS Health Effects Systematic Review*

Table 2Error! Not a valid bookmark self-reference. describes the PECO inclusion criteria used to screen the literature.

Table 2. Populations, Exposures, Comparator, and Outcome (PECO) Criteria for a Systematic Review on the Health Effects from Exposure to PFOA and PFOS

PECO Element	Inclusion Criteria
Population	<p>Human: Any population and life stage (occupational or general population, including children and other sensitive populations).</p> <p>Animal: Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages).</p> <p>In vitro/cell studies or in silico/modeling toxicity studies should be tagged as supplemental</p>
Exposure	<p>Relevant forms:</p> <p>PFOA (<i>CAS number 335-67-1</i>).</p> <p>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-</p>

PECO Element	Inclusion Criteria
	<p>PFOS (CAS number 1763-23-1). 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</p> <p>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.”</p> <p>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 or developmental studies, should be tagged as supplemental.</p>
Comparator	<p>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.”</p> <p>Animal: A concurrent control group exposed to vehicle-only treatment or untreated control.</p>
Outcome	All health outcomes (both cancer and noncancer).
PBPK Models	Studies describing physiologically-based pharmacokinetic (PBPK) models will be included

2.1.2.1 Human Epidemiological Study Design Considerations

Human epidemiological studies with cross-sectional, cohort, case-control, ecological, or controlled trial study designs were included. The following definitions were used for these studies:

- Cross-sectional: Exposure and outcome are examined at the same point in time in a defined study population. Researchers cannot determine if the exposure came before or after the 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.
- 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. Researchers 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.

2.2 Updated Literature Search Strategy

The updated literature searches targeted literature published since the 2016 HESD and Health Advisory. 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 toxicity, and *in vitro* studies that provide MOA information were included, along with data specifically useful for addressing risks to children and other susceptible populations (e.g., the elderly, pregnant or lactating women, genetically susceptible individuals) were identified. The searches likewise included ADME studies and models useful for dose-response assessment, such as dosimetry models and PBPK models.

Additionally, the literature searches included all literature related to physical and chemical properties, occurrence, and environmental fate of PFOA and PFOS. The literature search strategy included searches in literature databases (e.g., PubMed®, TOXLINE, Web of Science™) as well as relevant domestic and international non-periodical “gray” literature, such as books, 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)). The search strategy included the following secondary sources:

- Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles,
- National Toxicology Program (NTP),
- National Cancer Institute (NCI),
- National Institute for Environmental Health Sciences (NIEHS),
- National Center for Toxicological Research (NCTR),
- Toxic Substance Control Act Test Submissions (TSCATS) database,
- EPA (other appropriate health assessment documents and guidelines),
- Health Canada,
- California EPA (CalEPA), and
- International Agency for Research on Cancer (IARC).

These sources were reviewed for published as well as unpublished or interim research reports relevant to the search topics.

2.2.1 Database Search and Term Development

The current updated literature search focused on studies published since 2013, under the assumption that any critical studies published previously would have been considered in the public comment and external peer review processes used in developing the HESDs. This updated literature search focused only on the chemical name with no limitations on lines of evidence (i.e., human, animal, *in vitro*, *in silico*) or health outcomes. The databases listed below were searched

for literature containing the chemical search terms. Full details of the search strategy for each database are presented in Appendix A.

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- ToxLine (National Library of Medicine; only searched for the 2013-April 2019 literature search)
- TSCATS (only searched for the 2013-April 2019 literature search)

The database searches were conducted by an EPA information specialist on April 11, 2019 and September 3, 2020 and all records were stored in the Health and Environmental Research Online (HERO) database. Since the April 2019 search, Toxline was incorporated into PubMed. Because Toxline was defunct, TSCATS could not be searched after the April 2019 search. This wasn't identified as an issue because prior to being taken down, the most recent TSCATS reference in Toxline was from 2002. After deduplication in HERO, these studies were imported into SWIFT Review software (<https://www.sciome.com/swift-review/>) to identify those references most likely to be applicable to human health and ecotoxicological risk assessment. In brief, SWIFT Review has preset literature search strategies ("filters") developed and applied by information specialists to identify studies more likely to be useful for identifying human health content from those that likely are not (e.g., analytical methods). The filters function like a typical search strategy in which studies are tagged as belonging to a certain filter if the terms in the filter literature search strategy appear in title, abstract, keyword or medical subject headings (MeSH) fields content. The applied SWIFT Review filters focused on lines of evidence: human, 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). Studies not retrieved using these filters were not considered further. Studies that included one or more of the search terms in the title, abstract, keyword, or MeSH fields were exported as a RIS (Research Information System) file for screening in DistillerSR, as described below. Application of the SWIFT evidence stream filters reduced the number of studies for title and abstract screening from:

- 3,382 to 1,976 studies for the April 2019 search
- 1,153 to 868 studies for the September 2020 search

Additionally, in 2020, the National Toxicology Program (NTP) website was searched for PFOA and PFOS toxicity studies with final reports that could provide relevant health effects information. Three reports were identified and included as relevant: 1.) a 28-day PFOS study in rats, 2.) a 28-day PFOA study in rats and 3.) a two-year carcinogenicity study for PFOA in rats. These final reports are included in this literature search because these data have undergone standard NTP quality assurance/control processing, peer review, and are publicly available.

These studies were then imported into DistillerSR (Evidence Partners; <https://www.evidencepartners.com/products/distillersr-systematic-review-software>) and were screened by title and abstract with the goal of identifying health effects studies published since the development of the EPA's 2016 HESDs for PFOA and PFOS, which could influence the derivation of an oral RfD or CSF. Studies not meeting these inclusion criteria, but providing important supporting information, were categorized according to the type of supporting

information they provided. Studies that met the criteria were tagged as having relevant: human data, animal data in a mammalian model, or a PBPK model.

This updated literature search focused only on the chemical name with no limitations on lines of evidence (i.e., human, animal, in vitro, in silico) or health outcomes. The databases listed below were searched for the date range of April 2016 through September 2020. 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). Table 3 shows the search strings used for each of two databases: Web of Science and PubMed.

Table 3. Search String for Web of Science™ and PubMed®

Database	Search String	Results
WoS Batch: 39681	(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="Perfluorooctanoic 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="fluorotelomer*" 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=(2019-2020)	9/3/2020: 1,286 results
PubMed Batch: 39678	(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 "Perfluorooctanoic 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	9/3/2020: 811 results

Database	Search String	Results
	"EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR "perfluorooctane sulfonate"[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR ("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw])) AND (2019/04/01:3000[pdat])	
TOXLINE	TOXLINE taken down, cannot search.	
TSCATS	TOXLINE taken down, cannot search.	
Total number of references from all databases from April 2019 – September 2020		1,153 results
Total number of references after running SWIFT Review		868 results

2.2.2 Other Sources Consulted

The literature search strategies described above are designed to be broad, but like any search strategy, studies can be missed (e.g., cases where the specific chemical is not mentioned in title, abstract, or keyword content; “gray” literature that is not indexed in the databases listed above). Thus, in addition to the database searches, additional sources (described in this section) were used to identify studies that could have been missed based on the database search. Records that appear to meet the PECO criteria were uploaded into DistillerSR, annotated with respect to the source of the record, and screened. Searching of these sources was summarized to include the source type or name, the search string (when applicable), number of results present within the resource, and the URL (uniform resource locator, when available and applicable). The list of other sources consulted includes:

- Manual review of the reference list from final or publicly available final and draft assessments (e.g., ATSDR’s Toxicological Profile for Perfluoroalkyls {ATSDR, 2021, 9642134}; CalEPA’s First Public Review Draft of Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water {CalEPA, 2021, 9416932}) or published journal reviews specifically focused on human health. Reviews were identified from the database search.
- Manual review of the reference lists of studies screened as PECO-relevant after full-text review were reviewed at the title level for potentially relevant studies (backward citation search).
- Searches of the NTP database of study results and research projects (<https://ntp.niehs.nih.gov/data/index.html>).
- Manual review of studies collected by senior EPA staff scientists.

- Manual review of references identified during public comment periods, by technical consultants, and during peer-review.

2.3 Screening Process

The literature was screened at the title and abstract and full-text level by independent reviewers, with a process for conflict resolution using structured forms in DistillerSR. Literature inventories for PECO-relevant studies and studies tagged as “potentially relevant supplemental material” during full-text screening were created to facilitate review of studies by topic-specific experts by identifying evidence type and health effect system, in accordance with protocols used by Integrated Risk Information System (IRIS) risk assessments.

Table 2 **Error! Not a valid bookmark self-reference.** describes the PECO inclusion criteria used to screen the literature.

Table 2 describes the PECO inclusion criteria used to screen the literature. Studies that were not directly relevant to the PECO statement but contained potentially relevant supplementary information were inventoried during the literature screening process. Potentially relevant supplementary materials included:

- Mechanistic data (including in vitro/ex vivo/in silico studies),
- Non-mammalian model systems,
- Transgenic mammalian model systems,
- Non-oral or non-inhalation route of administration,
- ADME and toxicokinetic studies (including the application of PBPK models),
- Exposure characteristics (no health outcome assessment),
- Mixture studies (experimental studies or epidemiological studies that only report associations based on sum or total PFAS),
- 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.

2.4 Study Evaluation

When evaluating individual studies, two primary reviewers independently judged the reliability of the study results and one quality assurance (QA) reviewer (in accordance with IRIS protocol) made a final determination (reflected as study confidence ratings described below in Figure 1) regarding each health outcome or outcome grouping of interest; thus, different judgments were possible for different health outcomes within the same study. The results of these reviews were tracked within EPA’s version of the Health Assessment Workspace Collaborative (HAWC). To develop study quality ratings, each reviewer assigned a rating (listed from best to worst methodological conduct) of good, adequate, deficient (or “not reported,” which carried the same functional interpretation as deficient), or critically deficient. Reviewers also evaluated epidemiological and animal toxicological studies for potential risk of bias (systematic errors or deviations from the truth related to internal validity that affect the magnitude or direction of an

effect in either direction) or 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).

The domains descriptions are specified in Figure 1.

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 1. Study Confidence Determinations

The QA reviewer assessed the initial reviews and confirmed or modified the scores as needed. All reviews were maintained independently in HAWC. Reviewers also assigned an overall study confidence rating once the individual domains were rated (Figure 2). The identified strengths and limitations were considered and documented to reach an overall classification of *high*, *medium*, *low*, or *uninformative* for each PECO-relevant endpoint evaluated in the study.

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 2. Overall Study Quality Classifications

Using the HAWC platform (and conflict resolution by an additional reviewer, as needed), the reviewers reached a judgment regarding each evaluation domain and overall (study confidence) determination. The specific limitations identified during study evaluation were carried forward to

inform the synthesis findings within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform judgments in isolation).

Study quality evaluations for studies identified as key studies since the 2016 HESDs were summarized in HAWC heatmaps using the color scheme shown in Figure 2.

2.4.1 Dose-Response Studies

The evidence synthesis allowed evaluation of the most sensitive cancer and noncancer endpoints. Studies were evaluated for use in POD derivation on the basis of study design, study quality evaluation, and data availability. For human evidence, all study designs were considered; for animal evidence, only animal studies with at least two exposure groups and with *high* or *medium* for study quality were considered.

2.5 Data Extraction

2.5.1 Dose-Response Studies

Data extraction was conducted for most studies that were included in the literature inventory, except those excluded as described below. Extractions were conducted in DistillerSR (epidemiological studies) or HAWC (toxicological studies). Extractions were limited to outcomes of interest and/or the most sensitive LOAEL. An initial reviewer conducted the extraction, followed by a QA reviewer to confirm accuracy or edit the extraction. Discrepancies in data extraction were resolved by discussion or consultation within the evaluation team.

Not all studies that met the PECO criteria went through data extraction: studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction, and outcomes determined to be less relevant during PECO refinement did not go through data extraction. The same was true for *low* confidence studies when *medium* and *high* confidence studies (e.g., on an outcome) were available. All findings were considered for extraction, regardless of the statistical significance of the finding. The level of extraction for specific outcomes within a study could differ (i.e., ranging from a narrative to full extraction of dose-response effect size information).

Briefly, data extracted from epidemiology studies included the population, study design, year of data collection, exposure measurement, and quantitative data from statistical models. Data extracted from statistical models reported in the studies included the health effect category, endpoint measured, sample size, description of effect estimate, covariates, and model comments. All extracted epidemiology data are available via [Tableau Public](#).

Briefly, data extracted from toxicological studies included information on the experiment, animals used in the experiment, the dosing regime, and endpoints measured. All extracted data are available in the [public HAWC](#) site as exposure-response arrays, forest plots, and trees.

2.5.2 ADME Studies

Studies tagged as containing potentially relevant ADME data were screened using an ADME-focused PECO statement (Table 4) and underwent a light extraction of key study information in ICF's litstream™ software. Data were extracted, including general information on the study and separate forms for animal and human information, respectively.

Table 4. Populations, Exposures, Comparators, Outcomes (PECO) Criteria for Absorption, Distribution, Metabolism, and/or Excretion (ADME) Studies

PECO Element	Inclusion Criteria
Population	<p>Human: Any population and life stage (occupational or general population, including children and other sensitive populations): whole organism, tissues, individual cells, or biomolecules.</p> <p>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).</p>
Exposure	<p>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.</p> <p>PFOA (CAS number 335-67-1).</p> <p>Other names: perfluorooctanoate, perfluorooctanoic acid, 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, 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 perfluorooctanoate</p> <p>PFOS (CAS number 1763-23-1).</p> <p>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</p>
Comparator	<p>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.</p>
Outcome	<p>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 pharmacokinetic (PK) models for PFOA and/or PFOS will be included.</p> <p>Information and terms that are typically found in relevant ADME/PK modeling studies include the following:</p> <p>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.</p> <p>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).</p> <p>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.</p> <p>Metabolism: Metabolic/biotransformation pathway(s); enzymes involved; metabolic rate; V_{max}, K_m; ; metabolic induction; metabolic inhibition, K_i; 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.</p>

PECO Element	Inclusion Criteria
	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).

2.5.3 Mechanistic Studies

Studies that were tagged as containing potentially relevant mechanistic data were screened using a mechanistic-focused PECO statement (Table 5) and underwent a light extraction of key study information in ICF's litstream™ software. Data were extracted, including general information about the study and separate forms for animal, human, and mammalian cell information, respectively.

Table 5. Populations, Exposures, Comparators, Outcomes (PECO) Criteria for Mechanistic Studies

PECO Element	Evidence
Population	<p>Human: Any population and life stage (occupational or general population, including children and other sensitive populations).</p> <p>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).</p> <p>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.</p>
Exposure	<p>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.</p> <p>PFOA (CAS number 335-67-1). Other names: perfluorooctanoate, perfluorooctanoic acid, 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, 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 perfluorooctanoate</p> <p>PFOS (CAS number 1763-23-1). 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</p>
Comparator	<p>Human: Comparison to group with no exposure or lower exposure.</p> <p>Animal, ex vivo, in vitro, in silico: Comparison to an appropriate vehicle or no treatment control.</p>
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.

2.6 Evidence Synthesis

For each health effect assessed, evidence that directly informed the integrated assessment was synthesized to draw an overall judgment for each health effect. The available human and animal evidence pertaining to potential health effects was synthesized separately. Each synthesis provided a summary discussion that addressed considerations regarding causation as adapted from Hill {Hill, 1965, 71664}. Mechanistic evidence was also synthesized, as necessary, to help inform key decisions regarding the human and animal evidence.

The syntheses of human and animal health effects evidence focused on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies. Evidence synthesis was based primarily on studies of *high* and *medium* confidence. *Low* confidence studies were used if few or no studies with higher confidence were available to help evaluate consistency, or if the study designs of the *low* confidence studies addressed notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect. If *low* confidence studies were used, a careful examination of the potential study quality effects on the evidence synthesis conclusions was included in the narrative.

The animal and human, evidence summaries were combined to draw an overall judgment that incorporates inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed.

3.0 Hazard Identification

3.1 Literature Search Results

Studies referenced in the white paper are cited as “Author Last Name, Publication Year, HERO ID.” The HERO ID is a unique identifier for studies available in the EPA Health and Environmental Research Online: A Database of Scientific Studies and References. Additional study meta-data are publicly available by searching for the HERO ID on the public facing webpage available here: <https://hero.epa.gov/>.

3.1.1 General Results

The database searches yielded 2,868 unique records, with 24 records identified from additional sources, such as manual reviews from ATSDR, CalEPA, and NTP (Section 2.2.2). Of the 2,868 identified, 1,567 were excluded during title and abstract screening, and 658 were reviewed at the full-text level. Of the 658 screened at the full-text level, 378 were considered to meet PECO eligibility criteria (Table 2) and included information on PFOS. The studies meeting PECO criteria at the full-text level included 338 epidemiological (human) studies, 29 animal studies, and 37 PBPK studies. Additional details of the literature search and screening process are shown in Figure 3.

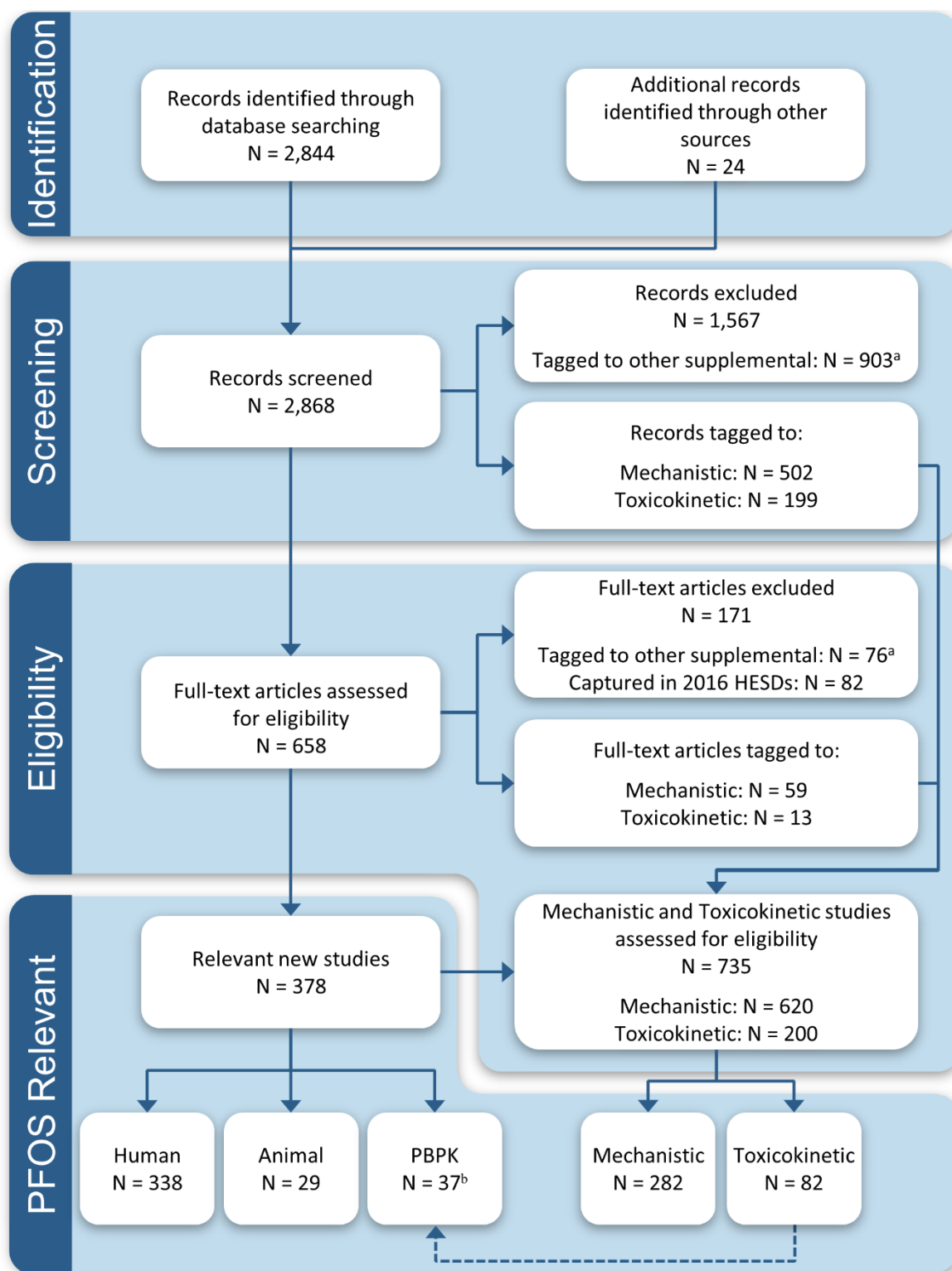


Figure 3. Summary of Literature Search and Screening Process for PFOS

^aNumber does not include studies that were also tagged to mechanistic and/or toxicokinetic.

^bNumber includes those studies identified during initial review as well as those identified during screening of toxicokinetic studies.

3.1.2 Literature Inventory for Epidemiology Studies of PFOS

Of the 337 epidemiological studies that met the inclusion criteria shown in Figure 4, most studies had a cohort ($n = 145$) study design. Of the remaining studies, 133 had a cross-sectional design, 34 had a case-control design, and 25 had other study designs (i.e., nested case-control). Epidemiological studies were categorized into 18 health systems. Most studies reported on the metabolic ($n = 69$), developmental ($n = 63$), cardiovascular ($n = 56$), or female reproductive systems ($n = 51$). Additional study details are shown in Figure 4.

Health System	Study Design				Grand Total
	Case-control	Cohort	Cross-sectional	Other	
Cancer	3	1	2	5	11
Cardiovascular	3	11	37	5	56
Dermal	0	1	0	0	1
Developmental	5	40	15	3	63
Endocrine	1	7	19	7	34
Gastrointestinal	1	4	0	0	5
Hematologic	0	0	8	0	8
Hepatic	1	3	13	2	19
Immune	5	23	12	3	43
Metabolic	7	31	27	4	69
Musculoskeletal	0	0	6	2	8
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	1	3	15	0	19
Reproductive, Male	0	7	14	2	23
Reproductive, Female	10	21	18	2	51
Respiratory	1	3	1	0	5
Other	0	2	3	0	5
Grand Total	34	145	133	25	337

Figure 4. Summary of Epidemiology Studies of PFOS^a

Interactive figure and additional study details available on [Tableau](#).

^aAttanasio, 2019, 5918605 is not included in the study counts as it is a data brief for the original Attanasio, 2019, 5412069 study.

3.1.3 Literature Inventory for Toxicology Studies of PFOS

Of the 35 animal studies that met the inclusion criteria shown in Figure 5, most studies had either short-term ($n = 15$) or developmental ($n = 12$) study designs. Of the animal studies, most reported on rats ($n = 19$). The rat studies included short-term ($n = 10$), developmental ($n = 5$), chronic ($n = 2$), and reproductive ($n = 2$) study designs. The remaining studies reported on mice ($n = 16$) using subchronic, short-term, developmental, or reproductive study designs, monkeys ($n = 1$) using a chronic study design, or rabbits ($n = 1$) using a developmental study design. Animal studies were categorized into 13 health systems. Most studies reported on the reproductive ($n = 18$), whole body ($n = 18$), hepatic ($n = 15$), or developmental ($n = 12$) systems. Additional study details are shown in Figure 5.

Health System	Study Design & Species										Grand Total
	Subchronic	Short-term		Chronic		Developmental			Reproductive		
	Mouse	Mouse	Rat	Monkey	Rat	Mouse	Rabbit	Rat	Mouse	Rat	
Cancer	0	0	0	0	1	0	0	0	0	0	1
Cardiovascular	2	1	1	1	1	0	0	0	0	1	7
Developmental	0	0	0	0	0	6	1	4	0	2	12
Endocrine	0	0	3	1	0	1	0	3	0	1	8
Hematologic	0	0	2	1	0	0	0	0	0	0	3
Hepatic	3	2	5	1	2	2	0	1	0	1	15
Immune	1	1	2	1	1	1	0	0	0	0	7
Metabolic	1	0	2	0	1	0	0	0	0	1	4
Nervous	0	1	5	0	0	1	0	2	0	1	10
Renal	1	0	2	1	1	1	0	0	0	0	5
Reproductive	2	1	3	1	0	4	1	3	1	2	18
Respiratory	0	0	1	0	0	0	0	0	0	0	1
Whole Body	3	3	6	1	1	1	1	1	0	2	18
Grand Total	4	5	10	1	2	6	1	5	1	2	35

Figure 5. Summary of Toxicology Studies of PFOS^a

Interactive figure and additional study details available on [Tableau](#).

^aStudy counts include key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}.

3.2 Toxicokinetics

3.2.1 ADME

Due to strong carbon-fluorine bonds, PFOS is stable to metabolic and environmental degradation. It is not readily eliminated and can have a long half-life in humans and animals. However, the toxicokinetic profile and the underlying mechanism for the chemical's long half-life are not completely understood. In the case of another perfluorinated compound, PFOA, membrane transporter families appear to play an important role in ADME. The transporter families identified for PFOA include organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), multidrug resistance-associated proteins (MRPs), and urate transporters. Transporters play a critical role in GI absorption, uptake by the tissues, and excretion via bile and the kidney. Limited data are available regarding the transporters for PFOS, however, the toxicokinetic properties of PFOS suggest tissue uptake and renal resorption through facilitated uptake. Some inhibition studies suggest that PFOS, with its similar chain length, renal excretion properties, and liver accumulation, could involve the same transporters; however, transporter-specific data related to PFOS are minimal.

Animal studies indicate that PFOS is well-absorbed orally and distributes to many tissues and organs. High levels of PFOS are consistently observed in blood and liver. While PFOS can form as a metabolite from other perfluorinated compounds, PFOS itself does not undergo further metabolism after absorption takes place. PFAS are known to activate peroxisome proliferator activated receptor (PPAR) pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, as well as sterol and bile acid biosynthesis. Based on transcriptional activation of many genes in PPAR α -null mice, other gene products likely modify toxicokinetics of PFOS {Andersen, 2008, 3749214}.

3.2.1.1 Absorption

Absorption data are available in laboratory animals for oral {Chang, 2012, 1289832} and inhalation {Rusch, 1979, 7561179} exposures, and extensive data are available from humans demonstrating the presence of PFOS in serum. Limited in vitro absorption data are also

available. Detailed study descriptions of literature informing absorption of PFOS in humans and animals are provided in Section D.1.

Since PFOS is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across interface membranes was thought to be dominated by transporters or mechanisms other than simple diffusion across the lipid bilayer. Recent mechanistic studies, however, support transporter-independent uptake through passive diffusion processes. Ebert and colleagues {Ebert, 2020, 6505873} determined membrane/water partition coefficients ($K_{\text{mem/w}}$) for PFOS and examined possible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. In this system, the partition coefficients were considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes.

Uptake by cells may be influenced by interactions with lipids and serum proteins. PFOA exhibited lower levels of binding to lipids and phospholipids relative to PFOS, which correlated with uptake into lung epithelial cells {Sanchez Garcia, 2018, 4234856}. Phospholipophilicity correlated 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.

While there are no studies available that quantify absorption in humans, extensive data on serum PFOS demonstrate uptake from the environment but do not establish exposure route. Studies that provide the basis for human half-life estimates rely on changes in PFOS serum levels over time.

Bioavailability of PFOS after oral exposure is very high in rats. Comparison of serum concentrations after oral dosing were $> 100\%$ of levels measured after intravenous (IV) dosing, which may reflect enterohepatic absorption that occurs after gavage but not IV administration {Kim, 2016, 3749289; Huang, 2019, 5387170}.

3.2.1.2 Distribution

3.2.1.2.1 PFOS Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature informing distribution of PFOS in humans and animals are provided in Section D.2. Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. Distribution of PFAS to plasma is chain-length dependent {Jin 2016, 3859825}. Increasing chain length correlated with an increased mass fraction in human plasma from C6 to C11. Within human blood fractions, PFOS accumulates to the highest levels in plasma, followed by whole blood and serum {Forsthuber, 2020, 6311640; Jin, 2016, 3859825; Poothong 2017, 4239163}. Poothong et al. (2017, 4239163) found median PFOS concentrations in plasma, serum, and whole blood were 5.24, 4.77 and 2.85 ng/mL, respectively. These findings suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum will not provide accurate estimates for PFOS.

PFOS is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOS interactions with human serum albumin (HSA) {Zhang, 2009, 2919350; Salvalaglio et al., 2010, 2919252; Chen and Guo, 2009, 1280480; D'Alessandro, 2013, 5084740; Liu, 2017, 3856708}. *In vitro* analyses {Kerstner-Wood, 2003, 4771364} found that plasma proteins can bind PFOS in plasma from humans, cynomolgus monkeys, and rats. 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%). HSA-PFOS intermolecular interactions are mediated through van der Waals forces and hydrogen bonds {Zhang, 2009, 2919350; Chen and Guo, 2009, 1280480}. Beesoon and Martin (2015, 2850292) determined that linear PFOS bound more strongly to calf serum albumin than the branched chain isomers in the order of 3m < 4m < 1m < 5m < 6m (iso) < linear.

PFOS binding to HSA results in alterations in the albumin secondary structure and can diminish esterase activity {Liu, 2017, 3856708}, though the extent to which this influences the physiological functions of albumin is unknown. PFOS-mediated conformational changes may also interfere with albumin's ability to transport its natural ligands and drugs including vitamin B₂ (riboflavin) and ibuprofen, {D'Alessandro, 2013, 5084740}, and may interfere with PFOS uptake into cells {Sheng, 2020, 6565171}.

Binding to albumin and other serum proteins may affect transfer of PFOS from maternal blood to the fetus {Gao et al., 2019, 5387135}. Since there is effectively a competition between PFOS binding in maternal serum versus 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 whereas maternal serum albumin concentration was associated with reduced transfer efficiency.

3.2.1.2.2 PFOS Binding to Intracellular Proteins and Transporters

Within cells, PFOS has been shown to bind to liver fatty acid binding protein (L-FABP) {Luebker, 2002, 1291067; Zhang, 2013, 5081488; Yang, 2020, 6356370}. 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.

PFOS entry from serum into tissues appears to be controlled by several families of membrane transporters based on extrapolation from PFOA studies and several PFOS-specific studies. Yu et al. (2011, 1294541) observed that PFOS exposure in rats increased hepatic OATP2 and MRP2 messenger RNA (mRNA) expression. Transporters responsible for PFOS transport across the placenta are not well understood though preliminary studies examining transporter expression identified OAT4 as a candidate receptor {Kummu, 2015, 3789332}. Thus far, no functional studies demonstrating a role for these transporters in PFOS uptake in liver or placenta have been identified.

3.2.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOS distributes to a wide range of tissues, organs, and matrices throughout the body. Blood and liver are major sites of PFOS accumulation {Olsen, 2001, 9641811}. Two studies measured PFOS levels 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 in the serum, suggesting PFOS does not easily cross the adult human blood-brain barrier in humans.

In a study of autopsy tissues collected in the first 24 hours after death, Pérez et al. (2013, 2325349) measured PFOS levels in tissue samples (liver, kidney, brain, lung, and bone) and

found PFOS primarily in the liver (104 ng/g), kidney (75.6 ng/g), 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, 2020, 6356899}, raising the possibility of reproductive toxicity in humans.

Studies of tissue distribution are available for several species of animals including non-human primates, rats, and mice. Studies of non-human primates indicate that levels of PFOS in serum accumulate in a dose-dependent manner {Seacat, 2002, 757853; Chang, 2017, 3981378}. While data are limited on liver accumulation of PFOS in monkeys, PFOS levels in the liver were similar or slightly lower than serum levels.

Several rodent studies identified high levels of PFOS in blood and liver across a range of dosing regimens and study durations. While monkeys had nearly a 1:1 liver to serum ratio, rodent models were observed to accumulate far more PFOS in liver than serum {NTP, 2019, 5400978}. For PFOA, striking sex-differences in rodents were observed with much higher levels observed in males compared to females. In contrast, plasma PFOS concentrations were generally similar in males and females. For example, in a 28-day toxicity study, dose-normalized plasma concentrations ($\mu\text{M}/\text{mmol}/\text{kg}/\text{day}$) in males and females were within 1.5-fold across the dose groups {NTP, 2019, 5400978}. Additional studies in rats and mice documented PFOS distribution to a wide range of tissues including kidney, heart, lungs, and spleen. Interestingly, in rodents, PFOS has been measured in moderate quantities in the brain and testicles, indicating that PFOS does cross the blood-brain barrier and blood-testis barrier in rats {Qui, 2013, 285095} and mice {Bogdanska, 2011, 2919253} {Cui, 2009, 757868}.

3.2.1.2.4 Distribution During Reproduction and Development

Several studies in humans, rats, and mice quantified distribution of PFOS from pregnant females to placenta, cord blood, and amniotic fluid, which demonstrate pathways of distribution to and elimination from fetuses. Accumulation of PFOS in fetal tissues was found to vary by gestational age. New studies also confirm that distribution of PFOS from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding. Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching.

The ratio of PFOS in placenta relative to maternal serum (R_{PM}) ranged between 0.048 to 0.749 {Zhang, 2013, 3859792; Chen, 2017, 3859806}. T. Zhang et al. (2015, 2851103) observed differential accumulation of PFOS by branching characteristics. Specifically, R_{PMs} of branched PFOS isomers increased as the branching points away from the sulfonate group in the order of iso-PFOS < 4m-PFOS < 3+5m-PFOS < 1m-PFOS. Mamsen et al. (2019, 5080595) demonstrated that gestational age can affect PFOS concentrations in maternal serum and placentas, estimating a placenta PFOS accumulation rate of 0.13% increase per day during gestation.

Several studies reported a strong positive correlation between maternal and cord serum levels of PFOS {Kato, 2014, 2851230; Porpora, 2013, 2150057}. The ratio of PFOS in cord serum relative to maternal serum ranged from 0.22 to 0.98 (Table D-8) and generally increased with gestational age {Li, 2020, 6505874}. Li et al. (2020, 6505874) also showed a 6% increase in branched PFOS accumulation compared to linear PFOS isomers. Zhao et al. (2017, 3856461) observed higher transplacental transfer efficiencies (TTEs) for 1m-, 4m-, 3+5m-, and m2-PFOS compared to n-PFOS. Together, these findings indicate that branched isomers of PFOS transfer more efficiently from maternal blood to cord blood compared to linear isomers. In addition to

PFOS branching, maternal factors including exposure sources, parity, and other maternal demographics are postulated to influence observed variations in cord:maternal serum ratios {Eryasa, 2019, 5412430; Jusko, 2016, 3981718; Brochot, 2019, 5381552}.

Lower PFOS concentrations were measured in amniotic fluid compared to placenta and cord blood {Zhang, 2013, 3859792}. The mean concentration ratio between amniotic fluid and maternal blood (AF:MB) was lower for PFOS (0.0014) than for PFOA (0.13). The mean concentration ratio between amniotic fluid and cord blood (AF:CB) was lower for PFOS (0.0065) than for PFOA (0.023). Authors attributed the differences in ratios between the two compartments to the solubility of PFOS and PFOA and their respective protein binding capacities in the two matrices.

PFOS also distributes widely 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. The concentration of PFOS decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. In a second study, PFAS levels were measured in embryos and fetuses at gestational weeks 7–42 and in serum from their matched maternal pairs {Mamsen, 2019, 5080595}. PFOS accumulated at higher levels in fetal tissues compared to other PFAS chemicals examined in fetal tissues and across trimesters. 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.

New studies also confirm that distribution of PFOS from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding {Mondal, 2014, 2850916; Cariou, 2015, 3859840; Mogensen, 2015, 3859839; Gyllenhammar, 2018, 4778766}. Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching. In the Mondal study {Mondal, 2014, 2850916}, the mean maternal serum PFOS concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOS than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding {Mondal, 2014, 2850916; Mogensen, 2015, 3859839}. 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 . The authors measured four PFAS chemicals in maternal serum and breastmilk and observed that PFOS was the main contributor in maternal serum, while PFOA and PFOS were found to be the main contributors in breastmilk.

Developmental studies in rodents confirmed distribution from rat and mouse dams to fetuses and pups, as well as variable PFOS level across many fetal tissues {Luebker, 2005, 1276160; Chang, 2009, 757876; Ishida, 2017, 3981472; Zeng, 2011, 1326732; Chen, 2012, 1276152; Borg, 2010, 2919287; Liu, 2009, 757877}.

3.2.1.2.5 Volume of Distribution in Humans and Animals

In humans, a single value of 239 mL/kg has been uniformly applied for most PFOS studies {Thompson, 2010, 2919278}. Gomis et al. (2017, 3981280) used a volume of distribution (V_d) of 235 mL/kg by averaging V_d values estimated for both humans and animals. V_d values may be

influenced by differences in distribution between males and females, between pregnant and non-pregnant females, and across serum, plasma, and whole blood fractions.

V_d estimates derived in monkeys, mice, and rats vary by species, age, sex, and dosing regimen. For example, Huang et al. (2019, 5387170) calculated the apparent volume of central and peripheral distribution in rats. 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. V_d values in females after IV administration were lower than that observed in males in both the central and peripheral compartments. For the oral route, striking sex differences were noted between the central and peripheral compartments. While V_d values were quite similar in males for both compartments, they were notably higher in the central compartment compared to the peripheral compartment in females. Interestingly, another study found that for PFOS, a classical compartment model was not applicable. Rather, the body organs behaved as an assortment of independent one-compartment with a longer elimination half-life in liver than serum in the elimination phase (Iwabuchi, 2017, 3859701).

3.2.1.3 Metabolism

Consistent with other reports and reviews {U.S. EPA, 2016, 3603279; ATSDR, 2018, 9642134; Pizzuro, 2019, 5387175}, there is no evidence that PFOS is metabolized in humans, primates, and rodents.

3.2.1.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Most studies have investigated the elimination of PFOS in humans, cynomolgus monkeys, and rats. Available evidence supports urine as the primary route of excretion in most species, though fecal elimination is prominent in rats. In rats, hair is another route of elimination in both males and females. In females, elimination pathways include menstruation, pregnancy (cord blood, placenta, amniotic fluid, and fetal tissues) and lactation (breast milk). Detailed study descriptions of literature informing excretion of PFOS in humans and animals are provided in Section D.4.

3.2.1.4.1 Urinary and Fecal Excretion

Urinary excretion is considered the main route of PFOS excretion in humans. Zhang et al. (2015, 2851103) estimated daily urinary excretion rate of 16% of the estimated total daily intake for PFOS for adults. Zhang et al. (2013, 3859849) 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. In a later study, Fu et al. (2016, 3859819) estimated a urinary clearance rate 0.010 mL/kg/day (geometric mean for men and women). These studies showed that PFOS daily renal clearance values were significantly lower in males compared to females.

Several studies in rats suggest that the fecal route is as or more important than the urinary route of excretion for PFOS. In a study by Chang et al. (2012, 1289832), excretion in urine and feces were approximately equivalent when examined 24 and 48 hours after oral gavage administration of ^{14}C -PFOS. A study 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 IV administration (Kim et al., 2016, 3749289) and examined excretion

over 70 days. PFOS levels in urine and feces were similar in both males and females, which correlated 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 IV routes, respectively).

Another study compared concentrations in urine and feces of male and female Wistar rats {Gao, 2015, 2851191}. A mixture of PFOA/perfluorononanoic acid (PFNA)/PFOS were administered to rats by drinking water for 90 days. Fecal concentrations in were consistently about 5 times greater than in the urine. It is unclear whether the higher levels of PFOS in feces reflected rat strain, differences in dose amount, route, or frequency among the various studies or is driven by differential excretion pathways in rats exposed to a mixture of PFAS.

In summary, evidence supports excretion through the fecal route in both animals and humans. Human studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. In rats, however, both urinary and fecal routes play prominent roles in PFOS elimination. 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 IV dosing. Finally, exposures to mixtures of PFAS 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.

3.2.1.4.2 Renal and 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 ¹⁴C elimination in feces by 9.5-fold for male CD rats administered 3.4 mg/kg ¹⁴C-PFOS. CSM is a bile acid sequestrant, and its facilitation of PFOS gastrointestinal clearance suggests enterohepatic circulation.

Several studies present evidence of enterohepatic excretion and potential resorption in humans {Genuis, 2010, 2583643; Harada, 2007, 2919450}. Harada and colleagues estimated a biliary resorption rate of 0.97, which could contribute to the long half-life in humans. Genuis et al. (2010, 2583643) described a case report of excretion analyzed after inhalation PFOS exposure. After treatment with a bile acid sequestrant CSM for 1 week, PFOS serum levels decreased from 23 ng/g to 14.4 ng/g. Additionally, stool PFOS concentrations increased from undetectable before treatment (LOD = 0.5 ng/g) to 9.06 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 via bile.

Zhao and colleagues (2015, 3856550; 2017, 3856461) evaluated enterohepatic transporters identified in liver hepatocytes and intestinal enterocytes in humans and rats. Using in vitro transfection assays, PFOS was found to be a substrate of both sodium-dependent and -independent enterohepatic transporters involved in recirculation of bile acids. With the exception of rat apical sodium-dependent bile salt transporter (ASBT), PFOS was demonstrated to be a substrate for all tested transporters (sodium/taurocholate cotransporting polypeptide (NTCP), OATP1B1, OATP1B3, OATP2B1) as well as organic solute and steroid transporter alpha/beta. Binding efficiency to the enterohepatic transporters was chain-length dependent. NTCP transported PFAS with decreasing affinity but increasing capacity as the chain length increased {Zhao, 2015, 3856550}. The opposite trend was seen for OATP-mediated uptake {Zhao, 2017a, 3856461}. 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.

3.2.1.4.3 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 3.2.1.4.4, females may eliminate PFOS through routes not available to males. The total daily elimination of PFOS in pregnant females was estimated to be 30.1 ng/day, higher than the 11.4 ng/day for PFOA (Zhang and Qin, 2014, 2850251). The ratio of branched:total PFOS isomers in cord blood was 0.27 and was higher in cord blood compared to maternal blood and placenta. These findings suggest branched PFOS isomers may transfer to the fetus more readily than linear forms. In another study in humans (Zhang, 2013, 3859792), the mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.1%, respectively, of levels found in the mother's blood, demonstrating that cord blood, placenta and amniotic fluid are additional routes of elimination in pregnant females. Blood loss during childbirth could be another source of excretion. 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 lower 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).

Mamsen and colleagues (2017, 3858487) measured placental samples and fetal organs in relation to maternal plasma levels of 5 PFAS in 39 Danish women (Mamsen, 2017, 3858487). 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 the 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.

After birth, women can also eliminate PFOS via lactation (Tao, 2008, 1290895; Lee, 2017, 3983576; Thomsen, 2010, 2186079) and it was shown that PFOS levels in breastmilk are affected by parity (Lee, 2017, 3983576; Jusko, 2016, 3981718). In one study, mean PFOS concentrations were 3.67, 1.38 and 0.040 ng/mL in maternal serum, cord serum, and breast milk, respectively (Cariou, 2015, 3859840). 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, essentially the same as 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.

3.2.1.4.4 Other Routes of Elimination

Menstruation may be an important factor in the sex-specific differences observed in PFOS elimination. Wong et al. (2014, 2851239) estimated that menstrual serum loss is 432 mL/year, which could account for > 30% of the difference in the elimination half-life between females and males.

Two 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 these 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) suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may 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.

Gao et al. (2015, 2850134) found that hair is potential route of PFAS elimination in rats. A dose-dependent increase in hair PFOS concentration was observed in all exposed animals. PFOS did not exhibit the sexual dimorphic pattern in hair noted for PFOA. While hair PFOS levels were lower in males compared to females in the low dose group, there were no significant differences in hair PFOS concentrations between males and females in the higher dose groups.

3.2.1.4.5 Half-Life Data

There have been several studies of half-lives in humans all supporting a long residence time for serum PFOS with estimates measured in years rather than months or weeks (Section D.4.5). Because there is no evidence that PFOS is metabolized in mammals, half-life determinations are governed by excretion. The calculation of PFOS half-lives reported in the literature vary considerably, posing challenges in predicting both the routes and rates of excretion. Half-life estimates vary considerably by species, being most rapid in rodents (measured in hours to days), followed by primates (measured in days to weeks) and humans (measured in years). Half-life estimates were shorter in human females relative to males, but sex differences were less clear in animal studies.

Human PFOS half-life estimates range from less than 1 year in a single male child of 16 years {Genuis, 2014, 2851045} to up to 60.9 years for males occupationally exposed in a plant in China {Fu, 2016, 3859819} (Table D-25). With one exception {Genuis, 2014, 2851045}, half-lives estimated for males are longer than those estimated for females and show an age-related increase {Zhang, 2013, 3859849}. Also, linear isoforms exhibit longer half-lives than branched isoforms {Zhang, 2013, 3859849; Xu, 2020, 6781357}. While most studies were conducted in adults and/or adolescents, at least one study estimated a PFOS half-life of 4.1 years in newborns {Spliethoff, 2008, 2919368}.

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 D-24). PFOS half-life values among these 4 studies varied dramatically 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 much longer half-lives compared to females which may, at least in part, relate to menstruation as an important route of elimination. Second, Xu et al. (2020, 6781357) suggest that linear PFOS molecules exhibit longer half-lives than branched forms, which may reflect differential affinities of linear versus branched forms for resorption transporters. Third, the relationships between blood and urine concentrations are not obvious,

underscoring the role of non-urinary routes of excretion and 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.

In animals, half-life values are reported in days rather than in years. Values in cynomolgus monkeys ranged from 88 to 200 days {Chang, 2012, 1289832; Seacat, 2002, 757853} and were generally longer than those observed in rodents, but much shorter than values observed in humans. Depending on the experimental conditions, half-lives in rats ranged from 14.5 to 43 days {Chang, 2012, 1289832; Huang, 2019, 5387170; Kim, 2016, 3749289}. In contrast to sex-specific differences in half-lives for PFOA, PFOS half-lives showed only minor differences between males and females.

3.2.2 *Pharmacokinetic Models*

Pharmacokinetic (PK) models are tools for quantifying the relationship between external measures of exposure and internal measures of dose. For this assessment, PK models were evaluated for their ability to allow for cross-species PK extrapolation of animal studies of both cancer and noncancer effects and to allow for the estimation of the external dose associated with an internal dose metric that represents the POD calculated from animal toxicological or epidemiological studies. The following sections first describe and evaluate previous and current PK modeling efforts and then present conclusions as to the utility of the model to predict internal doses for use in dose-response assessment.

Numerous PK models for PFOS have been developed over the years to characterize the unique ADME described in Section 3.2.1. These approaches can be classified into three categories: classical compartmental models, modified compartmental models, and PBPK models. With classical compartmental modeling, the body is defined as either a one or two compartment system with volumes and intercompartmental transfer explicitly fit to the PFAS PK dataset. Modified compartmental models are more physiologically based in that they attempt to characterize unique aspects of in vivo ADME through protein binding, cardiac output, and known renal elimination. However, these models still rely on explicit fitting of data to the non-physiological parameters. Finally, PBPK models describe the tissues and organs of the body as discrete, physiologically based, compartments with transport between compartments informed by physiologically relevant quantifications of blood flow and tissue perfusion. Determining additional, non-physiological, parameters typically requires explicitly fitting the PBPK model to time-course concentration data. However, the number of estimated parameters through data fitting are generally fewer than for classical PK or modified compartmental models. Below, we review the availability of each type of PK model for predicting PFOS ADME.

3.2.2.1 *Classical Compartmental Analysis*

The most common approach for the prediction of serum levels of PFOS is to apply a relatively simple one-compartment model. This type of model describes the toxicokinetics of the substance with a single differential equation that describes the rate of change in the amount or concentration of the substance over time as a function of the exposure rate and the clearance rate. This type of model describes the relationship between exposure, serum concentration, and

clearance and can be used to predict one of these values when the other two values are set. Additionally, because the model can produce predictions of changes in exposure and serum concentration over time, these models can be applied to fill the temporal gaps around, or between measured serum concentrations or exposures.

Some examples of one-compartment models used to predict exposure from serum concentrations include the work of Dassuncao et al. (2018, 4563862) who used a model to describe historical changes in exposure in seafood and consumer products, Hu et al. (2019, 5381562) who used paired tap water and serum concentration to estimate the proportion of total exposure that originates from drinking water, and Balk et al. (2019, 5918617) who used measured concentration in drinking water, dust and air samples, and serum concentrations at several time points in developing children to assess the relative proportion of exposure that originates from dietary exposure. Zhang et al. (2019, 5080526) performed a similar study using community tap water measurements and serum concentrations to estimate the proportion of PFOS exposure that originates from drinking water.

Other applications are used to better understand the toxicokinetics of PFOS by combining estimated exposure values and serum values to estimate clearance and half-life in a population of interest. One example was presented by Worley et al. (2017, 3859800) who estimated the half-life of PFOS using exposure predicted from drinking water PFAS concentration in a community with contaminated drinking water. Fu et al. (2016, 3859819) used paired serum and urine samples from an occupational cohort to estimate the half-life separately from renal clearance (in urine) and in the whole body (in serum). One of the largest challenges in the estimation of half-life is the problem of estimating exposure to PFOS.

One common modification of the one-compartment model is to perform a “steady-state approximation,” that is to assume that the rate of change of the serum concentration is zero. This scenario occurs when an individual experiences constant exposure, constant body habitus, and constant clearance over a timespan of several half-lives. Due to the long half-life of PFOS, steady state is a reasonable assumption for adults starting from the age of 25 and above. However, the steady state approximation cannot be applied for ages younger than 21 years of age (EPA’s defines childhood as <21 years of age; US EPA, 2021; available online at: <https://www.epa.gov/children/epas-policy-childrens-health>) due to ongoing development during childhood and adolescence. This development, including growth, effectively decreases chemical concentrations in the body and results in lower levels than would be seen in adults of similar body size. Even though development, including growth, has usually completed prior to the age of 25, it varies across individuals and there is a period after growth ceases where PFOS levels increase until the adult steady-state level is reached. The general acceptability of the steady-state assumption in adults has the caveat that pregnancy or breastfeeding will result in changes in serum concentration and will not be accounted for in the steady-state approximation.

When adopting a steady-state assumption, the rate of change in serum levels over time is zero. It follows that the ratio between exposure to the substance and clearance determines the serum concentration. This is the approach used in the 2016 HESD to determine the constant exposure associated with a serum concentration {U.S. EPA, 2016, 3603365}. A similar approach was used in the recent risk assessment performed by CalEPA {CalEPA, 2021, 9416932}. Publications reporting applications of similar models include the work of Zhang et al. (2015, 2851103) who used paired urine and serum data to estimate the total intake of PFOS and compare that to the

rate of urinary elimination, and Lorber et al. (2015, 2851157) who examined the effects of regular blood loss due to phlebotomy on PFOS levels and extrapolated that finding to clearance via menstruation.

In animals, two classical PK models for PFOS have been published since the original 2016 HESD for PFOS. In Huang et al. (2019, 5387170), male and female Sprague-Dawley rats were dosed via oral gavage at 2 or 20 mg/kg, through multiple administrations of PFOS at 2 mg/kg/day for five days, or intravenously at 2 mg/kg. Following the administration of PFOS, rats were sacrificed from five minutes up to 140 days post-dosing to characterize the biphasic PK curve. Using plasma data from these exposure scenarios, Huang and coworkers developed a two-compartment model to characterize PK parameters of interest such as the alpha- and beta-phase half-life, central and peripheral compartment volumes, and total PFOS clearance. For each dosing scenario, a single set of PK parameters were fit, making extrapolation to other dosing scenarios difficult. However, the authors demonstrate no significant difference between males and females in beta-phase half-life and overall clearance which is in agreement with previous studies of PFOS PK in rat {Kim, 2016, 3749289}.

Gomis et al. (2017, 3981280) utilized the functional form of a two-compartment model with oral gavage to predict internal dosimetry of PFOS in rats using PK data from Seacat et al. (2003, 1290852). However, because the scope of the Gomis et al. (2017, 3981280) study involved predicting internal dose points-of-departure, PK parameters are not presented.

3.2.2.2 Modified Compartmental Models

In addition to the common one-compartment models above, several models have been developed to extend the simple one-compartment model to describe the PK during pregnancy and lactation. The key factors that must be introduced into the model are the changes in body habitus that occur during pregnancy (increases in blood plasma volume and body weight), the distribution and transfer of the substance between the maternal and fetal tissues, the transfer from the mother to the infant during nursing, and the postnatal development, including growth of the infant during the early period of life. The mathematical formulation of this type of model requires two differential equations, one describing the rate of change in amount or concentration in the mother and one describing the rate in infants. One such developmental model with a lactational component was used to predict the maternal serum concentrations and exposure from measurements of PFOS concentrations in breast milk {Abdallah, 2020, 6316215}. Verner et al. (2016, 3299692) presented another developmental model to predict the PFOS serum concentrations in the mother and child and previous exposure using mother/child paired serum measurements at different times. This model included all the key aspects previously mentioned for developmental PK models. Another unique approach that extended the one-compartment framework was a publication by Shan et al. (2016, 3360127), who estimated the exposure to specific isomers of PFOS using measurements in food, tap water, and dust to estimate the isomeric profiles of the substances in human serum.

Toxicokinetic models that can accommodate half-life values that are longer than would be predicted based on standard ADME concepts have been published as tools to estimate internal doses for humans, monkeys, mice, and rats {Andersen, 2006, 818501; Wambaugh, 2013, 2850932; Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665; Chou, 2019, 5412429}. The underlying assumption for all of the

models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

One of the earliest PK models {Andersen, 2006, 818501} was developed for PFOS using two dosing situations in cynomolgus monkeys. In the first, three male and three female monkeys received a single IV dose of potassium PFOS at 2 mg/kg {Noker and Gorman, 2003, 9642133}. For oral dosing, groups of four to six male and female monkeys were administered daily oral doses of 0, 0.03, 0.15, or 0.75 mg/kg PFOS for 26 weeks {Seacat, 2002, 757853}. This model was based on the hypothesis that saturable resorption capacity in the kidney would account for the unique half-life properties of PFOS across species. The model structure was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells.

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption and transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion from what it would be without the transfer. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOS concentrations than at low concentrations. In addition to decreased renal excretion due to the renal resorption, excretion is also reduced in the model by implementing a constant proportion of PFOS that is bound to protein in plasma and is not available for renal filtration. The model was parameterized using the body weight and urine output for cynomolgus monkeys {Butenhoff, 2004, 3749227} and a cardiac output of 15 L/h-kg from the literature {Corley, 1990, 10123}. A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were assumed or optimized to fit the PK data for monkeys. In the IV time course data, some time and/or dose-dependent changes occurred in distribution of PFOS between the blood and tissue compartments, and these changes were less noticeable in the females, therefore, only the female data were used. The simulation captured the overall time course scenario but did not provide good correspondence with the initial rapid loss from plasma and the apparent rise in plasma concentrations over the first 20 days. For oral dosing, the 0.15 mg/kg dose simulation was uniformly lower, and the 0.75 mg/kg dose simulation was higher than the data. When compared to PFOA, PFOS had a longer terminal half-life and more rapid approach to steady-state with repeated oral administration.

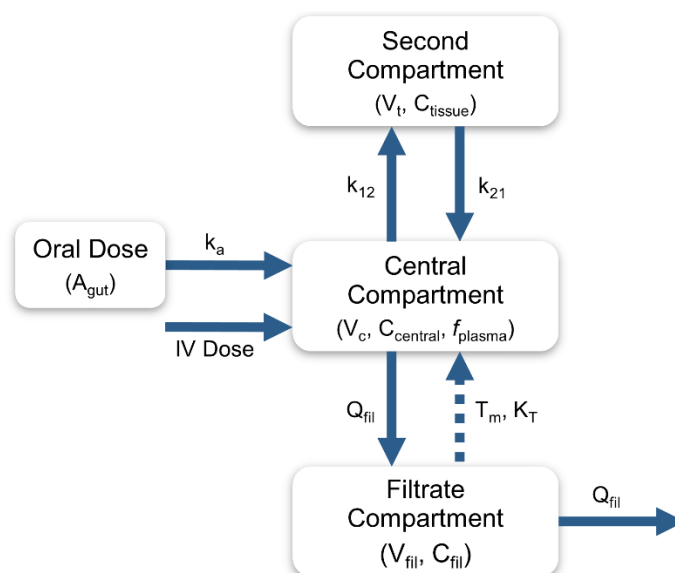


Figure 6. Schematic for a Physiologically Motivated Renal Resorption PK Model

Adapted from {Wambaugh et al., 2013, 2850932}.

Building on the work of other researchers, Wambaugh et al. (2013, 2850932) developed and published a PK model to support the development of an EPA RfD for PFOS {U.S. EPA, 2016, 3603365}. The model was applied to data from studies conducted in monkeys, rats, or mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption PK model was again used. This concept has played a fundamental role in the design of all of the published PFOS models summarized in this section. The model structure is depicted in Figure 6 with minor modifications.

Wambaugh et al. (2013, 2850932) placed bounds on the estimated values for some parameters of the Andersen et al. (2006, 818501) model to support the assumption that serum carries a significant portion of the total PFOS body load. The Andersen et al. (2006, 818501) model is a modified *two-compartment model* in which a primary compartment describes the serum and a secondary deep tissue compartment acts as a specified tissue reservoir. Wambaugh et al. (2013, 2850932) constrained the total V_d such that the amount in the tissue compartment was not greater than 100 times that in the serum. As a result, the ratio of the two volumes (serum versus total) was estimated in place of establishing a rate of transfer from the tissue to serum.

A nonhierarchical model for parameter values was assumed. Under this assumption a single numeric value represents all individuals of the same species, sex, and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters to vary. Measurement errors were assumed to be log-normally distributed. Table 17, in Section 4.1.3.1.1, provides the estimated and assumed PK parameters applied in the Wambaugh et al. (2013, 2850932) model for each of the species evaluated.

The PK data that supported the analysis were derived from two *in vivo* PFOS PK studies. The monkey PK data were derived from Seacat et al. (2002, 757853) and Chang et al. (2012, 1289832). Data for the rats (male/females) and mice were both from Chang et al. (2012, 1289832). The data were analyzed within a Bayesian framework using a Markov Chain Monte

Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and identify serum levels associated with the NOAEL and LOAEL external doses. Prior distributions for the parameters were chosen to be vague, uniformed distributions, allowing them to be significantly informed by the data. The values were assumed to be log-normally distributed constraining each parameter to a positive value.

3.2.2.3 PBPK Models

An alternative approach to the use of a classical or modified compartmental model is a PBPK model, which describes the changes in substance amount or concentration in a number of discrete tissues. One of the main advantages of a PBPK model are the ability to define many parameters based on physiological data, such as organ volumes and the blood flow to different organs, that can be measured relatively easily and that are chemical independent. Another advantage is that amount and concentration of the substance can be predicted in specific tissues, in addition to blood. This can be valuable for certain endpoints where it is expected that a tissue concentration would better reflect the relevant dosimetry compared to blood concentration.

The first PBPK model developed for this chemical was reported in a series of publications by Loccisano et al., which together describe the PK of PFOS in rats, monkeys and humans, in both adult and developmental (for rat and human) scenarios {Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665}. These models were developed based on an earlier “biologically motivated” model that served as a bridge between a one-compartment model and PBPK by implementing a tissue compartment (similar to a two-compartment model), an absorption compartment, and a renal filtrate compartment with saturable renal resorption {Tan, 2008, 2919374}. The PBPK model then extended this “biologically motivated” model by the addition of discrete tissue compartments, rather than a single compartment representing all tissues. The work of Tan et al. (2008, 2919374) was a development of the earlier work of Andersen et al. (2006, 818501).

A series of follow-up studies applied the Loccisano and coauthors’ model structure, with extensions, to address how PK variation in epidemiologic populations could bias the result of the study. This consisted of the work of Wu et al. (2015, 3223290) who developed a detailed model of adolescent development during puberty and menstrual clearance of PFOS to investigate the interaction between chemical levels and the timing of menarche, Ruark et al. (2017, 3981395) who added a detailed description of menopause to evaluate how that affects serum levels and the epidemiological association between early menopause and PFOS levels, Ngueta et al. (2017, 3860773) who implemented a reduction in menstrual clearance in individuals using oral contraceptives and the interaction between oral contraceptive use, endometriosis, and serum PFOS levels, and Dzierlenga et al. (2020, 6315786) and Dzierlenga et al. (2020, 6833691) who implemented a model of thyroid disease {Dzierlenga, 2019, 7947729} to describe changes in PFOS renal clearance due to disease state.

In addition to this chain of studies, Fabrega et al. (2014, 2850904) updated the model of Loccisano et al., for humans by modeling a population using regional food and drinking water measurements and human tissue data collected from cadavers in that region in Spain. The use of human tissue data is relatively rare due to the challenges in sourcing this data but may prove to be advantageous over the assumption that human distribution is similar to distribution in an animal model. However, Fabrega et al. (2014, 2850904) estimated their tissue:blood partition

coefficients from the ratio of tissue concentrations in the cadavers to the average serum concentrations in live volunteers who lived in the same region but were sampled several years earlier {Ericson, 2007, 3858652} and provide no details on how their renal resorption parameters were estimated from the epidemiological blood concentrations. This model was further applied to a population in Norway and extended to other PFAS {Fabrega, 2015, 3223669}.

Brochot et al. (2019, 5381522) presented the application of a PBPK model for PFOS with gestation and lactation phases to describe development and predicted maternal, infant, and breastmilk concentrations over a variety of scenarios including the prediction of maternal levels across multiple pregnancies.

One of the major challenges in the parameterization of PBPK models for PFOS are the estimation of the chemical-dependent parameters such as those involved in protein binding and renal clearance. One way to investigate this issue is to perform in vitro experiments to help to inform the parameters. Worley et al. (2015, 3981311) used in vitro measurements of renal transporter activity to describe in detail the various steps involved in the renal filtration, resorption, and excretion of PFOS.

Chou and Lin (2019, 5412429) developed a PFOS PBPK model for rat, mouse, monkey, and human. Using the model structure of Worley and Fisher (2015), parameters were determined using a hierarchical Bayesian framework to pool datasets across studies for each species. This model reflects saturable resorption in the proximal tubule cells of the kidney and fecal elimination through the bile. While the Bayesian approach is ideal for handling multiple datasets, the method for implementing the Bayesian inference raises questions about the final posterior parameter distributions. Priors for the hierarchical model were determined using a least-squares fitting method on the most sensitive parameters as opposed to defining priors using information from previous studies and letting the data update those priors to determine the joint posterior distribution of the parameter space. In a subsequent study, Chou and Lin (2021, 7542658) added a gestation/lactation element to the model and parameterized the gestation/lactation components for rats and humans. This model structure used a three-compartment fetal model during gestation and a physiologically motivated PK model, similar to Wambaugh et al. (2013, 2850932) with renal resorption, for the infant. Using this model, the authors developed HEDs using inter-species extrapolation of the average serum concentration point-of-departure derived from the rat model. While the fits demonstrated good agreement with the evaluation dataset, parameters for only the rat are available for developmental endpoints.

3.2.3 Half-Life Data

Many half-life values have been reported for the clearance of PFOS in humans, as reviewed in Section D.4.5. The slow excretion of PFOS requires measurement of a small change in serum concentration over a long time, which may be one reason for the variance in reported values. Another challenge is the ubiquity of PFOS exposure. Ongoing exposure will result in a positive bias in observed half-life values if not considered {Russell, 2015, 2851185}. In studies that calculate the half-life in a population with greatly decreased PFOS levels, typically due to the end of occupational exposure or the introduction of drinking water filtration, the amount of bias due to continuing exposure will be related to the ratio of the prior and ongoing exposure. That is, a high exposure will be less prone to bias than a lower exposure, e.g., when the ongoing exposure is at a similar level. On the other hand, a half-life value determined from a population

with very high exposure may not be informative of the half-life in typical exposure, due to non-linearities in PK that may occur due to the saturation of PFAS-protein interactions. This will likely take the form of a decreased half-life due to saturation of renal resorption, and increased urinary clearance.

Our approach for selection of half-life was to select a reported value from an exposure to the general population, with a clear decrease in exposure, with a high number of individuals and a long follow-up time. With these criteria, a half-life of 3.4 years for PFOS was selected {Lin, 2018, 4238434}. This value for PFOS comes from a community with contaminated drinking water with serial samples of 106 individuals for a relatively short follow-up time of 2 years.

A summary of PFOS half-life values is presented in Section D.4.5 for comparing model predicted half-lives to values reported in the literature.

3.2.4 Volume of Distribution Data

The value for human V_d , 230 mL/kg, was sourced from Thompson et al. (2010, 2919278). To estimate the V_d for PFOS, Thompson et al. (2010, 2919278) applied a creative approach and scaled the value they obtained for PFOA by the ratio of V_d s obtained by Andersen et al. (2006, 818501) in the parameterization of that PK model using PK data in monkey. That is, $V_d \text{ PFOA, human} = V_d \text{ (PFOA, human} * V_d \text{ (PFOS, monkey)} / V_d \text{ (PFOA, monkey)}$, where V_d is the volume of distribution. V_d is a parameter that is relatively easily obtained from an analysis of PK data from a controlled experimental study, as it is related to the peak concentration observed after dosing and is expected to be similar between human and non-human primates {Mordenti, 1991, 9571900}. For comparison, the optimized V_d value from oral dosing in monkeys was 220 mL/kg for PFOS {Andersen, 2006, 818501}.

Because the derivation of the V_d for PFOS relied on the value for PFOA, it is important to consider alternate values for V_d for PFOA. For PFOA, the V_d calculation depended on the half-life. Thompson et al. (2010, 2919278) used 2.3 years, which was estimated within their population. If the EPA chosen half-life of 2.7 years was used instead, the V_d for PFOA would be 200 mL/kg, which results in a PFOS value of 271 mL/kg. As mentioned in the PFOA draft document, EPA did not update the V_d values based on the updated half-life because the value of 2.3 years was calculated based on the same data as the V_d and this half-life may be more representative of that population at that specific time.

Gomis et al. (2017 3981280) calculated V_d by taking the average of reported animal and human values and estimated values of 235 mL/kg for PFOS. This calculation included the value from Thompson et al. (2010, 2919278) and did not include additional values derived from human data. This average value shows that the value from Thompson et al. (2010, 2919278) which was selected based on the fact that it was derived only from human and non-human primate data, is reasonable.

A summary of PFOA V_d values is presented in Section D.2.4 for comparing model predicted half-lives to values reported in the literature.

3.3 Health Effects Evidence Synthesis and Integration

3.3.1 Developmental

3.3.1.1 Human Evidence

3.3.1.1.1 Introduction and Study Evaluation Considerations

This section describes studies of PFOS exposure and potential in utero and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. The latter includes all studies where exposure is limited to gestation and/or early life up to 2 years of age.

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

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFOS in humans as described in Section 3.2.3, samples collected during all three trimesters, before birth or and shortly after birth) were considered adequately representative of the most critical in utero exposures for fetal growth and gestational duration measures. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where in utero perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in {De Boo, 2009, 6937194} and {Perng, 2016, 6814341}). There is some evidence that birth weight deficits can be followed by increased weight gain that may occur especially among those with rapid growth catch-up periods during childhood {Perng, 2016, 6814341}. Therefore, the primary critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero for study evaluation purposes, and studies of this outcome were downgraded in the exposure domain if exposure data were collected later during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (e.g., for sex-stratified results). In the Outcome domain, specific considerations address validation and accuracy of specific endpoints and adequacy of case ascertainment for some dichotomous (i.e., binary) outcomes. For example, birthweight measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and

incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias towards the null if ascertainment of fetal loss is not associated with PFOS exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null and can manifest as an apparent protective effect. Fetal and childhood growth restriction were examined using several endpoints including low birth weight, small for gestational age (SGA), ponderal index [i.e., birth weight grams)/birth length (cm³) x 100], abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher quality endpoints (i.e., classified as good in the Outcome domain) that were available in multiple studies to allow for an evaluation of consistency and other considerations across studies. However, even when databases were more limited, such as for spontaneous abortions, the evidence was evaluated for its ability to inform developmental toxicity more broadly, even if available in only one study.

Overall, mean birth weight and birth weight-related measures are considered very accurate and were collected predominately from medical records; therefore, more confidence was placed in these endpoints in the Outcome domain judgments. Some of the adverse endpoints of interest examined here included fetal growth restriction endpoints based on birth weight such as mean birth weight (or variations of this endpoint such as standardized birthweight z-scores), as well as binary measures such as SGA (e.g., lowest decile of birthweight stratified by gestational age and other covariates) and low birth weight (i.e., typically < 2500 grams; 5 pounds, 8 ounces) births. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary to be classified as good for these endpoints in this domain. In contrast, other measures of fetal growth that are subject to more measurement error (e.g., head circumference and body length measures such as ponderal index) were given a rating of adequate {Shinwell, 2003, 6937192}. These sources of measurement error are expected to be non-differential with respect to PFOS exposure status and, therefore, would not typically be a major concern for risk of bias but could impact study sensitivity.

Gestational duration measures were presented as either continuous (i.e., per each gestational week) or binary endpoints such as preterm birth (typically defined as gestational age <37 weeks). Although changes in mean gestational age may lack some sensitivity, especially given the potential for measurement error, many of the studies were based on ultrasound measures early in pregnancy, which should increase the accuracy of estimated gestational age and the ability to detect associations that may be present. Any sources of error in the classification of these endpoints would also be anticipated to be non-differential with respect to PFOS exposure. While they could impact precision and study sensitivity, they were not be considered a major concern for risk of bias.

3.3.1.1.2 Study Inclusion

Sixty-one studies of developmental outcomes not included in the 2016 HESD report on PFOS {U.S. EPA, 2016, 3603365} have been identified based on a literature search dated September 2020. Although every study is included in the study quality evaluation heat maps for

comprehensiveness, six developmental epidemiological studies identified in the literature search were excluded for consideration in this synthesis because other studies report results for the same health outcomes and from the same study cohorts (i.e., were considered duplicative). For example, the Rokoff et al. (2018, 4238310) study overlapped with the Project Viva study by Sagiv et al. (2018, 4238410), as did the Bjerregaard-Olesen et al. (2019, 5083648) study with Aarhus birth cohort detailed in Bach et al. (2016, 3981534). Similarly, the Li et al. (2017, 3981358) Guangzhou Birth Cohort Study overlapped with a more recent study by Chu et al. (2020, 6315711).

Three studies (Kishi et al. (2015, 2850268), Kobayashi et al. (2017, 3981430) and Minatoya et al. (2017, 3981691)) were also not considered in this synthesis, because they provided overlapping data from the same Hokkaido Study on Environment and Children's Health birth cohort population as Kashino et al. (2020, 6311632). For those Japanese studies with the same endpoints such as mean birthweight (BWT), the analysis with the largest sample size was used in forest plots and tables (e.g., Kashino et al., 2020, 6311632). Although the Kobayashi et al. (2017, 3981430) study included a unique endpoint called ponderal index, this measure is more prone to measurement error and was not considered in any study given the wealth of other fetal growth restriction data. Similarly, the Costa et al., (2019, 5388081) study that examined a less accurate in utero growth estimate was not considered in lieu of their more accurate birth outcomes measures reported in the same cohort (Manzano-Salgado et al., 2017). In general, to best gauge consistency and magnitude of reported associations US EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Two other studies with overlapping cohorts were included in the synthesis, as each study provided some unique data for different endpoints. For example, the Woods et al. (2017, 4183148) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018, 4619944) but has additional mean BWT data (communication with author). The mean BWT results for singleton and twin births from Bell et al. (2018, 5041287) are included in forest plots here as are the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019, 5080619) as they target different developmental windows.

Following exclusion of the six studies noted above, 55 developmental epidemiological studies were included in the synthesis that were not included in the 2016 HESD report. Five additional studies {Alkhalawi, 2016, 3859818; Jin, 2020, 6315720; Lee, 2013, 3859850; Lee, 2016, 3981528; Maekawa, 2017, 4238291} were considered uninformative due to critical study deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies and are not further examined here.

Thus, 50 studies were included across various developmental endpoints for further examination and synthesis. Thirty-five of the 50 different studies examined PFOS in relation to fetal growth restriction measured by the following endpoints: small for gestational age (SGA), low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Sixteen studies examined gestation duration, three examined fetal loss, and four examined birth defects.

3.3.1.1.3 Growth Restriction: Fetal Growth

3.3.1.1.3.1 Study Characteristics

Of the 32 included studies that examined BWT measures in relation to PFOS exposures, 29 studies examined mean BWT, ten studies examined BWT z-scores and seven {Ashley-Martin,

2017, 3981371; Bach, 2016, 3981534; Gyllenhammar, 2018, 4238300; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Wang, 2019, 5080598; Wikström, 2019, 6311677} reported results for both.

Twenty of the 29 non-overlapping and informative mean BWT studies shown in Figure 7 and Figure 8 were prospective birth cohort studies, and the remaining nine were cross-sectional analyses {Bell, 2018, 5041287; Callan, 2016, 3858524; de Cock, 2016, 3045435; Gao, 2019, 5387135; Gyllenhammar, 2018, 4238300; Kwon, 2016, 3858531; Shi, 2017, 3827535; Wang, 2019, 5080598; Xu, 2019, 5381338}. Overall, eight of the PFOS studies relied on umbilical cord measures {Cao, 2018, 5080197; de Cock, 2016, 3045435; Govarts, 2016, 3230364; Kwon, 2016, 3858531; Shi, 2017, 3827535; Wang, 2019, 5080598; Workman, 2019, 5387046; Xu, 2019, 5381338}, and one collected blood samples in infants 3 weeks following delivery {Gyllenhammar, 2018, 4238300}. Results from the Bell et al. (2018, 5041287) study were based on infant whole blood taken from a heel stick and captured onto filter paper cards at 24 hours or more following delivery, and one study used both maternal serum samples collected 1–2 days before delivery and cord blood samples collected immediately after delivery {Gao, 2019, 5387135}. One study examined pre-conception maternal serum samples {Robledo, 2015, 2851197}. Sixteen studies had maternal exposure measures that were sampled during trimesters one {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}, two {Lauritzen, 2017, 3981410}, or three {Callan, 2016, 3858524; Chu, 2020, 6315711; Kashino, 2020, 6311632; Valvi, 2017, 3983872}, or across multiple trimesters {Hjermitslev, 2019, 5880849; Lenters, 2016, 5617416; Marks, 2019, 5081319; Starling, 2017, 3858473; Wikström, 2019, 6311677; Woods et al., 2017, 4183148}. The study by Meng et al. (2018, 4829851) pooled exposure data from two study populations, one which measured PFOS in umbilical cord blood and one which measured PFOS in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean BWT, only one biomarker measure was used (e.g., preferably maternal samples when collected in conjunction with umbilical cord samples). In addition, other related publications (e.g., {Gyllenhammar, 2017, 7323676}) or additional information or data (e.g. {Woods et al., 2017, 4183148}) provided by study authors (communication with author) were used.

Thirteen of the 29 mean BWT studies included in the synthesis were rated *high* in overall study confidence {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Bell, 2018, 5041287; Chu, 2020, 6315711; Govarts, 2016, 3230364; Lauritzen, 2017, 3981410; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Valvi, 2017, 3983872; Wikström, 2019, 6311677; Woods et al., 2017, 4183148}, while nine were rated *medium* {de Cock, 2016, 3045435; Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Kwon, 2016, 3858531; Lenters, 2016, 5617416; Meng, 2018, 4829851; Robledo, 2015, 2851197; Wang, 2019, 5080598}, and seven were classified as *low* {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2019, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Xu, 2019, 5381338} (Figure 7, Figure 8).

Eighteen of the twenty-two *high* or *medium* confidence studies detailed in this synthesis were classified as having good {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Lauritzen, 2017, 3981410; Lenters, 2016, 5617416; Lind, 2017, 385812; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Robledo, 2015, 2851197; Sagiv, 2018, 4238410;

Starling, 2017, 3858473; Wikström, 2019, 6311677; Valvi, 2017, 3983872; Woods et al., 2017, 4183148} or adequate study sensitivity {Chu, 2020, 6315711; Govarts, 2016, 3230364}, while four had deficient study sensitivity {Bell, 2018, 5041287; de Cock, 2016, 3045435; Kwon, 2016, 3858531; Wang, 2019, 5080598} (Figure 7, Figure 8). The median exposure values across all of the studies were quite variable and ranged from 0.38 ng/mL {Kwon, 2016, 3858531} to 30.1 ng/mL {Meng, 2018, 4829851}.

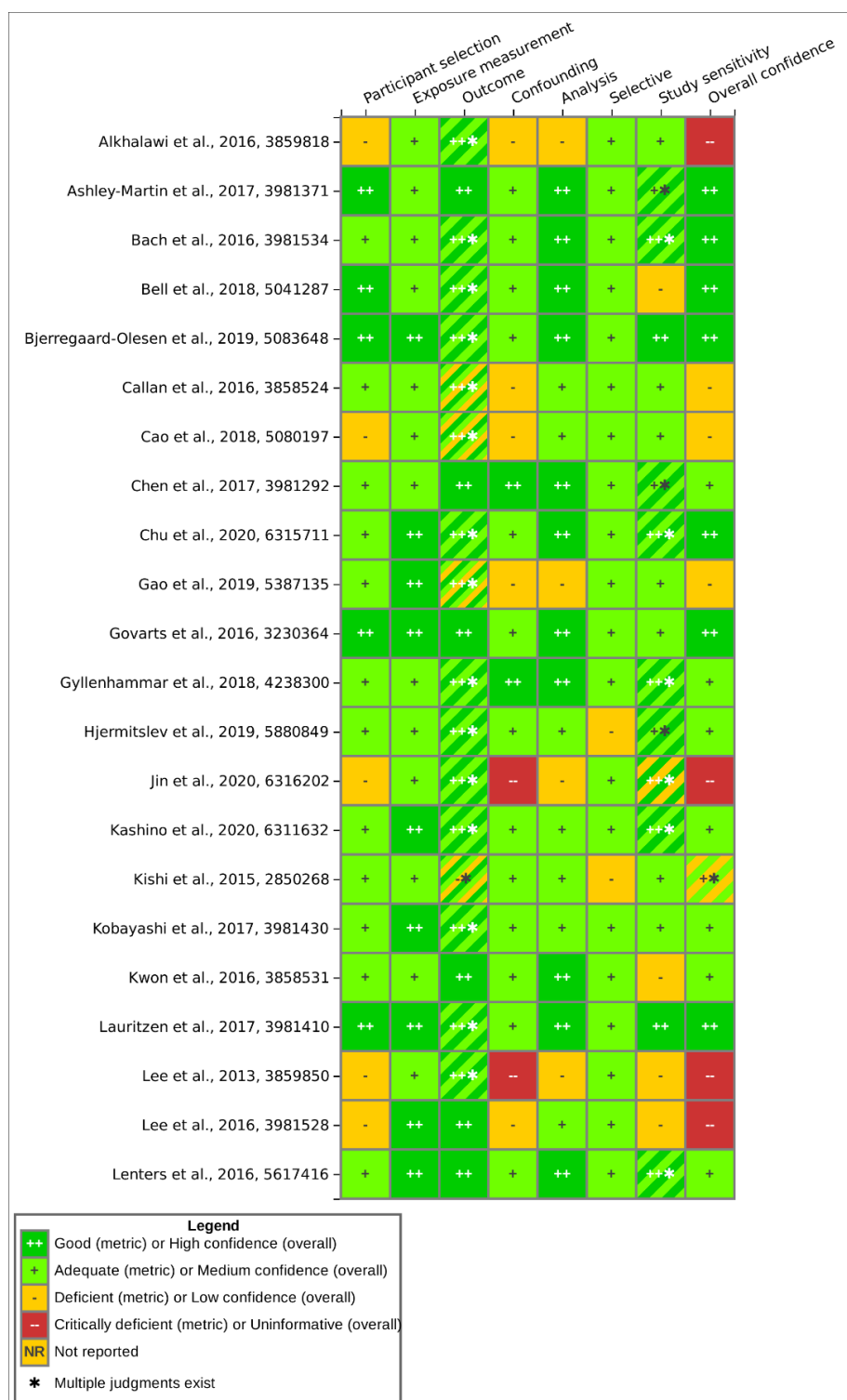


Figure 7. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects^a

Interactive figure and additional study details available on [HAWC](#).

^a Includes six overlapping studies (Bjerregaard-Olesen, 2019, 5083648; Kishi, 2015, 2850268; Kobayashi, 2017, 3981430; Li, 2017, 3981358; Minatoya, 2017, 3981691; Rokoff, 2018, 4238310).

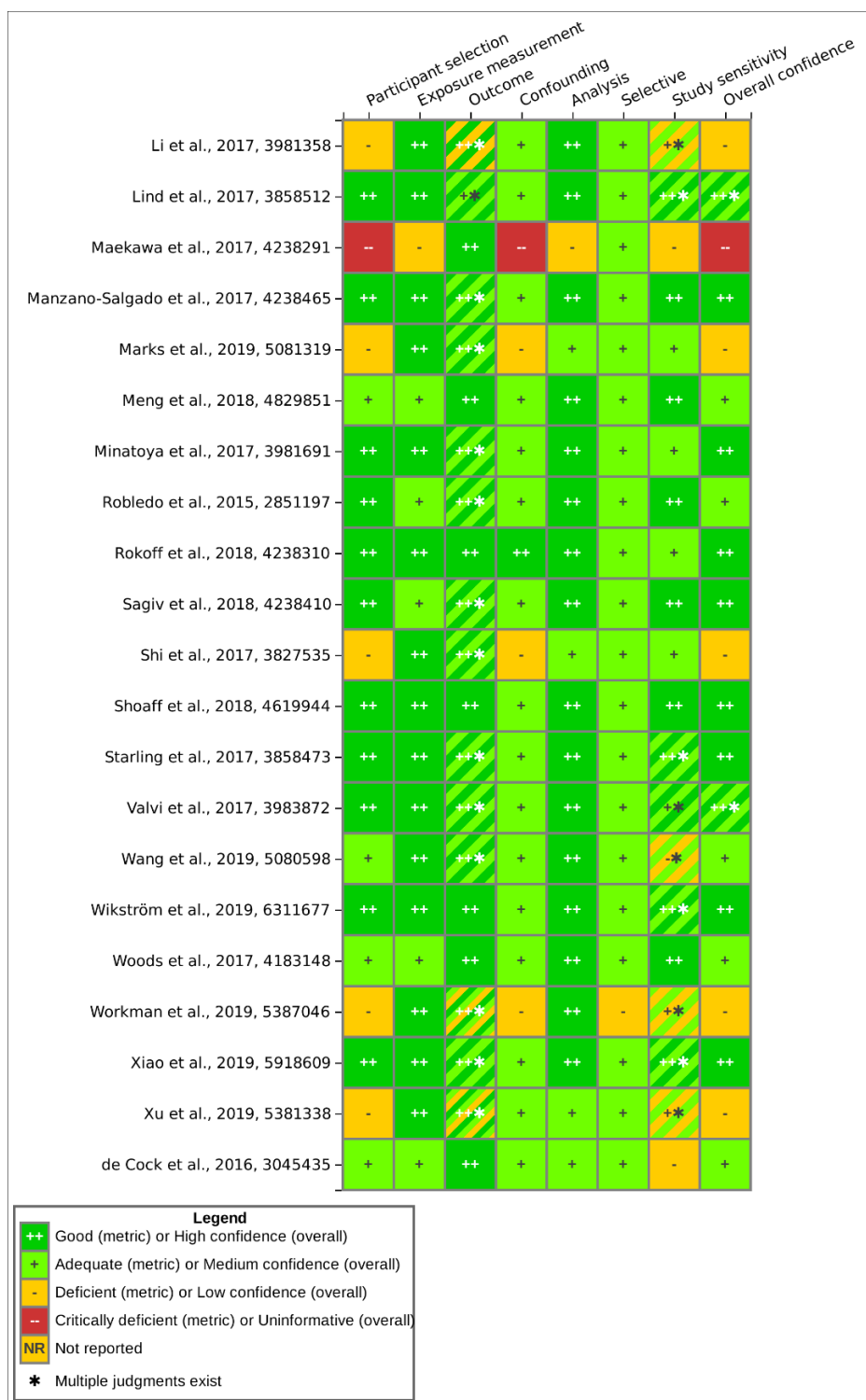


Figure 8. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)^a

Interactive figure and additional study details available on [HAWC](#).

^a Includes six overlapping studies (Bjerregaard-Olesen, 2019, 5083648; Kishi, 2015, 2850268; Kobayashi, 2017, 3981430; Li, 2017, 3981358; Minatoya, 2017, 3981691; Rokoff, 2018, 4238310).

3.3.1.1.3.2 Mean Birth Weight Study Results: Overall Population Studies

As shown in Figure 7 and Figure 8, 38 BWT studies with BWT measures were considered as part of the study quality evaluation. Among the 29 non-overlapping and informative studies with mean BWT data, four examined sex-specific data only {Ashley-Martin et al. 3981371; Lind et al., 2017, 3858512; Marks et al., 2019, 5081319; Robledo, 2015, 2851197}. Eighteen of the 25 PFOS studies with analyses based on an overall population reported some mean BWT deficits, although some of these were not statistically significant. Five mean BWT studies in the overall population reported null associations {Bell, 2018, 5041287; Gao, 2019, 5387135; Hjermitsev, 2020, 5880849; Manzano-Salgado, 2017, 4238465; Woods, 2017, 4183148}, while two reported increased mean BWT deficits {de Cock, 2016, 3045435; Shi, 2017, 3827535}. Among the 11 studies showing some adverse associations in the overall population, there was a wide distribution of deficits ranging from 11 to 417 grams across both categorical and continuous exposure estimates. Only two studies {Starling, 2017, 3858473; Sagiv, 2018, 4238410} out of seven studies which examined categorical data {Cao, 2018, 5080197; Gao, 2019, 5387135, Manzano-Salgado, 2017, 4238465, Meng, 2018, 4829851; Starling, 2017, 3858473; Sagiv, 2018, 4238410; Wikström, 2019, 6311677} showed monotonic exposure-response relationships.

Eleven of these 18 studies reported deficits based on either categorical or continuous from 45 to 95 grams including nine studies that were either *medium* or *high* confidence. Few patterns emerged when examining results by study characteristics and overall confidence although five of the studies with the smallest deficits were *high* confidence. Study sensitivity was not an explanatory factor of the null BWT studies as all three studies had large exposure contrasts and large sample sizes. A relationship between PFOS sample timing and magnitude of associations also was evident with nine of the largest deficits detected among studies that used umbilical cord data or maternal serum with some or all samples collected during trimester 3 (Figure 9, Figure 10).

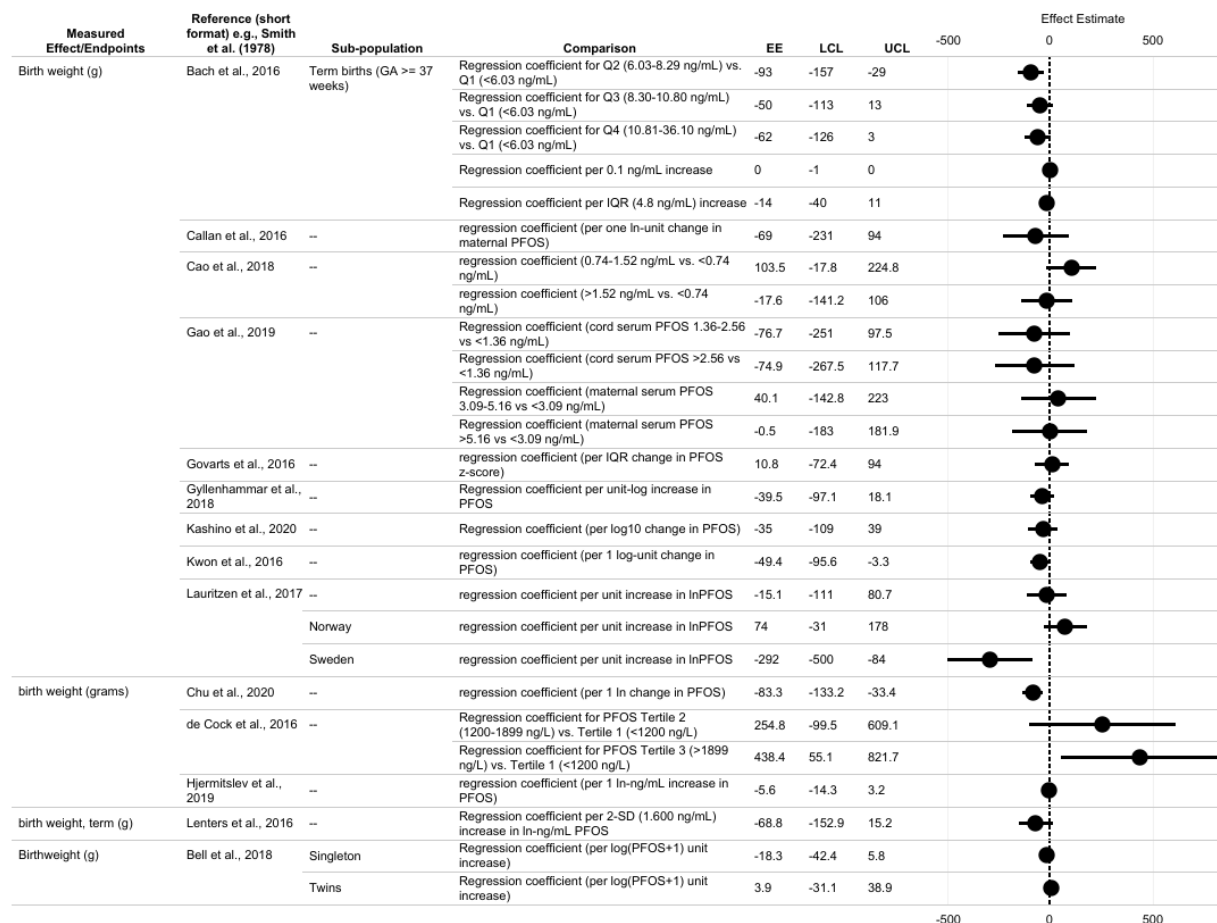


Figure 9. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

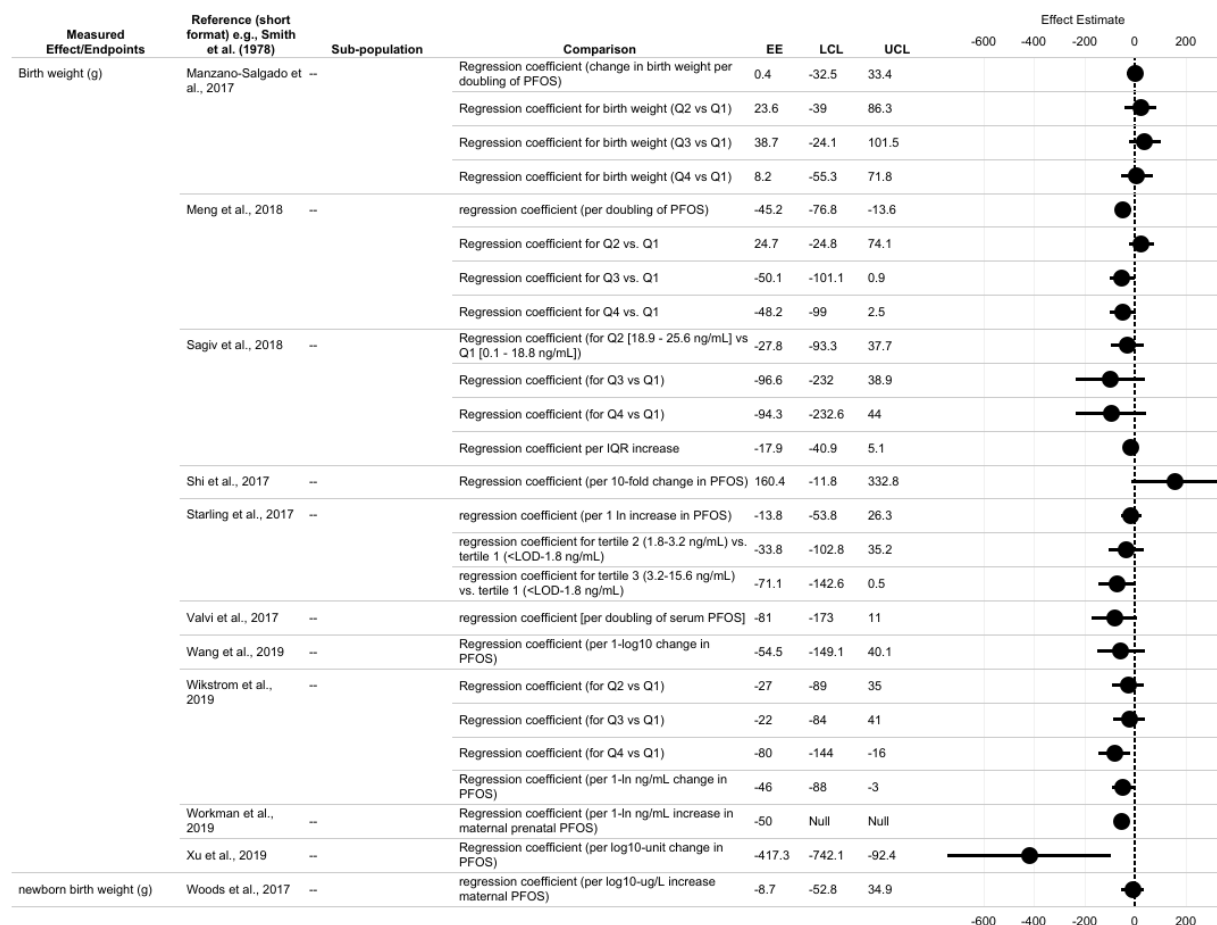


Figure 10. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.3.3 Mean Birth Weight Study Results: Sex Specific Studies

Sixteen epidemiological studies examined sex-specific results, with five studies {Ashley-Martin, 2017, 3981371; de Cock, 2016, 3045435; Hjermistlev, 2019, 5880849; Robledo, 2015, 2851197; Shi, 2017, 3827535} not showing any evidence of adverse associations (Figure 7, Figure 8). The *low* confidence study by Marks et al. (2019, 5081319), which only had data on male neonates, showed an exposure-response with reported large deficits in PFOS tertile 2 (-26.6 g; 95% CI: -147.3, 94.2) and tertile 3 (-83.9 g; 95% CI: -201.4, 33.7) compared to tertile 1 referent. Four other studies reported mean BWT deficits only in boys {Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872}; no studies reported deficits in girls only. Among the 15 studies examining mean BWT associations in both boys and girls, six studies detected some deficits in both sexes. Two of these six studies showed sex-specific deficits comparable in magnitude among boys and girls {Chu, 2010, 6315711; Wang, 2019, 5080598}. Three of these studies (Bach, 2016, 3981534; Meng, 2018, 4829851; Wikström, 2019, 6311677) showed larger deficits among girls and one showed larger deficits among boys {Kashino, 2020, 6311632}.

There was somewhat stronger evidence of more consistent results in boys, generally especially in the few studies that detected associations in both sexes. The magnitude of deficits in three studies in boys {Chu, 2010, 6315711; Valvi, 2017, 3983872; Wang, 2019, 5080598} showing adverse associations ranged from –62 to –150 g, although two studies {de Cock, 2016, 3045435; Shi, 2017, 3827535} reported large increased BWT in girls for either continuous or categorical PFOS exposure comparisons. These five studies with larger mean BWT differences were all based on later biomarker samples collected from either the mother in the third trimester or from the infant’s umbilical cord (Figure 11, Figure 12).

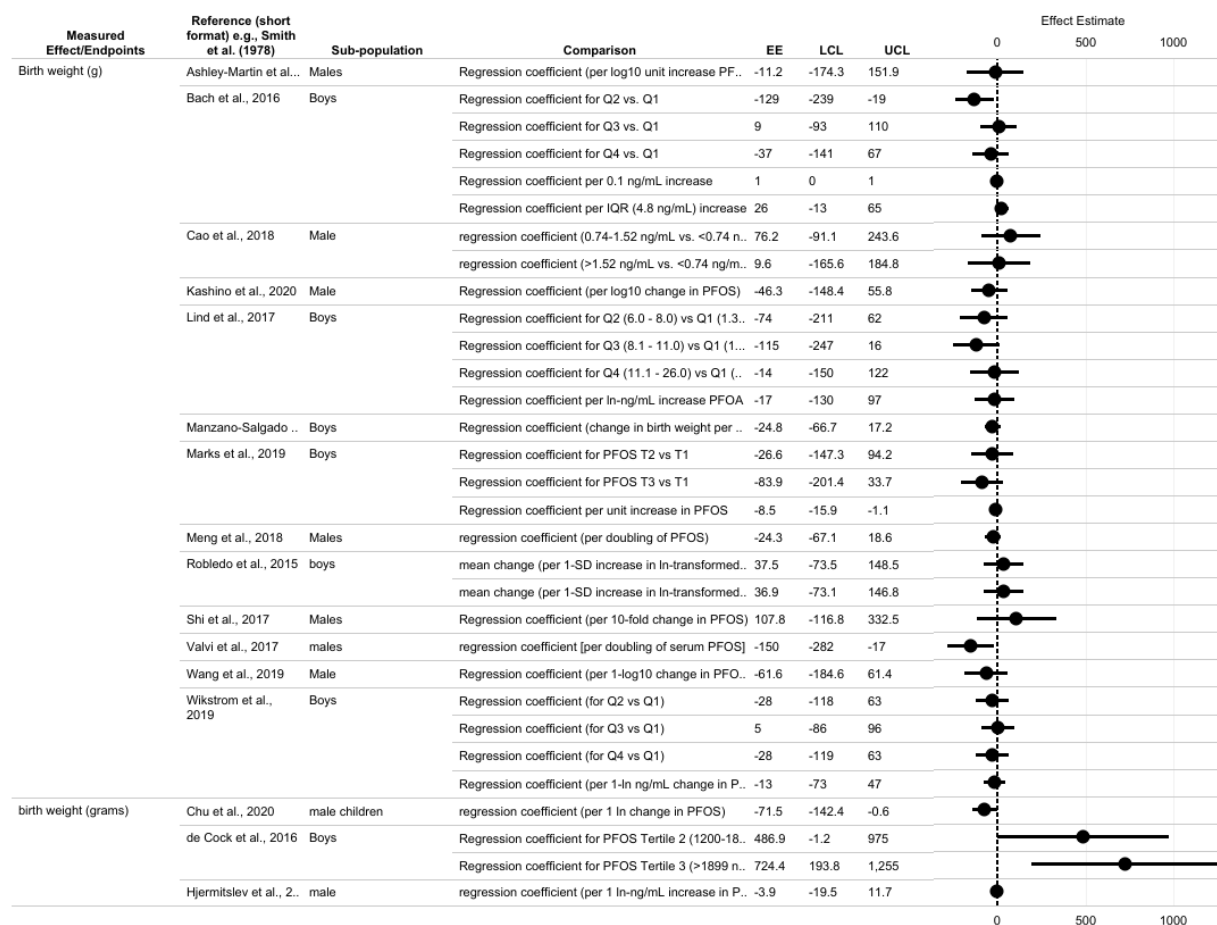


Figure 11. Mean Birth Weight in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

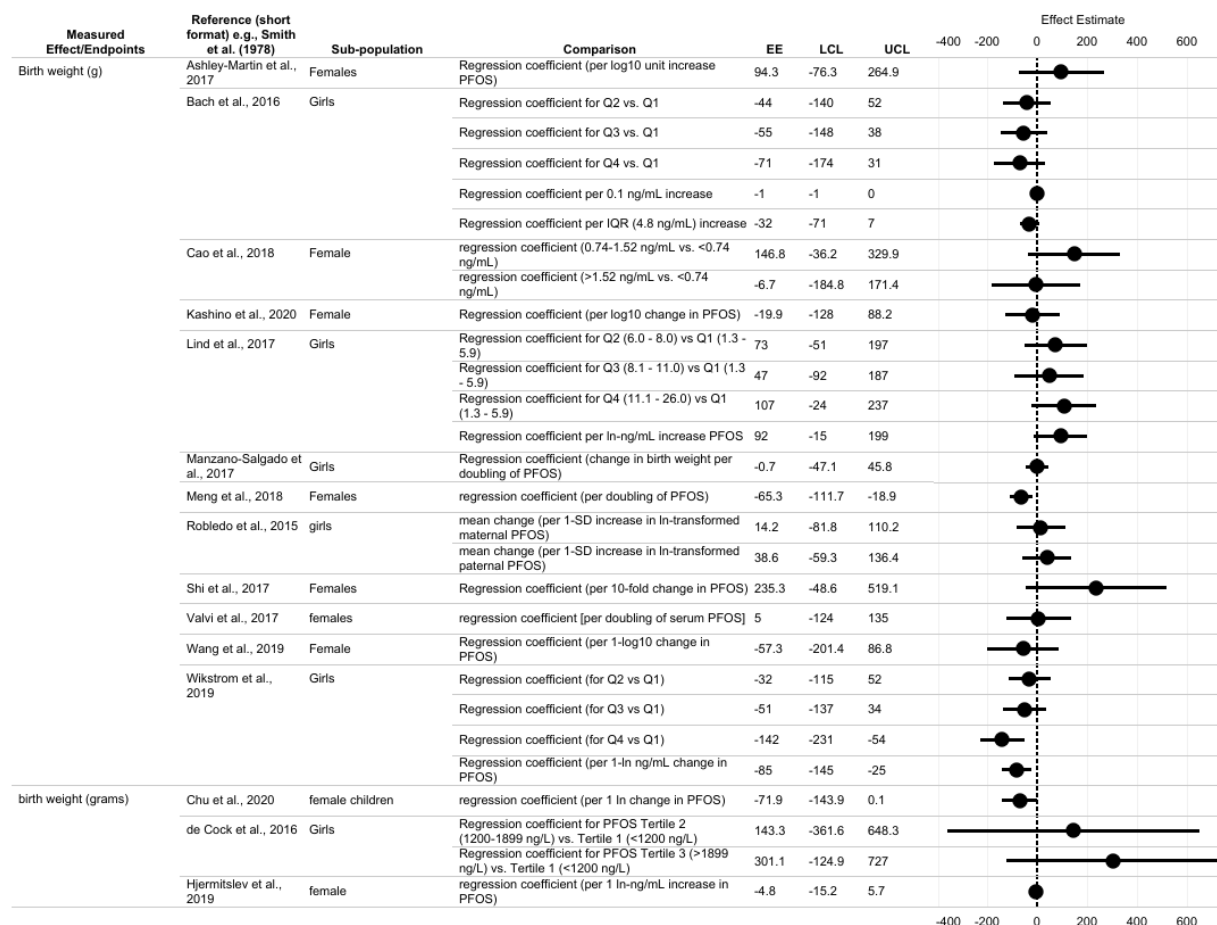


Figure 12. Mean Birth Weight in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.3.4 Birth Weight Z-Scores

Ten studies examined for BWT z-scores in relation to PFOS {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Shoaff, 2018, 4619944; Wang, 2019, 5080598; Wikström, 2019, 6311677; Xiao, 2019, 5918609} (Figure 7, Figure 8). Seven of the ten studies showed adverse associations between PFOS exposures and BWT z-scores, and of these five were *high* confidence {Bach, 2016, 3981534; Sagiv, 2018, 4238410; Shoaff, 2018, 4619944; Wikström, 2019, 6311677; Xiao, 2019, 5918609} and two were *medium* confidence {Chen, 2017, 3981292; Wang, 2019, 5080598}. Chen et al. (2017, 3981292) reported adverse associations in the overall population (-0.14; 95% CI: -0.26, -0.01) with comparable results in both male and female neonates (BWT z-score range: -0.13 to -0.15). Although not statistically significant, both Wang et al. (2019, 5080598) (-0.15; 95% CI: -0.41, 0.11) and Shoaff et al. (2018, 4619944) (-0.12; 95% CI: -0.36, 0.13) reported associations similar in magnitude for their overall population. Sagiv et al. (2018, 4238410) reported associations for PFOS quartile 4 in the overall population, while the largest association in this study was found for male neonates (-0.19; 95% CI: -0.33, -0.05) per each interquartile range (IQR) increase. Compared to quartile 1, Wikström et al. (2019, 6311677) reported adverse associations in quartile 4 in the overall population (-0.17;

95% CI: -0.37, -0.03); these results appeared to be driven by associations detected in female neonates (-0.36; 95% CI: -0.77, 0.05). Among the seven studies showing some deficits, the largest associations were detected in Xiao et al. (2019, 5918609) for the overall population (-0.47; 95% CI: -0.85, -0.09), male neonates (-0.40; 95% CI: -0.89, 0.08), and female neonates (-0.56; 95% CI: -1.12, 0). Bach et al. (2016, 3981534) reported a statistically significant association between mean BWT z-score and PFOS quartiles 2 (-0.15; 95% CI: -0.29, -0.02) and quartile 4 (-0.11; 95% CI: -0.25, 0.02) only, with no exposure-response relationship detected.

Overall, 7 out of 10 studies showed some associations between standardized BWT scores and PFOS exposures. All seven studies were all either *medium* or *high* confidence studies, and five of them had large exposure contrasts. No patterns by sample timing were evident as three of these studies had trimester one maternal samples. Study sensitivity did not seem to be an explanatory factor in the three null studies of standardized BWT as all three studies had large exposure contrasts and large sample sizes. None of the five studies with categorical data reported strong evidence of exposure-response relationships (Figure 13, Figure 14, Figure 15).

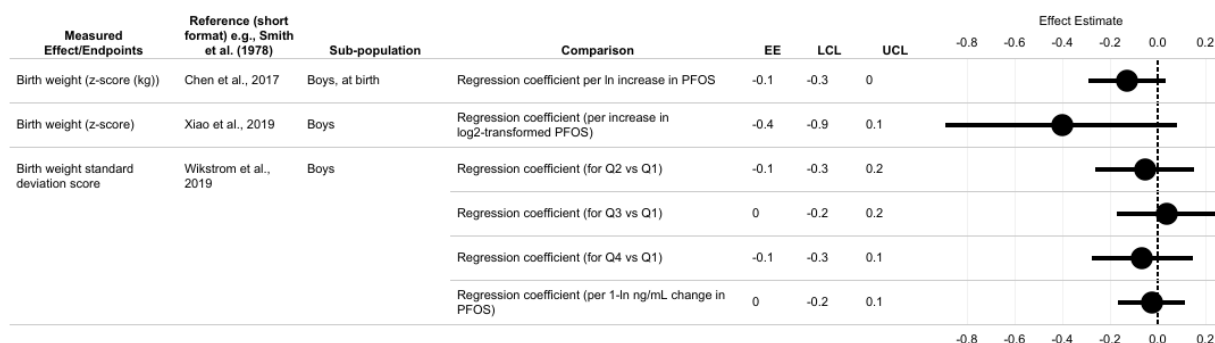


Figure 13. Birth Weight Z-scores in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

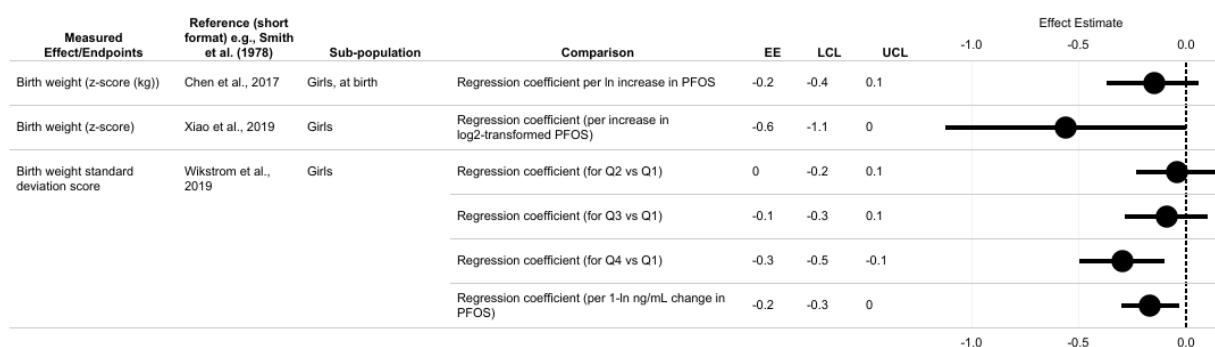


Figure 14. Birth Weight Z-scores in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

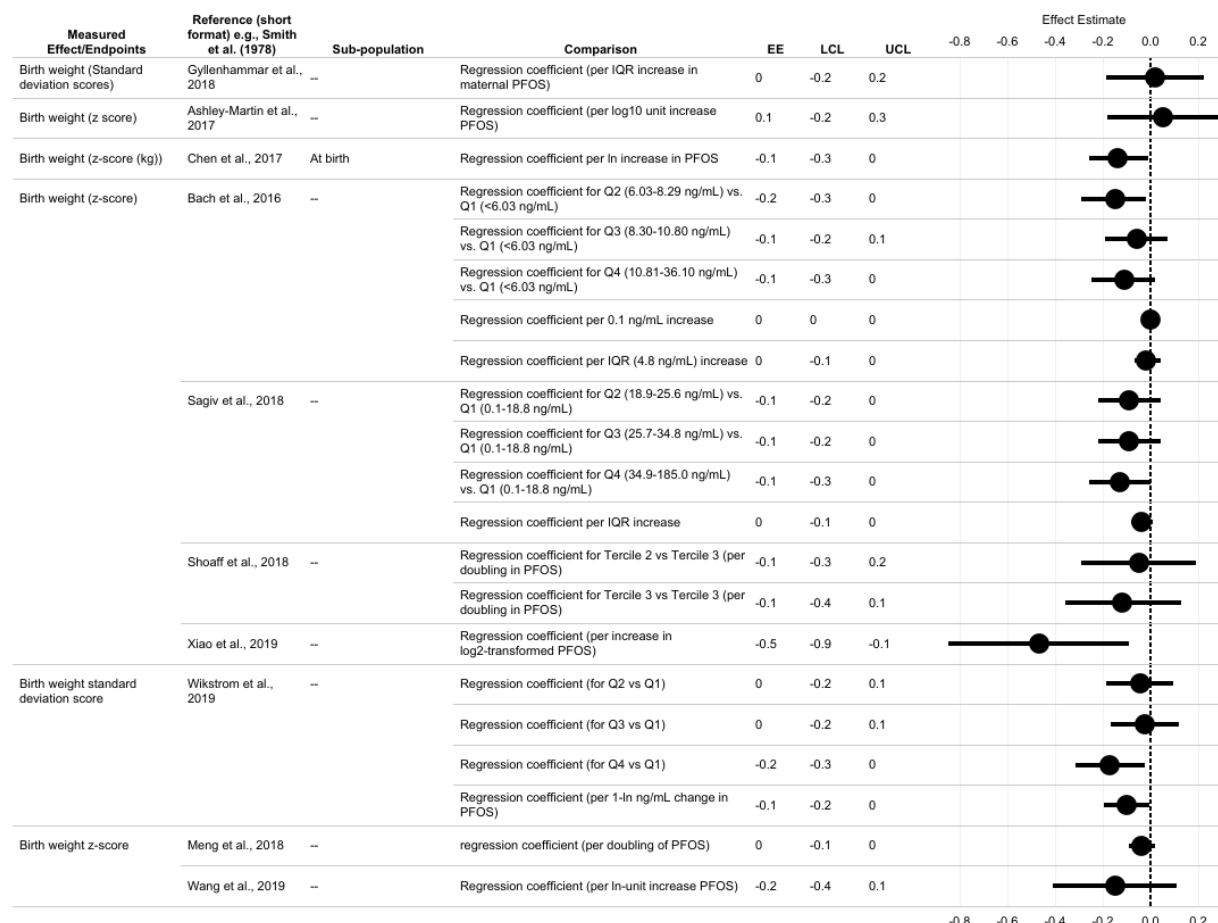


Figure 15. Overall Birth Weight Z-scores from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.3.5 Small for Gestational Age/Low Birth Weight

Nine epidemiological studies examined associations between PFOS exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), LBW, or both (i.e., {Manzano-Salgado, 2017, 4238465}) (Figure 16). Six studies examined SGA in relation to PFOS exposure with four classified as *high* confidence {Govarts, 2016, 3230364; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Wikström, 2019, 6311677} and two as *low* {Souza, 2020, 6833697; Xu, 2019, 5381338}. Three of these had *good* study sensitivity {Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Wikström, 2019, 6311677}, while three were considered *adequate* {Govarts, 2018, 3230364; Souza, 2020, 6833697; Xu, 2019, 5381338}.

Four {Lauritzen, 2017, 3981410; Wikström, 2019, 6311677; Souza, 2020, 6833697; Xu, 2019, 5381338} of the six SGA studies showed some adverse associations, while two studies were null {Govarts, 2018, 3230364; Manzano-Salgad, 2017, 4238465}. The magnitude of odds ratios (ORs) across the three studies showing adverse associations in the overall population (OR range: 1.19 to 4.14) was quite variable whether the effect estimates were based on either categorical or

continuous exposures (per each unit increase) Figure 16). Lauritzen et al. (2017, 3981410) did not show an increased risk in the overall population per each ln-unit PFOS increase, but they did show a larger association among participants from Sweden (OR = 2.51; 95% CI: 0.93, 6.77). None of the three studies examining categorical exposures showed any evidence of an exposure-response relationship. There were two *low* and two *high* confidence studies that reported increased risks for SGA with increasing PFOS exposures. Although the number of studies was small, few patterns were discernible across study characteristics or overall confidence for these SGA findings.

Four studies examined LBW in relation to PFOS including two each that were *high* confidence {Chu, 2020, 6315711; Manzano-Salgado, 2017, 4238465} and *medium* confidence {Hjermitslev et al. 2019, 5880849; Meng, 2018, 4829851}. All but one {Hjermitslev, 2019, 5880849} of the four LBW studies showed some associations with either the overall population, or in either boys or girls (Figure 16). Manzano-Salgado et al. (2017, 4238465) did not detect associations in the overall population but showed an increased risk for term LBW among boys only (OR = 1.6–8; 95% CI: 0.62, 4.54). Meng et al. (2018, 4829851) reported non-significant increased ORs (range 1.2–1.8) in the overall population across all quartiles but no evidence of an exposure-response relationship. The Chu et al. (2020, 6315711) study that examined associations with categorical PFOS exposures in the overall population reported limited evidence of an exposure-response relationship with imprecise increased risks shown for quartile 3 (OR = 1.41; 95% CI: 0.23, 8.82) and quartile 4 (OR = 3.70; 95% CI: 0.61, 22.6) exposures compared to the quartile one referent.

The three LBW studies that showed increased risks were all either *medium* or *high* confidence. Although the number of studies was small, few discernible patterns across study characteristics or confidence were evident across these LBW findings. No patterns by sample timing or exposure levels and study sensitivity were detected for either endpoint. Collectively, the majority of SGA and LBW studies were supportive of an increased risk with increasing PFOS exposures.

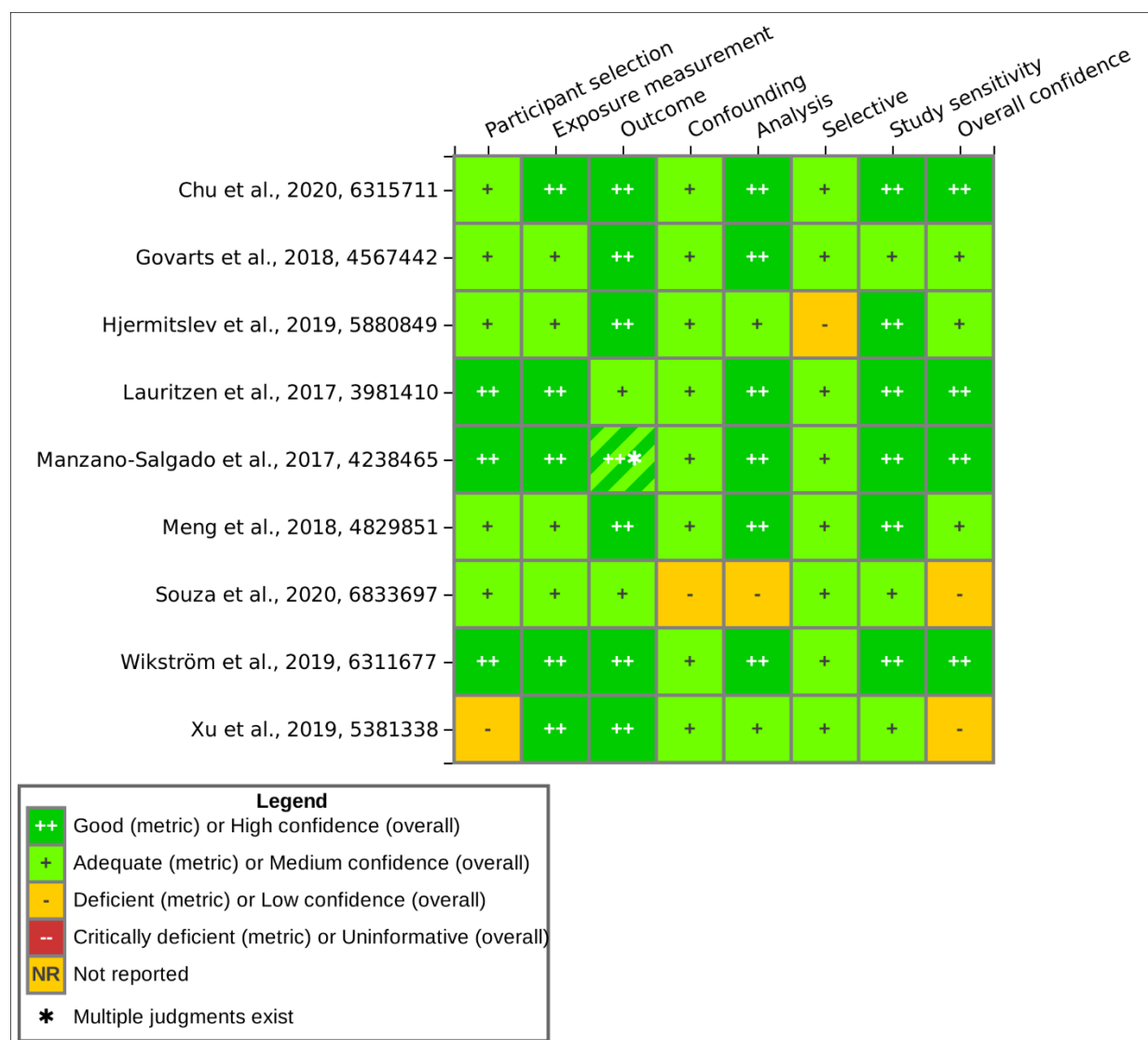


Figure 16. Summary of Study Evaluation for Epidemiology Studies of PFOS and Low Birth Weight or Small for Gestational Age Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.1.1.3.6 Birth Length

As shown in Figure 17, twenty-six birth length studies were considered as part of the study quality evaluation. Twenty non-overlapping and informative studies examined birth length in relation to PFOS with four examining standardized birth length measures only {Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Shoaff, 2018, 4619944; Xiao, 2019, 5918609}, and two evaluating both measures {Wang, 2019, 5080598; Workman, 2019, 5387046}. Nine studies examined sex-specific data with two studies {Marks, 2019, 5081319; Robledo, 2015, 2851197} reporting only sex-specific results (Figure 18, Figure 19). Fourteen studies examined mean birth length differences in the overall study population (Figure 20, Figure 21).

Seven of these 20 studies were *high* confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Shoaff, 2018, 4619944; Valvi, 2017, 3983872; Xiao, 2019, 5918609}, six were *medium* confidence {Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Robledo, 2015, 2851197; Wang, 2019, 5080598} and seven were *low* confidence studies {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2018, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Xu, 2019, 5381338}. Eleven PFOS studies had *good* study sensitivity, six had *adequate* sensitivity and three were considered *deficient*.

Six of the overall 20 birth length studies showed some adverse associations including three of the five studies that reported standardized birth length data. The *high* confidence study by Xiao et al. (2019, 5918609) reported reduced birth length z-scores (-0.33 ; 95% CI: $-0.69, 0.03$) in the overall population, and results comparable in magnitude for male (-0.41 ; 95% CI: $-0.87, 0.05$) and female neonates (-0.23 ; 95% CI: $-0.75, 0.30$) per each \log_2 increase in PFOS. Although smaller in magnitude, the *medium* confidence study by Chen et al. (2017, 3981292) also reported deficits in all three groups. They reported a birth length deficit of -0.16 gm (95% CI: $-0.31, -0.02$) in the overall population as well as male (-0.15 ; 95% CI: $-0.33, 0.03$) and female neonates (-0.20 ; 95% CI: $-0.44, 0.05$) per each ln unit PFOS increase. The other *high* confidence study by Shoaff et al. (2018, 4619944) of standardized birth length measures showed a deficit only for tertile 3 (-0.24 ; 95% CI: $-0.64, 0.15$) compared to tertile 1.

Fourteen of the studies examined mean birth length in the overall population with only two {Callan, 2017, 3858524; Lauritzen, 2017, 3981410} showing any adverse associations. The *high* confidence study by Lauritzen et al. (2017, 3981410) showed a small deficit in the overall population (-0.3 cm; 95% CI: $-0.7, 0.1$), but detected the strongest association when restricted to the Swedish population (-1.2 cm; 95% CI: $-2.1, -0.3$). The *high* confidence study by Valvi et al. (2017, 3983872) reported no associations in the overall population but did detect a non-significant birth length deficit in male neonates (-0.18 cm; 95% CI: $-0.60, 0.23$ per each PFOS \log_2 exposure increase). Among the two sex-specific only studies {Robledo, 2015, 2851197; Marks, 2019, 5081319}, the Marks et al. (2019, 5081319) *low* confidence study of boys only showed adverse associations (-0.52 cm; 95% CI: $-1.05, 0.01$ for tertile 3 vs. tertile 1).

In summary, only six of the 20 birth length studies showed some adverse associations. None of the five studies examining categorical data in either sex or the overall population showed any evidence of an exposure-response relationship. No patterns were evident across study characteristics or confidence levels. Overall, there is *limited* evidence for associations between PFOS and birth length.

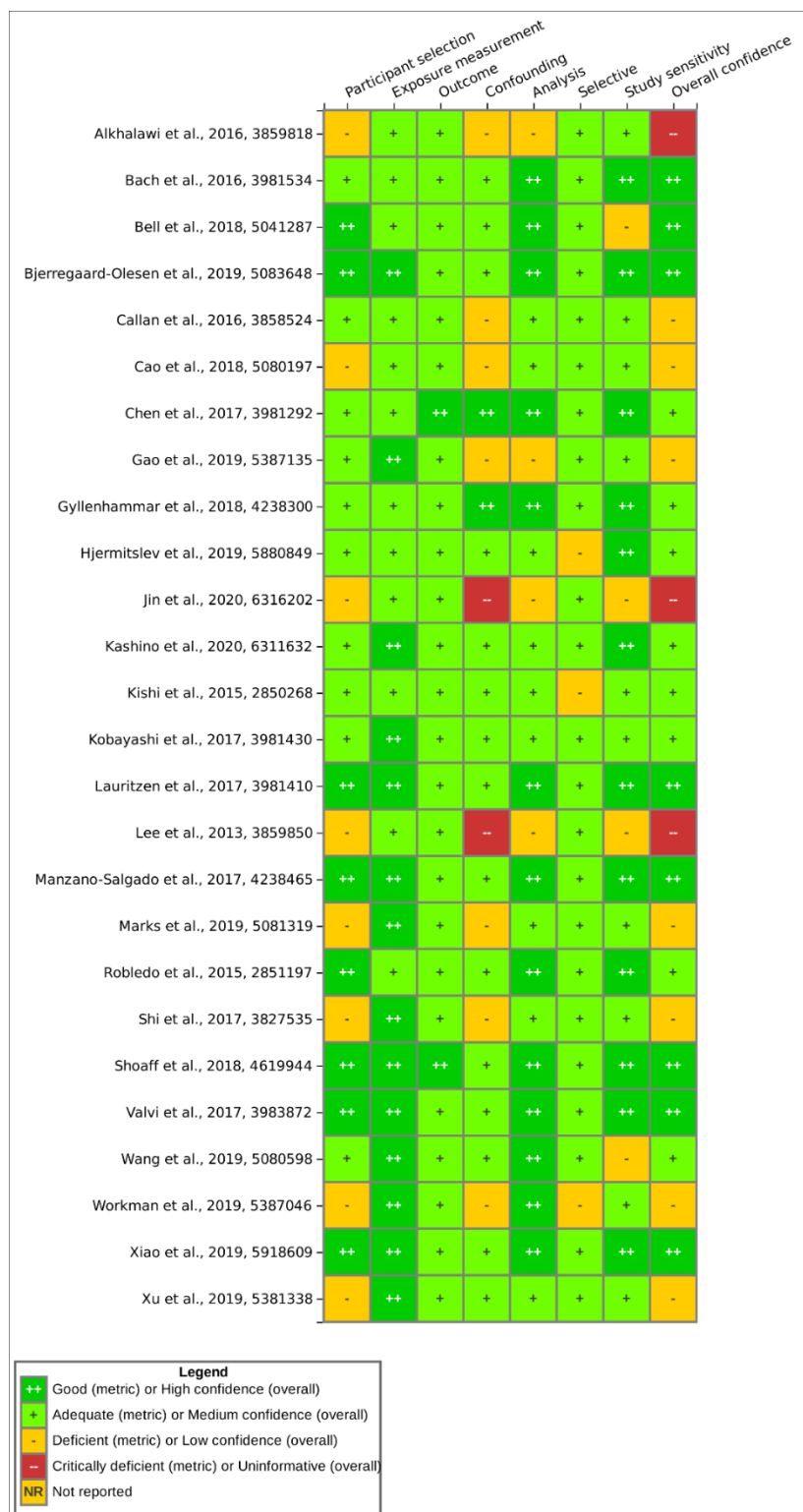


Figure 17. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Length Effects^a

Interactive figure and additional study details available on [HAWC](#).

^a Includes three overlapping studies (Bjerregaard-Olesen, Kishi, Kobayashi).

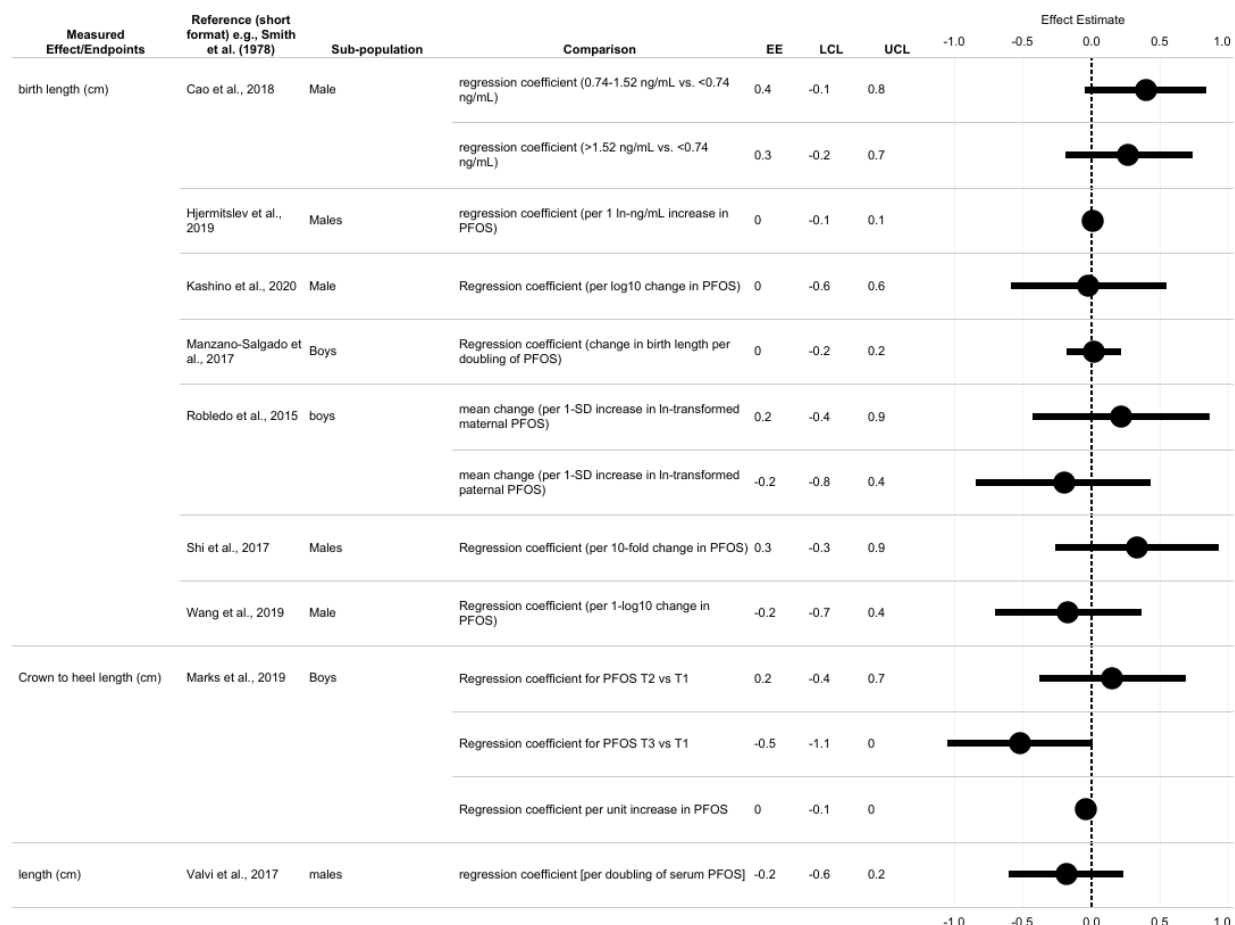


Figure 18. Mean Birth Length in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

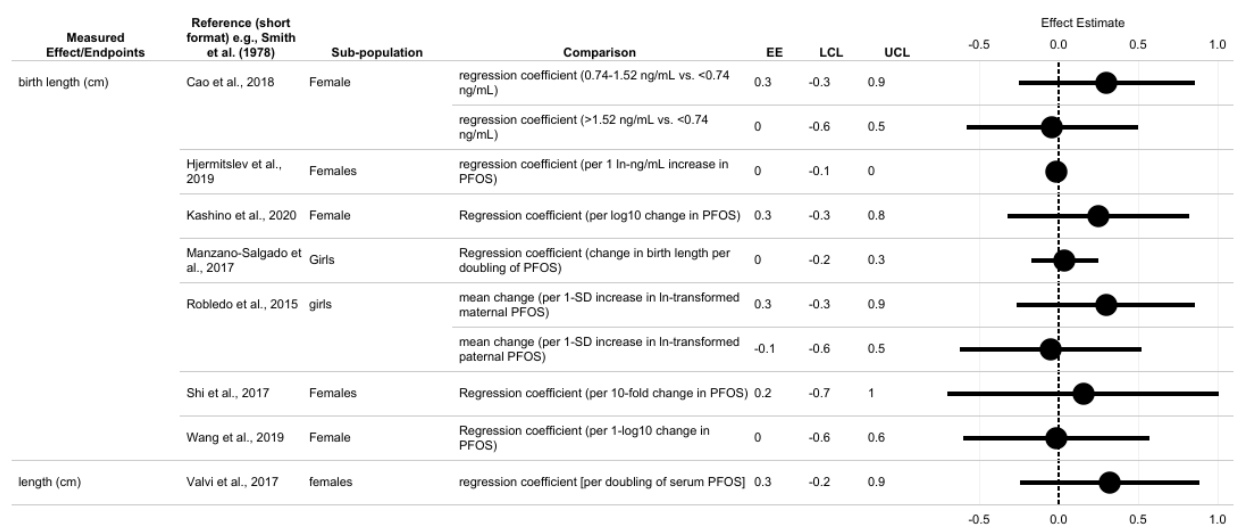


Figure 19. Mean Birth Length in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

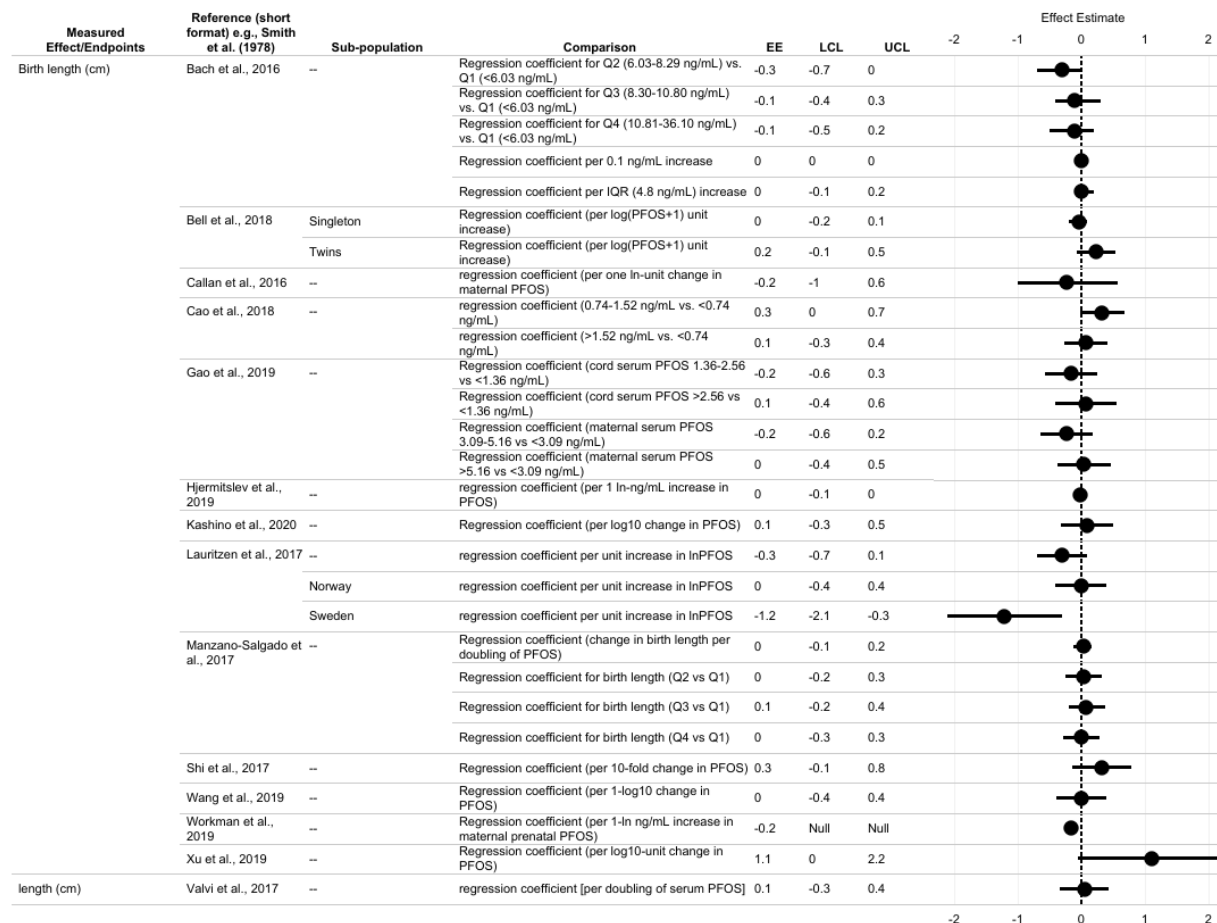


Figure 20. Overall Mean Birth Length from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

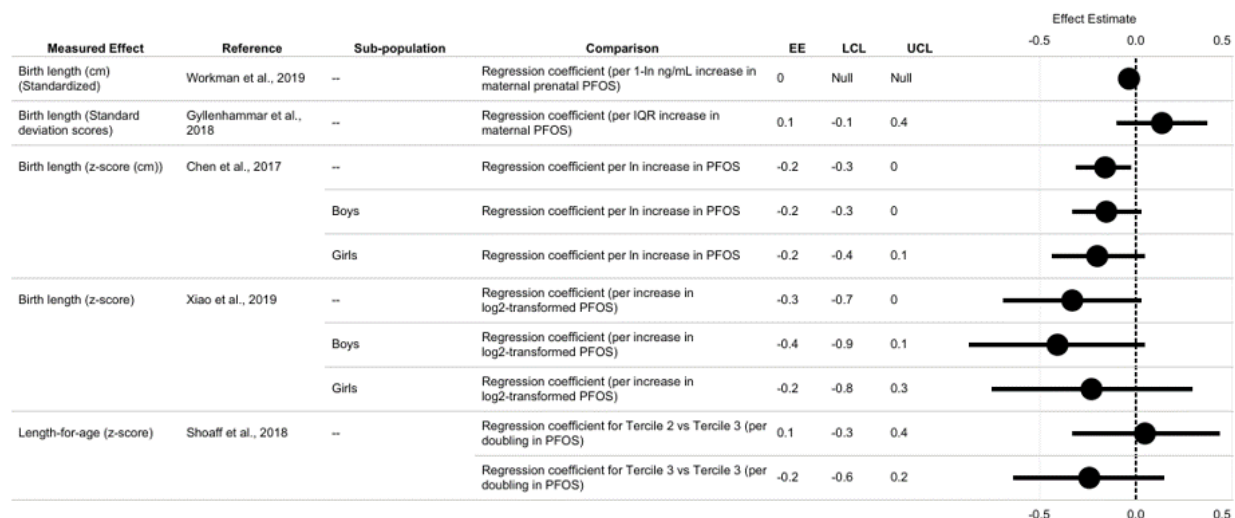


Figure 21. Overall Birth Length Z-scores from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.3.7 Head Circumference at Birth

As shown in Figure 22, 16 studies measured head circumference at birth. Four of the 16 studies were *low* confidence {Callan, 2016, 3858524; Marks, 2019, 5081319; Workman, 2019, 5387046; Xu, 2019, 5381338}, while studies six each were *medium* confidence {Gyllenhammar, 2018, 4238300; Hjermitsev, 2019, 5880849; Kashino, 2020, 6311632; Lind, 2017, 3858512; Robledo, 2015, 2851197; Wang, 2019, 5080598} and *high* confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872; Xiao, 2019, 5918609}. Four studies were *deficient* in study sensitivity, while six each had *good* and *adequate* study sensitivity. Fourteen of the 16 studies examined PFOS in relation to mean head circumference differences including eight studies with sex-specific data (Figure 23, Figure 24) and eleven studies with results in the overall population (Figure 25, Figure 26). Three of the mean head circumference studies {Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197} only reported sex-specific data, including the *low* confidence study by Marks et al. (2019, 5081319) which only examined male neonates. Two additional studies {Gyllenhammar, 2018, 4238300; Xiao, 2019, 5918609} examined unitless standardized measures not included on the forest plots.

Seven of the 16 studies reported some associations between PFOS and head circumference. Only one of eleven studies examining mean head circumference reported an association in the overall population with the *low* confidence study by Callan et al. (2016, 3858524) reporting a -0.39 cm (95% CI: $-0.98, 0.20$) difference per each \ln unit PFOS change. The *medium* confidence study by Lind et al. (2017, 3858512) reported deficits across all quartiles (range: 0.3 – 0.4 cm) but only in male neonates. The *high* confidence study by Valvi et al. (2017, 3983872) also reported deficits only in male neonates (-0.28 cm; 95% CI: $-0.65, 0.09$ per each doubling of serum PFOS exposures), while head circumference increases were found for female neonates (0.48 cm; 95% CI: $0.05, 0.90$). The *low* confidence study by Marks et al. (2019, 5081319), which only had data on male neonates with deficits in PFOS tertile 3 (-0.3 cm; 95% CI: $-0.6, 0.1$) compared to tertile

1 referent. Although the *medium* confidence study by Gyllenhammar et al. (2018, 4238300) (data not shown on figures) was null, the *high* confidence study by Xiao et al. (2019, 5918609) showed consistent head circumference z-score deficits across their overall population (-0.26 ; 95% CI: $-0.68, 0.16$), male (-0.15 ; 95% CI: $-0.68, 0.39$), and female neonates (-0.42 ; 95% CI: $-1.05, 0.21$) per each \log_2 increase in PFOS.

There was limited evidence of associations between PFOS and head circumference among the overall population in these epidemiological studies. Interestingly, mean head circumference deficits were detected in four out of eight sex-specific studies but only in male neonates. An additional standardized head circumference study showed deficits in both sexes, but larger deficits were noted among females. Although limited numbers across different study characteristic or overall confidence level sub-groups precluded a detailed assessments, few patterns were evident across the seven studies that showed adverse associations with head circumference. Only one of these seven studies had early pregnancy (i.e., trimester 1) samples, with six studies {Callan, 2016, 3858524; Lauritzen, 2017, 3981410; Marks, 2019, 5081319, Valvi, 2017, 3983872; Xiao, 2019, 5918609} based on either second and/or third trimester maternal samples. Overall, the evidence for head circumference was considered *limited* with seven of 17 total studies showing some evidence of adverse associations with some uncertainty as to what degree these results may be influenced by sample timing.

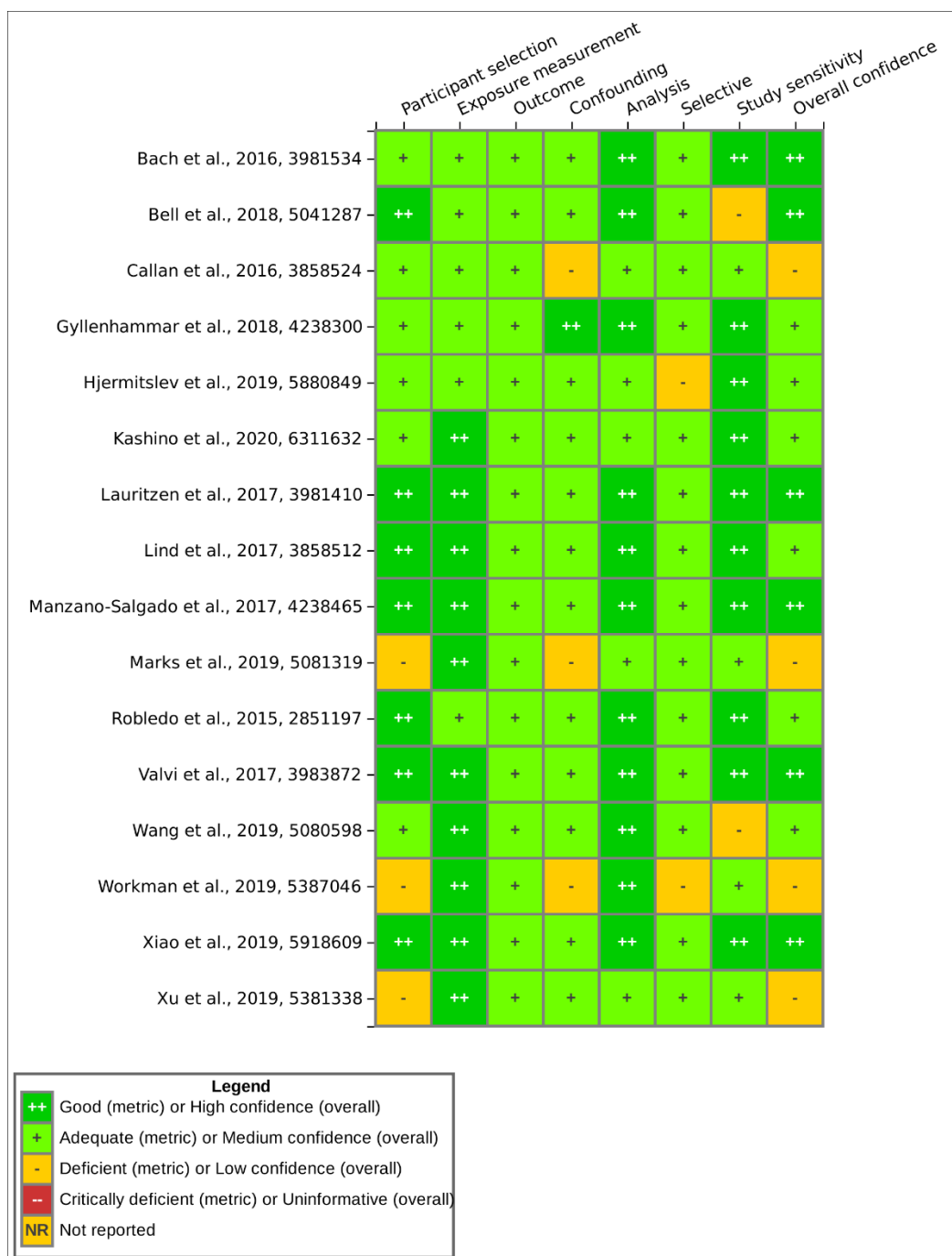


Figure 22. Summary of Study Evaluation for Epidemiology Studies of PFOS and Head Circumference Effects

Interactive figure and additional study details available on [HAWC](#).

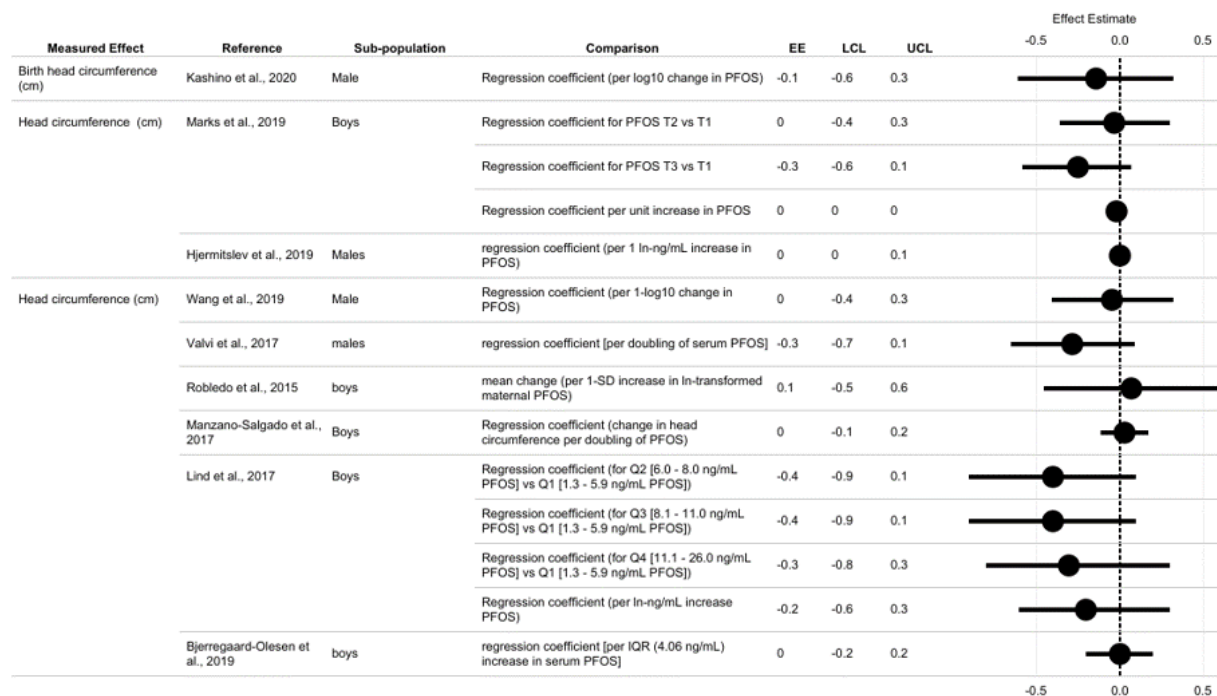


Figure 23. Head Circumference at Birth in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

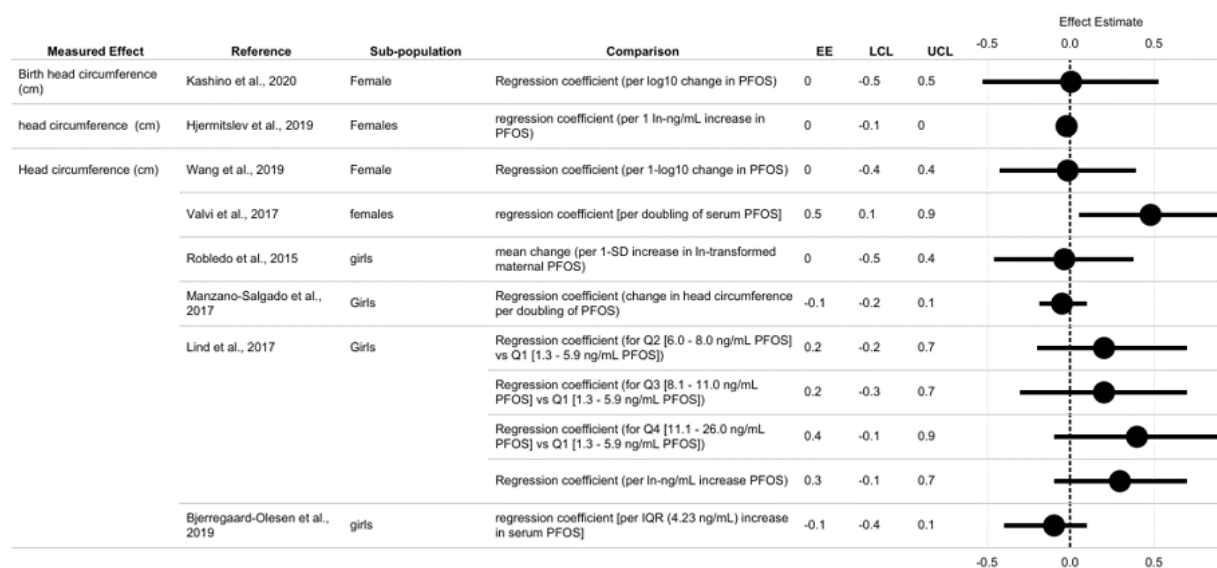


Figure 24. Head Circumference at Birth in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

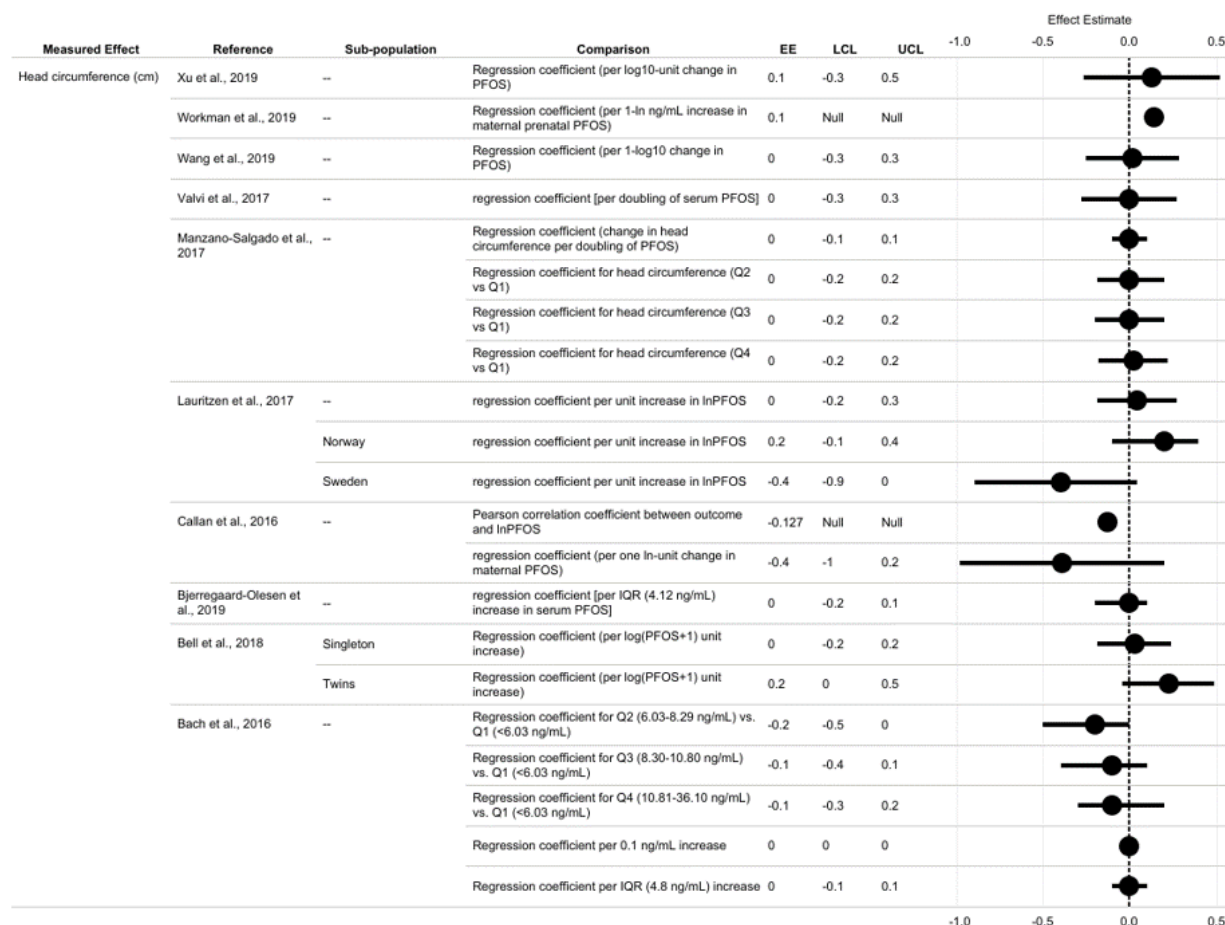


Figure 25. Overall Head Circumference at Birth from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

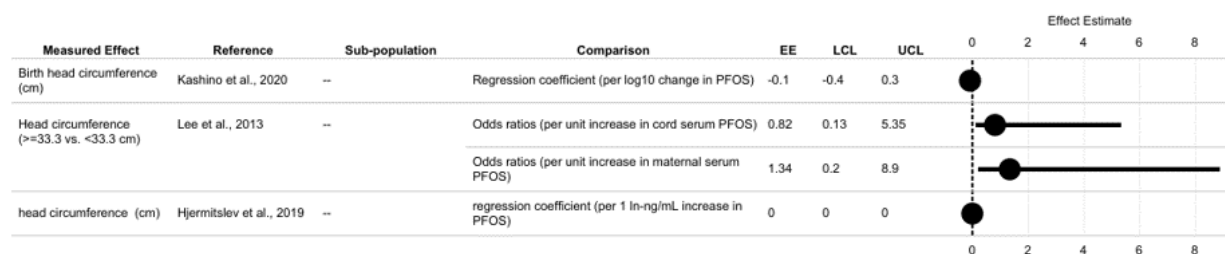


Figure 26. Overall Head Circumference at Birth from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.3.8 Fetal Growth Restriction Summary

The majority of studies examining fetal growth restriction showed suggestive evidence of associations with PFOS exposures especially those that included BWT data (i.e., SGA, low

BWT, as well as mean and standardized BWT measures). The evidence for two fetal growth measures such as head circumference and birth length were less consistent.

Among the most accurate fetal growth restriction endpoints examined here, there was generally consistent evidence for birth weight deficits across different measures and types of PFOS exposure metrics considered. BWT deficits were detected in the roughly three-fourths of included studies whether measured as mean BWT or standardized z-scores. This included 19 out of 26 mean BWT studies in the overall population but only 9 of 22 *medium* or *high* confidence studies. Most of the sex-specific mean BWT studies showed some adverse associations in either male or female neonates, and although it wasn't consistent across studies, more deficits were found in male neonates. There was a wide variety of deficits that were detected 11 to 417 grams across both categorical and continuous exposure estimates and may be in part due to vastly different exposure contrasts and comparisons being made across studies. As noted above, many of the individual study results lacked precision and were not statistically significant especially the sex-stratified results which may have been largely underpowered to detect sex-specific differences. There was limited evidence of exposure-response relationships across categorical data for the overall population or different sexes.

One strength of the current database (since the 2016 HESD) is that most studies considered here (e.g., 22 out of 29 mean BWT) were *high* or *medium* confidence and most of them had adequate or good study sensitivity, so the database is fairly robust. As noted earlier, one source of uncertainty is that previous meta-analyses of PFOS by Dzierlenga et al. (2020, 7643488) and PFOA by Steenland et al. (2018, 5079861) have shown that some measures like mean BWT may be prone to bias from pregnancy hemodynamics especially in studies with sampling later in pregnancy. Although a limited number of studies across some strata does not fully lend itself to differentiating patterns across different study characteristics, like study confidence and sample timing, a few patterns emerged across the study results. For example, there was some evidence of more consistent results for studies that sampled later in pregnancy for endpoints such as head circumference and some of the mean BWT data in both the overall population and across the sexes. This would seem to comport with the meta-analysis by Dzierlenga et al., (2020 7643488) that suggested that results for mean BWT may be impacted by some bias due to pregnancy hemodynamics. Thus, across these fetal growth endpoints there is evidence of an association between fetal growth restriction and PFOS exposure, although important uncertainties remain mainly around the degree that some of the results examined here may be influenced by sample timing.

3.3.1.1.4 Postnatal growth

Ten studies examined PFOS exposure in relation to postnatal growth measures {Cao, 2018, 5080197; Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Jensen, 2020, 6833719; Lee, 2018, 4238394; Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619} (Figure 27). The synthesis here is focused on postnatal growth measures including mean and standardized weight (all 10 studies above), height {Cao, 2018, 5080197; Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Lee, 2018, 4238394; Shoaff, 2018, 4619944; Yeung, 2019, 5080619}, body mass index (BMI)/adiposity measures {de Cock, 2014, 2713590; Jensen, 2020, 6833719; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619} and rapid growth during infancy { Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling,

2019, 5412449; Yeung, 2019, 5080619}. Five {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619} postnatal growth studies were *high* confidence, three {Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300} were *medium* confidence, and two {Cao, 2018, 5080197; Lee, 2018, 4238394} were *low* confidence. Seven postnatal growth studies had good study sensitivity, two were *adequate* and one was *deficient*.

In their *high* confidence study, Yeung et al. (2019, 5080619) reported statistically significant negative growth trajectories for BMI, BMI z-score, and weight-for-length z-scores in relation to each log SD increase in PFOS exposures among singletons followed for three years. No exposure-response relationship was detected for BMI z-scores across PFOS quartiles, and no associations were detected for infant length (i.e., height) measures. In their *high* confidence study of repeated measures at 4 weeks, 1 year and 2 years of age, Shoaff et al. (2018, 4619944) detected statistically significant deficits for infant BMI (−0.36; 95% CI: −0.60, −0.12), weight for age (−0.46; 95% CI: −0.78, −0.14), and weight-for-length z-scores (−0.34; 95% CI: −0.59, −0.08) in PFOS tertile 3 compared to tertile 1 with exposure-response relationships detected for infant weight for length z-scores. Small deficits that were not statistically significant were observed in tertile 3 for length for age z-score (−0.22; 95% CI: −0.49, 0.04). The *high* confidence study by Manzano-Salgado et al. (2017, 4238509) reported null associations for their overall population, female, and male neonates for weight gain z-score measured at 6 months per each log₂ PFOS increase. The *high* confidence study by Jensen et al. (2020, 6833719) reported null associations between adiposity and per each 1-unit increase in PFOS measured at 3 and 18 months.

The *low* confidence study by Lee et al. (2018, 4238394) reported statistically significant associations per each PFOS ln unit increase for height at age 2 years (−0.77 cm; 95% CI: −1.27, −0.15) as well as height change from birth to 2 years (−0.71 cm; 95% CI: −1.27, −0.15). Small differences were seen for mean weight differences at age 2 years (−0.17 cm; 95% CI: −0.38, 0.04) but not for weight change from birth to 2 years. Although a statistically significant birth length association was detected, the *medium* confidence study by Chen et al. (2017, 3981292) reported no association with infant height z-score up to 24 months. They did report statistically significant infant weight z-scores among girls comparable in magnitude for 6–12 months (−0.25; 95% CI: −0.47, −0.04) or 12–24 months (−0.25; 95% CI: −0.41, −0.06) per each ln unit PFOS increase. The *medium* confidence study by de Cock et al. (2014, 2713590) did not report effect estimates but indicated that there were no statistically significant associations between PFOS quartiles and infant BMI (p-value = 0.59), infant weight (p-value = 0.80), and infant height (p-value = 0.98) measures up to 11 months of age.

Compared to the tertile 1 referent, the *low* confidence study of infants followed up to a median age of 19.7 months by Cao et al. (2018, 5080197) reported slight increases in postnatal length (i.e., height) (1.37 cm; 95% CI: −0.5, 3.28), while large postnatal weight deficits were reported for PFOS tertiles 2 (−138 g; 95% CI: −574, 298) and 3 (−78 g; 95% CI: −532, 375). The *medium* confidence study by Gyllenhammar et al. (2018, 4238300) did not show standardized BWT deficits per each IQR PFOS change, but they showed slight deficits (~ −0.2) at 3 months that persisted throughout 60 months of age. In contrast, standardized birth length measures were null for increasing PFOS exposures regardless of time window examined. Associations at five months of age in the overall population (−0.28; 95% CI: −0.51, −0.05) and females (−0.56; 95%

CI: -0.87, -0.26) from the *high* confidence study by Starling et al. (2019, 5412449) were detected for weight-for-age z-scores, as well as weight-for-length z-scores (overall: -0.26; 95% CI: -0.53, 0.0; females; -0.52; 95% CI: -0.88, -0.17). They also detected decreased adiposity (-2.08; 95% CI: -3.81, -0.35) among girls in PFOS tertile 3 compared to the tertile 1 referent, and a small OR of 1.36 was detected for rapid growth in the overall population based on either weight for length-based z-scores. The other three *high* confidence studies were null for rapid infant growth {Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Yeung, 2019, 5080619}.

Seven of the 10 studies examining different infant weight measures showed some evidence of adverse associations with PFOS exposures either in the overall population or either/or both male or female neonates. No patterns by study characteristics or study confidence including study sensitivity were evident as there was one *low*, one *medium* and one *high* confidence studies among the three studies not showing adverse associations with infant weight. Only two (one *low* and one *high* confidence) of the eight studies examining different infant height measures showed some evidence of adverse associations with PFOS exposures. None of the five postnatal growth studies showed increased infant BMI or adiposity with increasing PFOS exposures, while three showed decreased risk of higher BMI or adiposity. Only one out of four *high* confidence studies showed any evidence of rapid growth among infants following PFOS exposures. Although the data for some endpoints was less consistent, the majority of infant weight studies indicated that PFOS may be associated with post-natal growth measures up to two years of age. Overall, the evidence for postnatal associations is *slight* predominately due to the infant weight results.

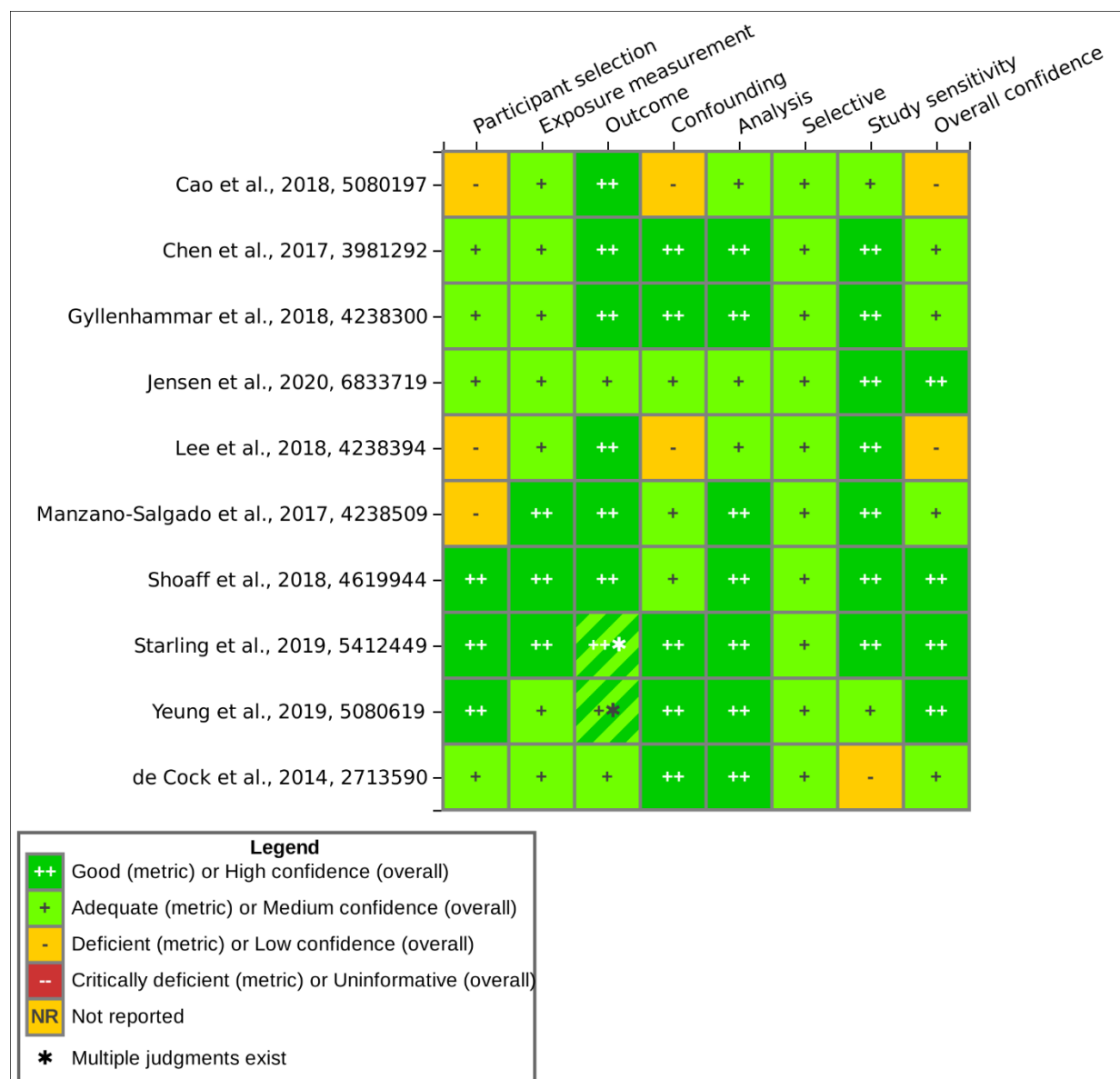


Figure 27. Summary of Study Evaluation for Epidemiology Studies of PFOS and Postnatal Growth Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.1.1.5 Gestational Duration

Seventeen studies examined gestational duration measures (i.e., preterm birth or gestational age measures) in total including one uninformative study {Lee, 2013, 3859850} and one overlapping cohort {Li, 2017, 3981358} (Figure 28). One additional study by Bangma et al. (2020, 6833725) did not examine gestational age as a dependent variable which precluded direct comparison to other results.

3.3.1.1.5.1 Gestational Age

Sixteen studies examined gestational age in relation to PFOS exposures including one uninformative study {Lee, 2013, 3859850} and one overlapping cohort {Li, 2017, 3981358}. 14 non-overlapping and informative studies examined mean gestational age (in weeks) in relation to PFOS exposures. Seven of the 13 studies examined here were *high* confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Chu, 2020, 6315711; Huo, 2020, 6835452; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Sagiv et al. 2018, 4238410}, four were *medium* {Gyllenhammar, 2018, 4238300; Hjerimitslev, 2019, 5880849; Lind, 2017, 3858512; Meng, 2018, 4829851} and three were *low* confidence {Gao, 2019, 5387135; Workman, 2019, 5387046; Xu, 2019, 5381338}. Nine of these studies had *good* study sensitivity, three were *adequate* and one was *deficient*.

Eight of the 14 studies showed some evidence of adverse impacts on gestational age. Among these, four were *high* confidence, and two each were *medium* and *low* confidence. The *high* confidence study by Sagiv et al. (2018, 4238410) reported (0.36 weeks; 95%CI: -0.64, -0.09) for PFOS quartile 4 versus quartile 1. The *high* confidence study by Chu et al. (2020, 6315711) reported similar deficits in the overall population (-0.32 weeks; 95% CI: -0.53, -0.11) which was driven by female neonates (-0.61 weeks; 95% CI: -0.90, -0.32). The *high* confidence study by Lauritzen et al. (2017, 3981410) only showed deficits among their Swedish population (-0.4 weeks; 95% CI: -0.9, 0.2). Compared to tertile 1, the *low* confidence study by Gao et al. (2019, 5387135) reported deficits in tertile 2 (-0.47 weeks; 95% CI: -0.95, 0.01) and tertile 3 (-0.15; 95% CI: -0.63, 0.33). The *high* confidence study by Manzano-Salgado et al. (2017, 4238465) reported deficits in quartile 4 among the overall population (-0.31 weeks; 95% CI: -0.55, -0.06) compared to quartile 1. The *medium* confidence study by Gyllenhammar et al. (2018, 4238300) reported a deficit similar in magnitude (-0.29 weeks; 95% CI: -0.59, 0.01). The *medium* confidence study by Meng et al. (2018, 4829851) also reported statistically significant gestational age deficits (range -0.16 to -0.29 weeks) across all quartiles but no evidence of an exposure-response relationship. The *low* confidence study by Workman et al. (2019, 5387046) reported a non-significant decrease (-0.17 weeks; p-value = 0.34) per each ln unit PFOS change. The seven studies that showed deficits in the overall population were fairly consistent in magnitude (range: 0.15–0.36 weeks for different PFOS contrasts) (Figure 29, Figure 30, Figure 31, Figure 32).

Overall, eight of 14 studies and six out of 11 *medium* and *high* confidence studies showed some adverse associations between PFOS and gestational age. Few patterns emerged based on study confidence or other study characteristics, although two of the five studies that did not show adverse associations had limited exposure contrasts and *deficient* study sensitivity.

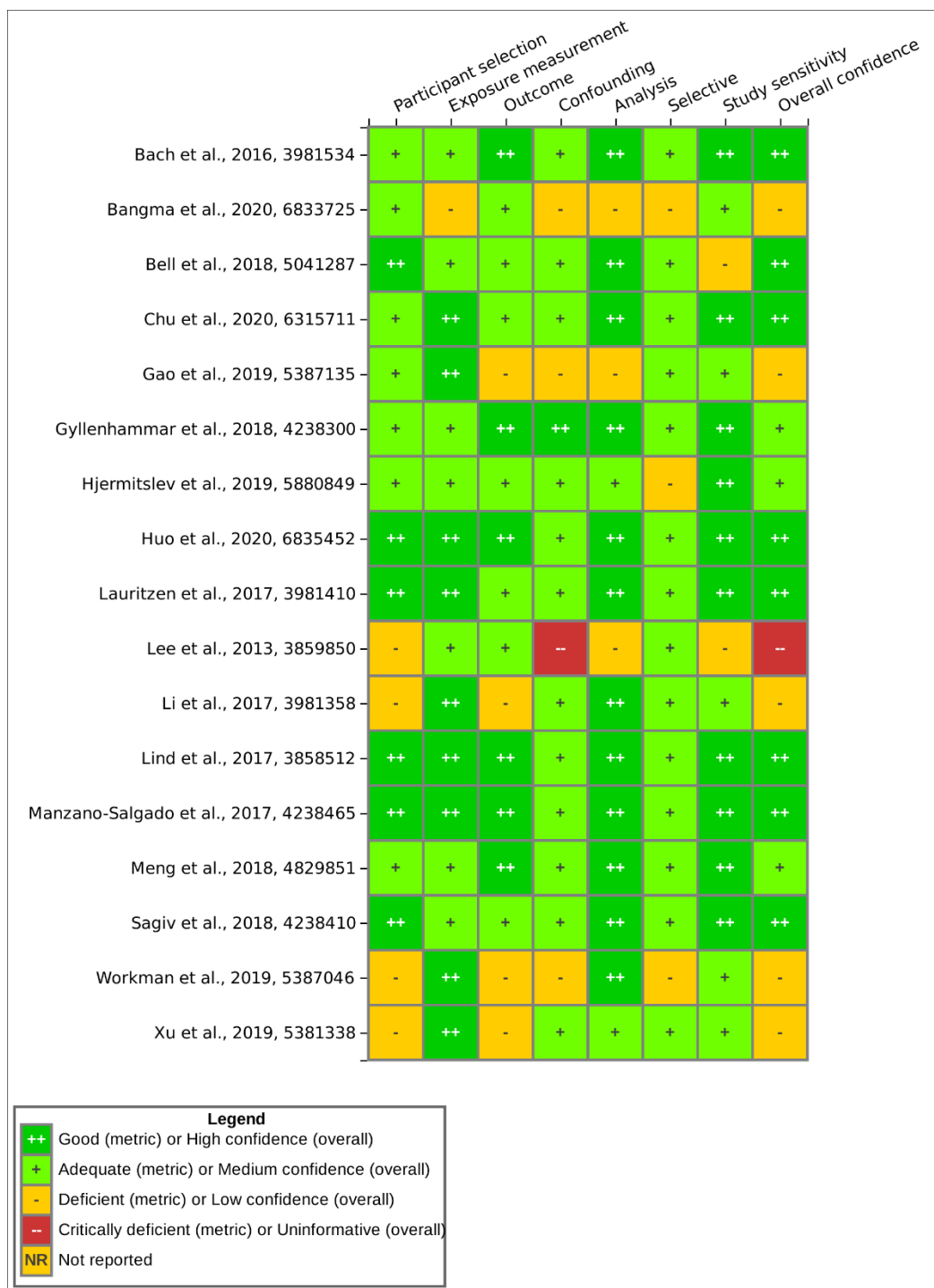


Figure 28. Summary of Study Evaluation for Epidemiology Studies of PFOS and Gestational Age Effects^a

Interactive figure and additional study details available on [HAWC](#).

^a Includes one overlapping study (Li et al., 2017).

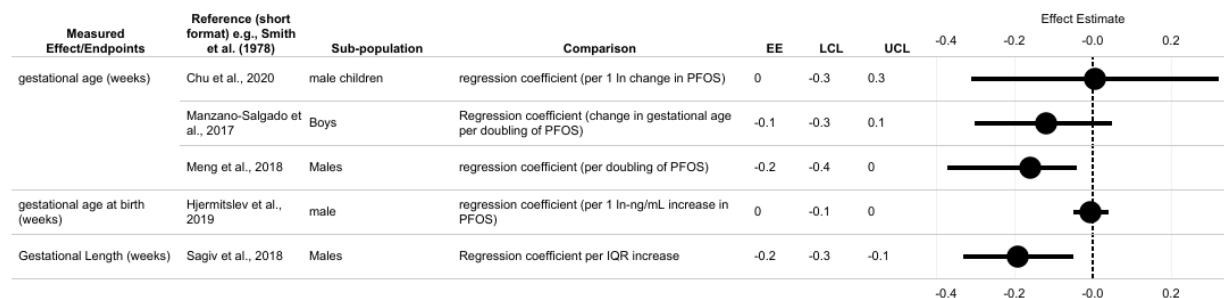


Figure 29. Gestational Age in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

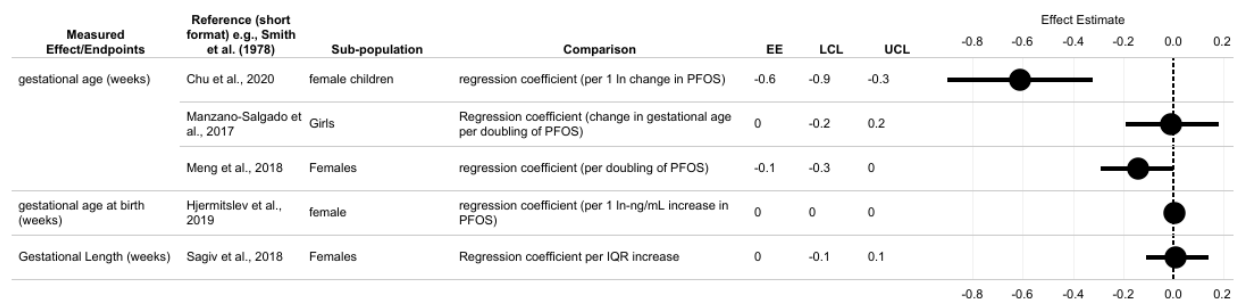


Figure 30. Gestational Age in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

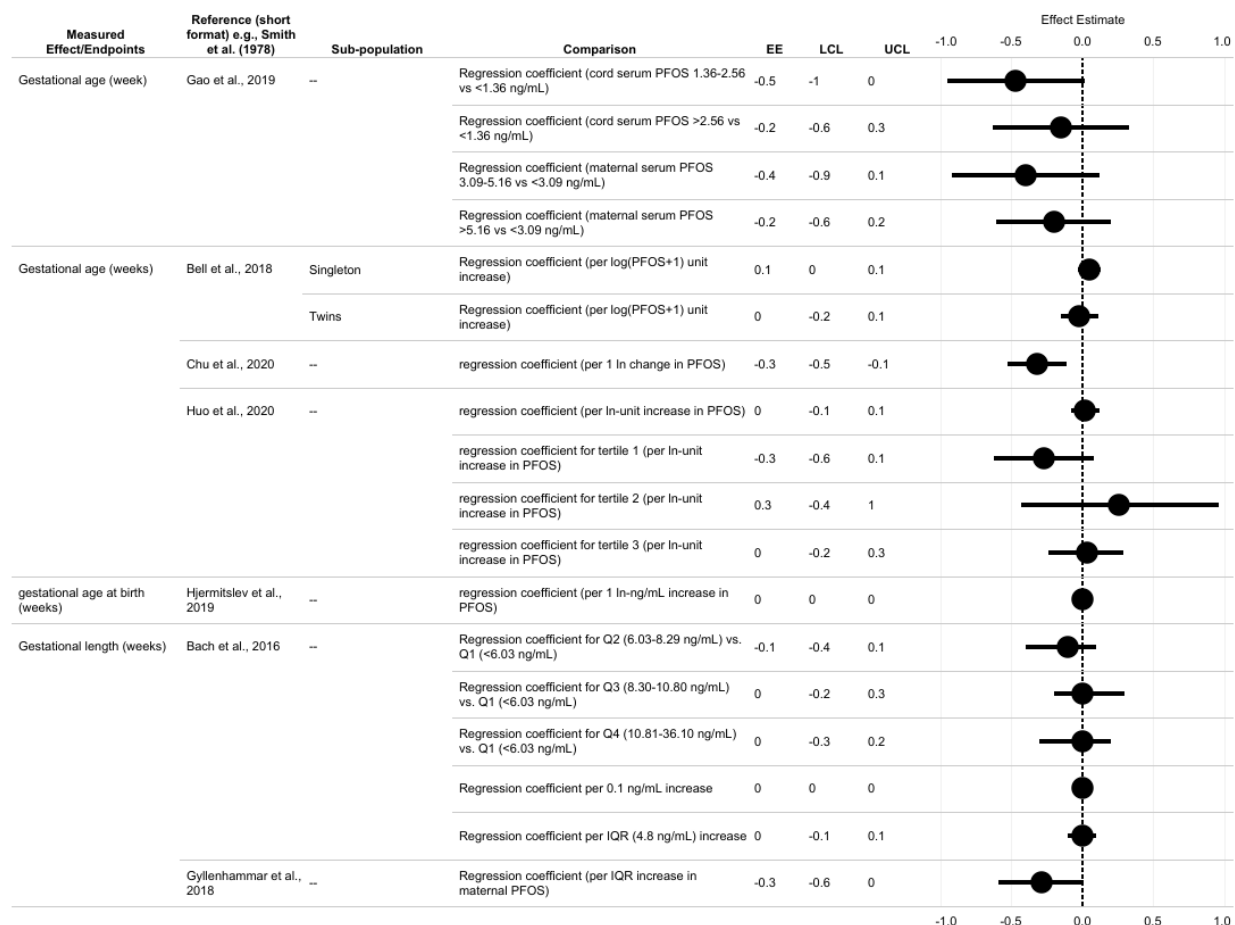


Figure 31. Overall Gestational Age from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

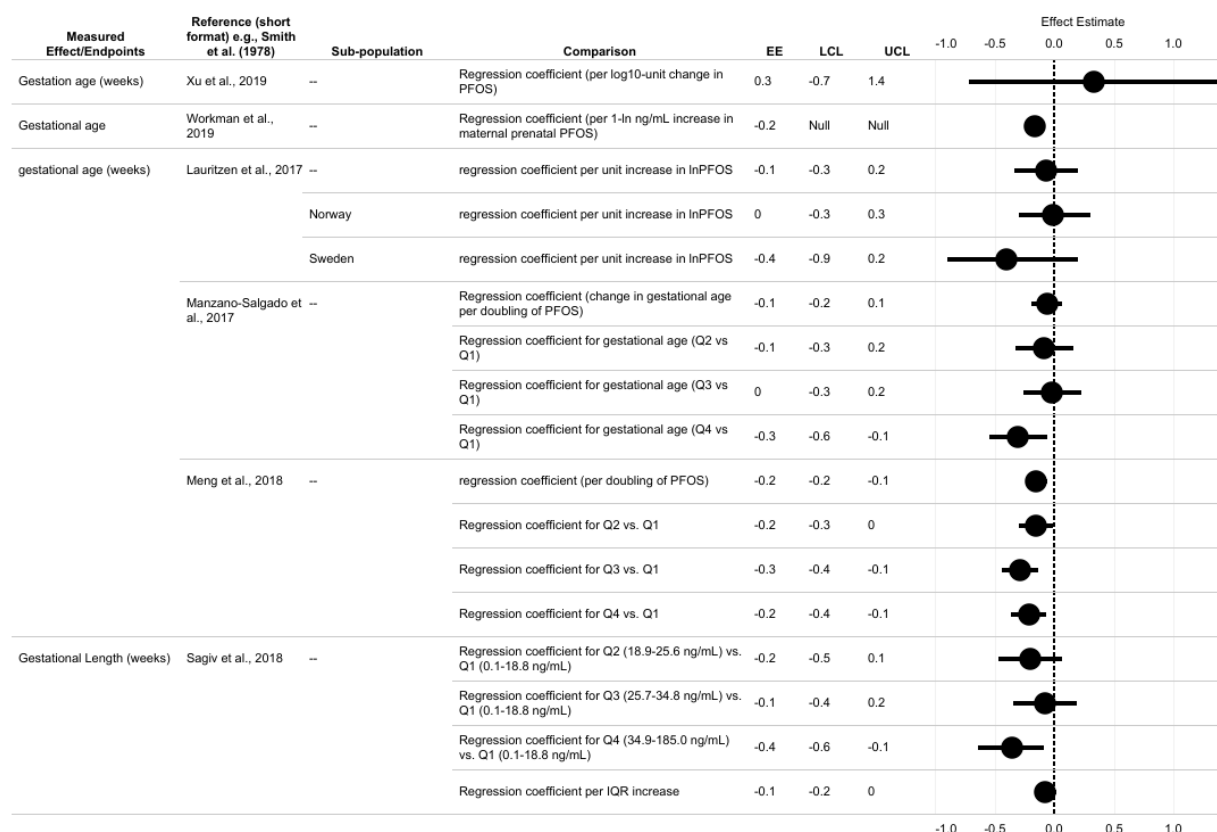


Figure 32. Overall Gestational Age from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.5.2 Preterm Birth

As shown in Figure 33, eight studies examined the relationship between PFOS and preterm birth (PTB); all of the studies were either *medium* {Hjermitslev, 2019, 5880849; Liu, 2020, 6833609; Meng, 2018, 4829851} or *high* confidence {Bach, 2016, 3981534; Chu, 2020, 6315711; Huo, 2020, 6835452; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}. Seven of the eight studies were prospective birth cohort studies, while the study by Liu et al. (2020, 6833609) was a case-control study. Three studies had maternal exposure measures that were sampled during trimester one {Bach, 2016, 3981534; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}, and one study sampled during the late third trimester or within three days of delivery {Chu, 2020, 6315711}. Three studies collected samples across multiple trimesters {Hjermitslev, 2019, 5880849; Huo, 2020, 6835452; Liu, 2020, 6833609} and Meng et al. (2018, 4829851) pooled umbilical cord blood and maternal serum (trimester 1 and 2) exposure data from two study populations. All but one of the studies had *good* study sensitivity (Chu et al. 2020, 6315711 which was *adequate*) with the median exposure values ranging from 1.79 ng/mL {Liu et al. 2020, 6833609} to 30.1 ng/mL {Meng, 2018, 4829851}.

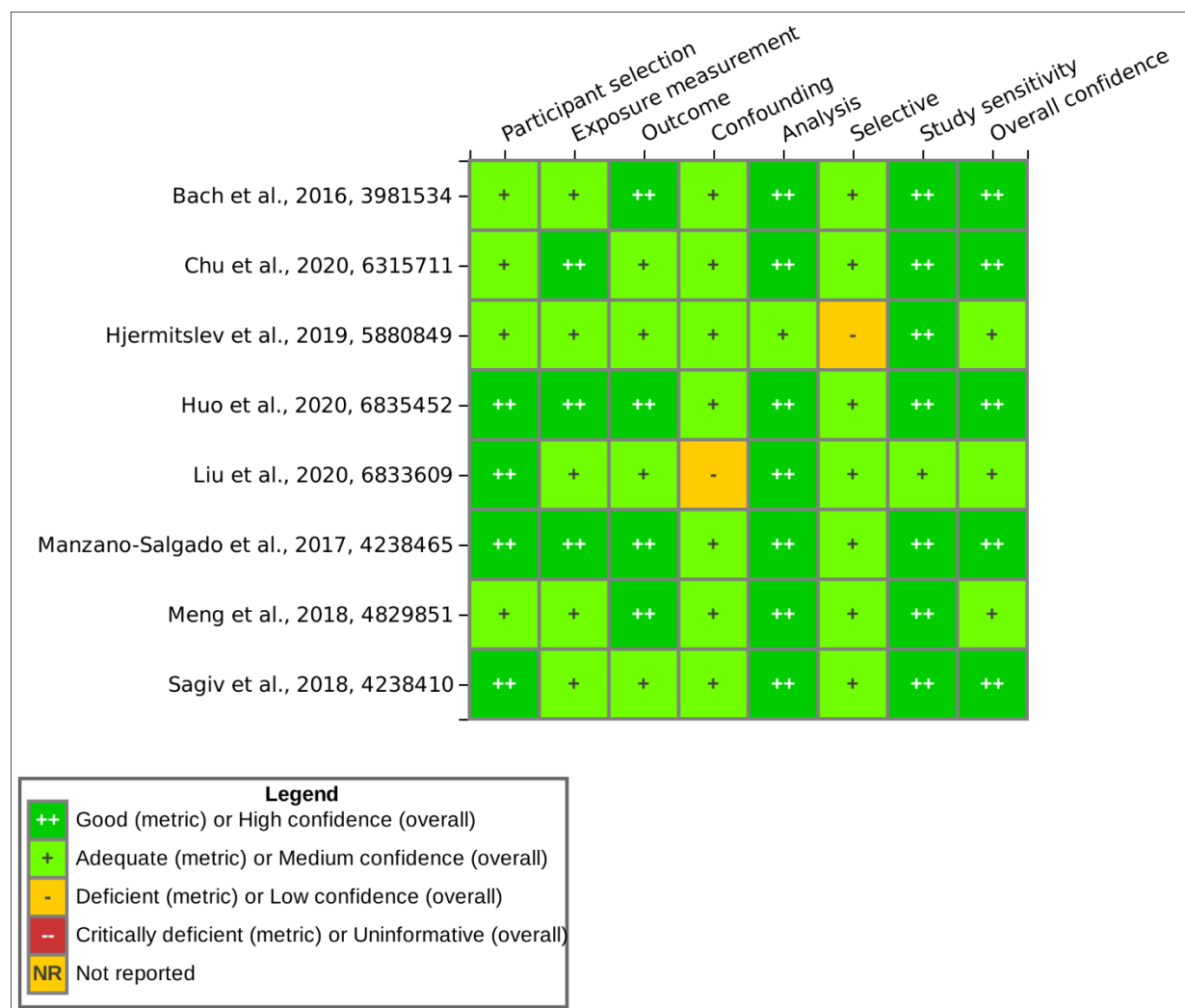


Figure 33. Summary of Study Evaluation for Epidemiology Studies of PFOS and Preterm Birth Effects

Interactive figure and additional study details available on [HAWC](#).

Adverse associations were reported in four of the eight PTB studies with ORs from 1.5- to 5-fold higher for elevated PFOS exposures. The *medium* confidence study by Meng et al. (2018, 4829851) study reported statistically significant non-monotonic increased ORs for PTB in the upper three PFOS quartiles (OR range: 1.9–3.3), as well as per each doubling of PFOS exposures (OR = 1.5; 95% CI: 1.1, 2.2). The *high* confidence study by Chu et al. (2020, 6315711) reported some statistically significant increased ORs per 1 ln ng/mL unit increase (OR = 2.03; 95% CI: 1.24, 3.32) as well as an exposure-response relationship across upper three quartile (OR range: 2.22–4.99) exposures when compared to the referent. Although they were not statistically significant, the *medium* confidence study by Liu et al. (2020, 6833609) reported increased ORs of similar magnitude per each log₁₀ unit increase (OR = 1.30; 95% CI: 0.76, 2.21) or when quartile 3 (OR = 1.51; 95% CI: 0.85, 2.69) and quartile 4 (OR = 1.35; 95% CI: 0.74, 2.45) exposures were compared to the referent. The *high* confidence study by Sagiv et al. (2018, 4238410) study reported consistently elevated non-monotonic ORs for PTB in the upper three

PFOS quartiles (OR range: 2.0–2.4), but smaller ORs when examined per each IQR increase in PFOS exposures (OR = 1.1; 95% CI: 1.0, 1.3). Null or inverse associations were reported by Bach et al. (2016, 3981534), Huo et al. (2020, 6835452), Manzano-Salgado et al. (2017, 4238465) and Hjermitsev et al. (2019, 5880849) (Figure 34, Figure 35, Figure 36).

Few patterns in the PTB results emerged based on study confidence or other study characteristics. Since nearly all studies had *good* study sensitivity, study sensitivity did not largely appear to be a concern in this database. In addition, only one out of the four studies that did not show adverse associations had limited exposure contrasts.

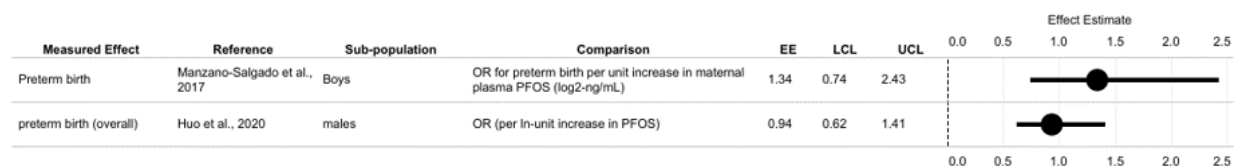


Figure 34. Preterm Birth in Males Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

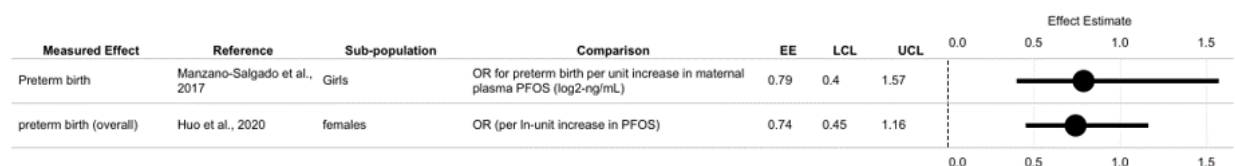


Figure 35. Preterm Birth in Females Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

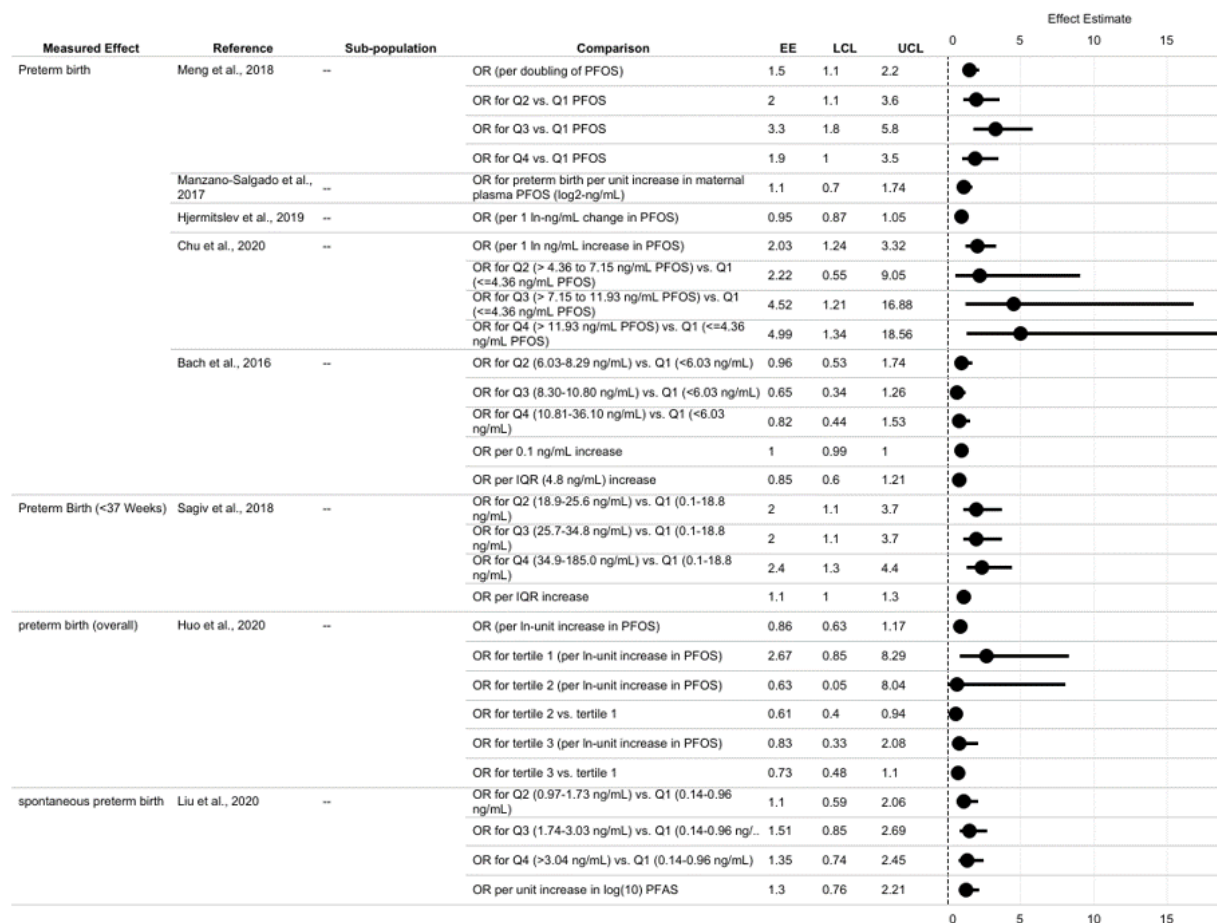


Figure 36. Overall Preterm Birth from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on [Tableau](#).

3.3.1.1.5.3 Gestational Duration Summary

Overall, there is *moderate* evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as most of studies showed some adverse associations. This was strengthened by consistency in the reported magnitude of gestational age deficits despite different exposure levels and metrics examined. Although they were not as consistent (one-half of the PTB studies showed some adverse associations), some of the effect estimates were large for preterm birth in relation to PFOS exposures with limited evidence of exposure-response relationships. Few patterns were evident as explanatory factors for heterogeneous results based on our qualitative analysis.

3.3.1.1.6 Fetal Loss

As shown in Figure 37, three (2 *medium* and 1 *low* confidence) studies examined PFOS exposure and fetal loss. Two of these had *deficient* study sensitivity {Buck Louis, 2016, 3858527; Jensen, 2015, 2850253}, while one had *adequate* {Liew, 2020, 6387285}. Although the ORs were not statistically significant in the *medium* confidence study by Liew et al. (2020, 6387285), there was some suggestion of an exposure-response for miscarriages across PFOS quartiles (OR range: 1.1–1.4). Similarly, the *low* confidence study by Jensen et al. (2015, 2850253) also showed some

suggestion of an exposure-response in tertiles 2 and 3 (OR range: 1.15–1.33) albeit with results that were not statistically significant. No association was detected in the *medium* confidence study by Buck Louis et al. (2016, 3858527) study (hazard ratio (HR) = 0.81; 95% CI: 0.65, 1.00) per each SD increase in PFOS exposures. The overall evidence was *limited* given the increased relative risk estimates were low in magnitude, although there was a suggestion of an exposure-response relationship in two of the three studies.

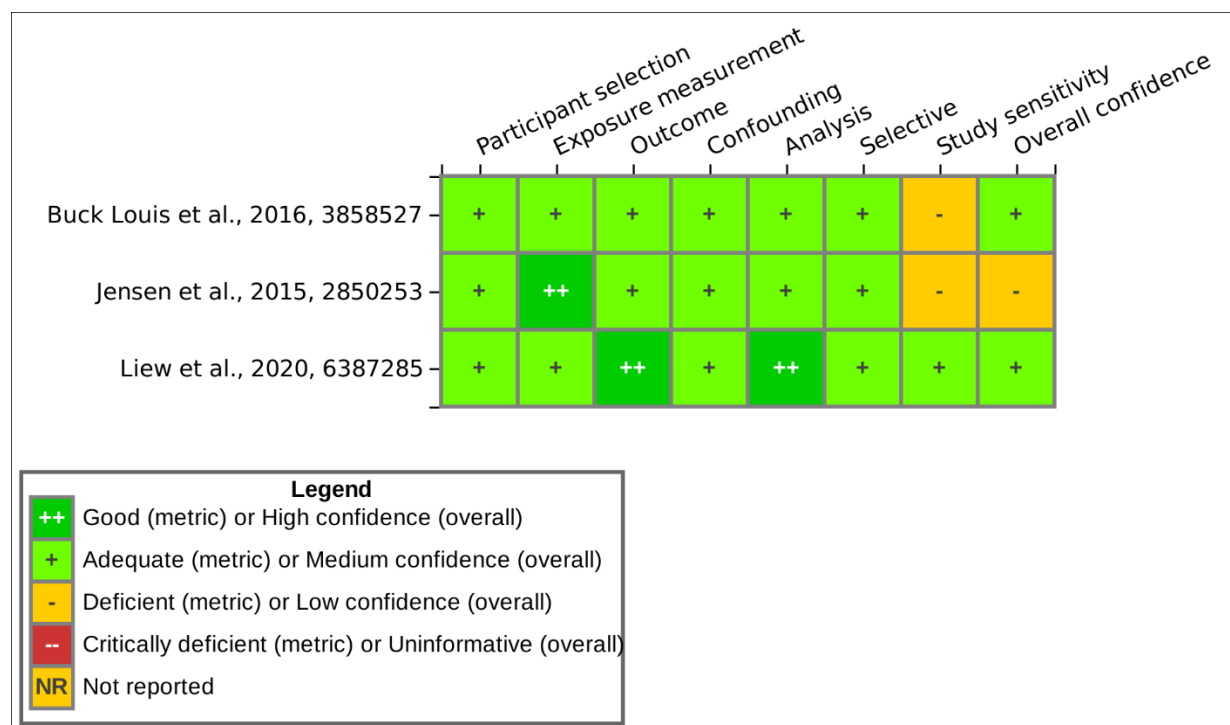


Figure 37. Summary of Study Evaluation for Epidemiology Studies of PFOS and Fetal Loss Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.1.1.7 Birth Defects

Four studies examined PFOS exposure and birth defects. As shown in Figure 38, four (2 *medium* and 2 *low* confidence) studies examined PFOS exposure. Three of the four had *adequate* study sensitivity. This included one *low* confidence study of a non-specific grouping of all birth defects {Cao, 2018, 5080197} that reported a small but imprecise increased risk (OR = 1.27; 95% CI: 0.59, 2.73). Three studies examined PFOS exposures in relation to cryptorchidism. The *medium* confidence study by Vesterholm Jensen et al. (2014, 2850926) detected an inverse association for cryptorchidism (OR = 0.51; 95% CI: 0.21–1.20) per each ln-unit increase in PFOS exposures. This risk seemed to be largely driven by boys from Finland. The *medium* confidence study by Toft et al. (2016, 3102984) reported null associations per each ln-unit increase in PFOS exposures and both cryptorchidism (OR = 0.99; 95% CI: 0.75–1.30) and hypospadias (OR = 0.87; 95% CI: 0.57–1.34). The *low* confidence study by Anand-Ivell et al. (2018, 4728675) did not find statistically significant PFOS exposure differences among cryptorchidism or hypospadias cases compared to controls, but they did not examine this in a multivariate fashion adjusting for

confounders. Overall, there was *very limited* evidence of associations between PFOS and birth defects based on the available epidemiological studies.

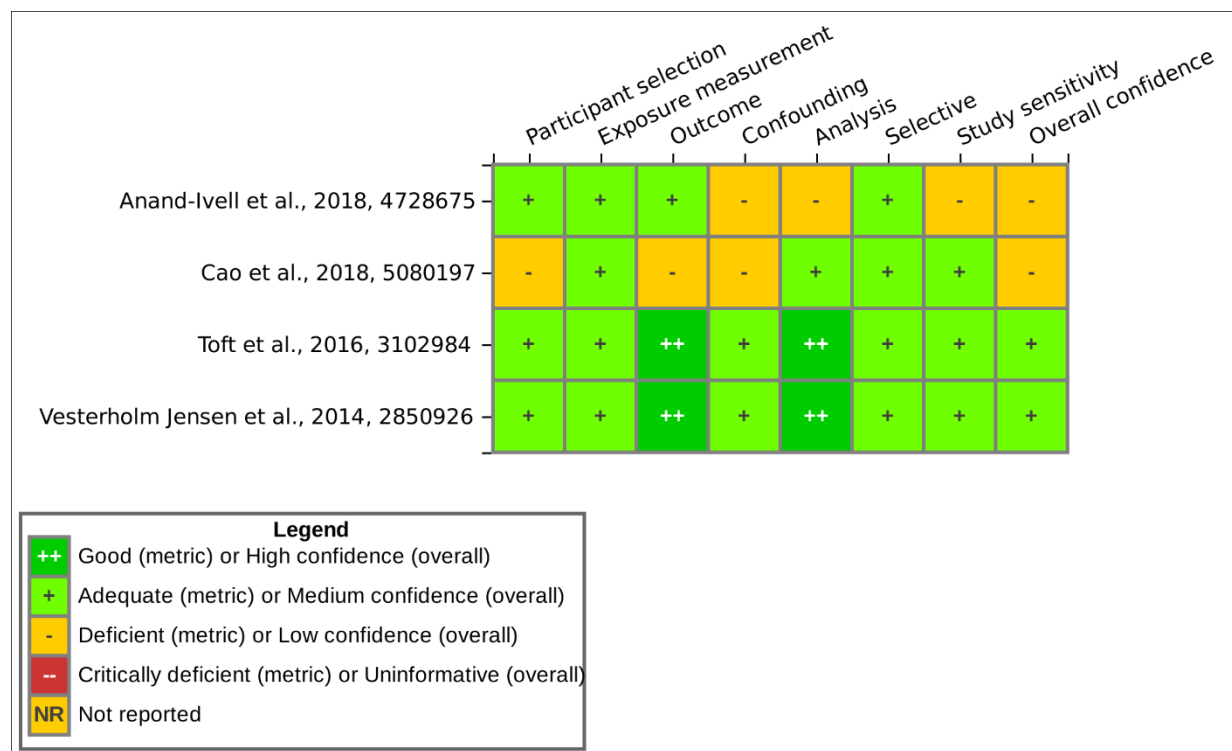


Figure 38. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Defect Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.1.2 Animal Evidence

There are 8 studies from the most recent literature search conducted in 2020 and 4 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and developmental effects. Study quality evaluations for these 12 studies are shown in Figure 39.

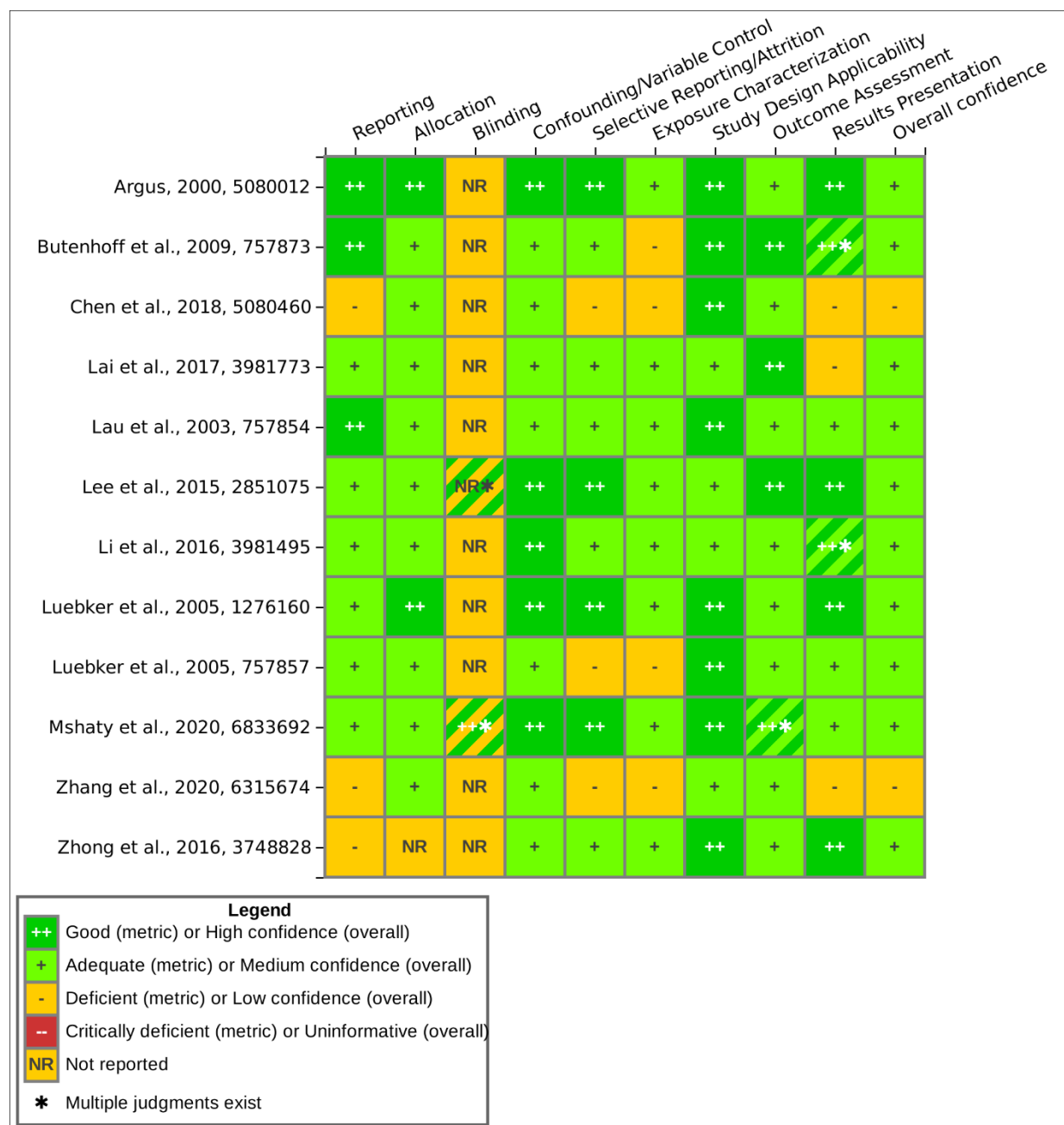


Figure 39. Summary of Study Evaluation for Toxicology Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on [HAWC](#).

Evidence suggests that PFOS exposure can adversely affect development. Oral studies in mice, rats, and rabbits report effects in offspring including decreased survival, decreased body weights, structural abnormalities (e.g., reduced skeletal ossification), histopathological changes in the lung, and delayed eye opening, among others. Effects in offspring primarily occurred at similar doses as those seen in the maternal animals. Adverse effects observed in dams include alterations in gestational weight and gestational weight gain, as well as evidence of altered placental

histology. In some cases, adverse developmental effects of PFOS exposure that relate to other health outcomes may be discussed in the corresponding health outcome section (e.g., fetal and neonatal pulmonary effects are discussed in Section 3.3.11.2 and neurodevelopmental effects are discussed in Section 3.3.8.2).

3.3.1.2.1 Maternal Effects

Multiple developmental studies evaluated maternal weight outcomes in rats, mice, and rabbits (Figure 40). Yahia et al. (2008, 2919381) observed a decrease in body weight in ICR mouse dams administered 20 mg/kg/day PFOS from gestational day 1 to 17 (GD1 to GD17) or GD18. The dams exhibited no clinical signs of toxicity. Thibodeaux et al. (2003, 757855) observed significantly decreased maternal body weight gain in CD-1 mice at exposed to 20 mg/kg/day PFOS (highest dose tested in the study); food and water consumption were not affected by treatment. Lee et al. (2015, 2851075) also reported reduced maternal body weight gain in CD-1 mice treated with 2 or 8 mg/kg/day PFOS (not 0.5 mg/kg/day) compared to controls. Dams in the 2 and 8 mg/kg/day dose groups had significantly lower mean body weights on GD14–GD17. In contrast, Lai et al. (2017, 3981773) did not observe a significant difference in maternal body weight in CD-1 mouse dams orally exposed to 0, 0.3, or 3 mg/kg/day throughout gestation (GD1–GD20). The authors determined that there were no observable maternal effects related to PFOS exposure at the relatively low doses evaluated. Mshaty et al. (2020, 6833692) orally administered PFOS to C57BL/6J mice from postnatal day 1 (PND1) to PND14, resulting in lactational exposure to pups. Mean maternal body weights were evaluated at PND21 and determined to be comparable between the control and the 1 mg/kg/day dose groups.

Thibodeaux et al. (2003, 757855) observed significant, dose-dependent decreases in maternal body weight, food consumption, and water consumption in Sprague Dawley rats dosed with ≥ 2 mg/kg/day PFOS from GD2 to GD20. In a 2-generation reproductive toxicity study with dams dosed pre-mating through lactation with 0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day PFOS, Luebker et al. (2005, 1276160) similarly observed dose-dependent decreases in maternal body weight in the parental generation (P_0) from the 3.2 mg/kg/day dose group from day 15 of the pre-mating exposure through lactation day 1 (LD1), the last recorded weight; this dose group also had significantly decreased maternal weight gain from GD0 to GD20. The 1.6 mg/kg/day dams experienced transient decreases in maternal weight compared to controls in the window between GD3 and GD11. There were no reported differences in the maternal weight of adult first generation (F_1) females during pre-cohabitation until the end of lactation, though the highest dose tested in these females was only 0.4 mg/kg/day. In another study with Sprague Dawley rats dosed with 0, 5, or 20 mg/kg/day PFOS from GD12 to GD18, Li et al. (2016, 3981495) also reported reduced mean maternal body weights in the 20 mg/kg/day dose group. Butenhoff et al. (2009, 757873) observed comparable maternal body weight and body weight gain during gestation in Sprague Dawley rat dams dosed with 0, 0.1, 0.3, or 1 mg/kg/day PFOS from GD0 to LD20 but observed significantly lower absolute body weights during lactation (PND4–PND20) in dams treated with 1 mg/kg/day PFOS. Transient decreases in food consumption were observed in the 0.3 and 1.0 mg/kg/day groups throughout the study, though these findings were not considered treatment-related or adverse.

In a single rabbit study, Argus (2000, 5080012) reported significantly decreased maternal body weight gain from GD7 to GD21 at PFOS doses ≥ 1 mg/kg/day (mean body weight change of 0.38, 0.3, 0.2, and -0.01 kg with 0, 1, 2.5, and 3.75 mg/kg/day PFOS, respectively); no

significant effect was observed from GD21 to GD29. There were observations of scant or no feces for some does in the 1.0, 2.5, and 3.75 mg/kg/day groups. Observations of scant feces were significant relative to control at 3.75 mg/kg/day. Significant reductions in absolute (g/day) and relative (g/kg/day) feed consumption was also observed in the 2.5 and 3.75 mg/kg/day dose groups.

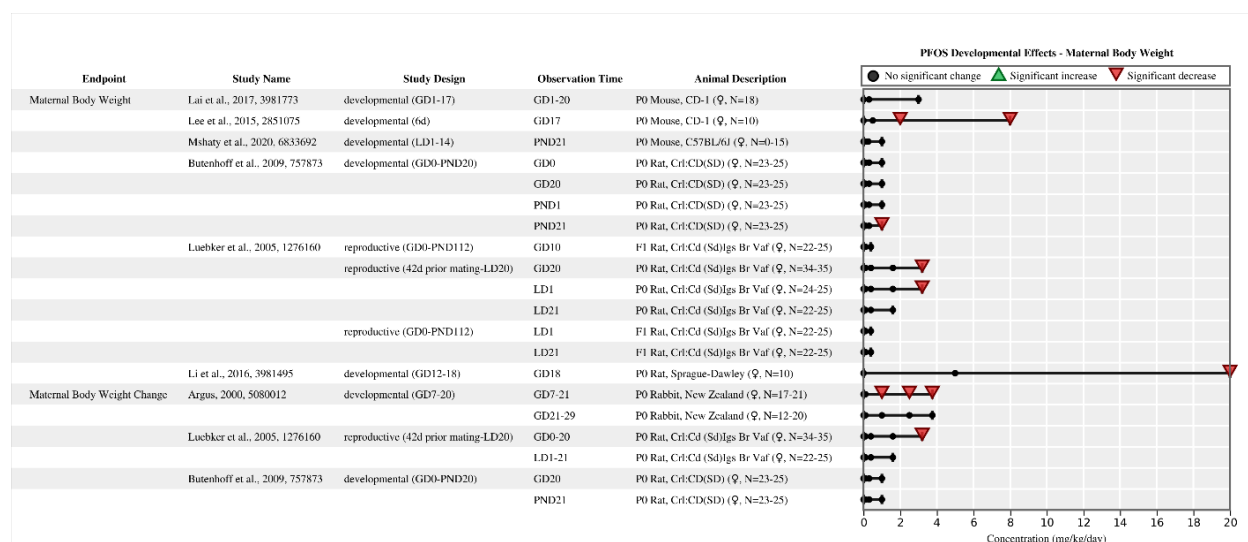


Figure 40. Maternal Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

GD = gestation day; PND = postnatal day; LD = lactational day; P0 = parental generation; F1 = first generation; d = day.

3.3.1.2.2 Viability

Decreases in both fetal and pup survival and viability with perinatal PFOS exposure were observed in multiple studies (Figure 41). Lee et al. (2015, 2851075) reported a significantly higher incidence of resorptions, post-implantation loss, and dead fetuses at GD17 after dosing pregnant CD-1 mice by gavage with 0.5, 2, or 8 mg/kg/day from GD11 to GD16; however, there was no significant difference in the mean number of implantations. A significant decrease in mean number of live fetuses was also observed in the 2.0 and 8.0 mg/kg/day dose groups versus controls. A decrease in the mean number of live fetuses was also reported in the 0.5 mg/kg/day dose group but this difference was not significant relative to control. Administration of 0, 1, 5, 10, 15, or 20 mg/kg/day PFOS to CD-1 mice from GD1 to GD17 did not affect the number of implantation sites but resulted in a significant increase in post-implantation loss, as measured by decrease in mean percentage of live fetuses, in dams administered 20 mg/kg/day {Thibodeaux, 2003, 757855}.

Mice appear to be more sensitive to alterations in fetal viability than rats. Thibodeaux et al. (2003, 757855) dosed pregnant Sprague-Dawley rats with 0, 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from GD2 to GD20. The number of implantations was not affected by treatment and there were no treatment-related effects observed on the live rat fetuses at terms. Butenhoff et al. (2009, 757873) also observed no treatment-related effects on the number of implantation sites or resorptions in pregnant Sprague-Dawley rats exposed to 0.1, 0.3, or 1.0 mg/kg/day by gavage from GD0 to PND20.

In pregnant New Zealand white rabbits cesarean sectioned on GD29 after gestational exposure to PFOS, Argus (2000, 5080012) reported no significant effects on implantations or resorptions. However, Argus (2000, 5080012) did report abortions among New Zealand white rabbits orally dosed with 2.5 mg/kg/day (1/17 does, 5.9%) or 3.75 mg/kg/day (9/21 does, 42.8%) from GD7 to GD20. The abortion rate was significantly greater relative to control for the 3.75 mg/kg/day dose group. Argus (2000, 5080012) reported no significant effects on the mean number of live fetuses/doe, number of dead fetuses/doe, mean litter size, and offspring viability.

Altered pup viability was observed in studies of both rats and mice. In one- and two-generation reproductive toxicity studies in Sprague Dawley rats, Luebker et al. (2005, 757857; 2005, 1276160) observed reduced pup viability index (ratio of the number of pups alive at PND5 to the number of live pups born) at higher maternal PFOS doses. A significant decrease in pup viability for the one-generation study was associated with a dose of 1.6 mg/kg/day {Luebker, 2005, 757857}; the number of dams with all pups dying between PND1 and PND5 was also significantly increased in the 2 mg/kg/day dose group. The dose associated with a decreased viability index in F₁ pups was also 1.6 mg/kg/day in the two-generation study {Luebker, 2005, 1276160}; between PND1 and PND4, 100% of dams had all pups dying in the 3.2 mg/kg/day dose group. Following gestational exposure to PFOS on GD19–GD20, Grasty et al. (2003, 5085464) observed survival of 98%, 66%, and 3% of rat pups in the control, 25, and 50 mg/kg/day groups, respectively, on PND5. Chen et al. (2012, 1276152) also observed decreased pup survival through PND3 in rat pups exposed to 2 mg/kg/day PFOS from GD1 to GD21. Thibodeaux et al. (2003, 757855) and Lau et al. (2003, 757854) similarly observed decreased pup survival in rats exposed to ≥ 2.0 mg/kg/day PFOS from GD2 to GD21.

Lau et al. (2003, 757854) also reported PFOS-related effects on survival in mice following gestational exposure to PFOS. Briefly, most mouse pups from dams administered 15 or 20 mg/kg/day did not survive for 24 hours after birth. Fifty percent mortality was observed at 10 mg/kg/day. Survival of pups in the 1 and 5 mg/kg/day treated dams was similar to controls. Yahia et al. (2008, 2919381) also observed significant effects on pup survival. In this study, pregnant ICR mice/group were administered 0, 1, 10, or 20 mg/kg of PFOS daily by gavage from GD1 to GD17 or GD18. All neonates in the 20 mg/kg/day dose group were born pale, weak, and inactive, and all died within a few hours of birth. At 10 mg/kg/day, 45% of those born died within 24 hours. Survival of the 1 mg/kg/day group was similar to that of controls. Of the developmental studies identified in the most recent literature search, only Mshaty et al. (2020, 6833692) evaluated the impact of lactational (PND1–PND14) PFOS exposure on pup survival. Mshaty et al. (2020, 6833692) observed no difference in C57BL/6J mouse pup survival through PND21 between control group pups and pups exposed to 1 mg/kg/day PFOS (quantitative data not provided).



Figure 41. Mortality and Viability in Mice, Rats, and Rabbits 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; PND = postnatal day; LD = lactational day; P0 = parental generation; F1 = first generation; d = day.

3.3.1.2.3 Skeletal, Soft Tissue, and Gross Effects

Skeletal defects in offspring, including bone ossification, are a known effect of gestational PFOS exposure. In one study, 0, 1, 10, or 20 mg/kg of PFOS was administered daily by gavage to pregnant ICR mice from GD1 to GD17 or GD18 {Yahia, 2008, 2919381}. Five dams/group were sacrificed on GD18 for fetal external and skeletal effects. In the fetuses from dams treated with 20 mg/kg/day, there were significant increases in the numbers of fetuses with cleft palates (98.56%), sternal defects (100%), delayed ossification of phalanges (57.23%), wavy ribs (84.09%), spina bifida occulta (100%), and curved fetus (68.47%). In mice, Thibodeaux et al. (2003, 757855) observed significantly increased incidences of cleft palate at 15 and 20 mg/kg/day PFOS, sternal defects at 5, 10, 15, and 20 mg/kg/day PFOS, and ventricular septal defects at 20 mg/kg/day PFOS. Thibodeaux et al. (2003, 757855) also observed significantly increased incidences of these deformities in rats. The authors reported incidences of cleft palate at 10 mg/kg/day PFOS and sternal defects at 2 and 10 mg/kg/day PFOS.

Skeletal malformations in fetal and neonatal rabbits were reported in Argus (2000, 5080012) at comparatively lower PFOS doses than those described in rat and mouse studies. A significant decrease in the mean number of isolated ossification sites of the metacarpal per fetus per litter was observed in the 3.75 mg/kg/day dose group versus control (4.82 versus 4.98, respectively); no significant change in mean number of ossification sites per fetus per litter was reported in the 0.1 (4.97), 1 (4.99), or 2.5 mg/kg/day (4.97) dose groups. A significant decrease in the mean number of sternal center ossification sites per fetus per litter was observed in the 2.5 and 3.75 mg/kg/day dose groups relative to control (3.81 and 3.82, respectively, relative to 3.98 for the control group); no significant change in the mean number of sternal center ossification sites per fetus per litter was detected in the 0.1 (3.92) and 1 mg/kg/day (3.95) dose groups. A significant difference in fetal incidence of irregular ossification of the skull was reported in both the 2.5 and 3.75 mg/kg/day dose groups relative to control (0.8% and 9.2% incidence respectively, relative to 4% in the control); no significant difference was observed in the 0.1 (5.6%) and 1 mg/kg/day

(2%) dose groups. There were no significant differences in litter incidence of irregular ossification of the skull in the 0.1, 1, 2.5, and 3.75 dose groups versus control (38.9%, 15.8%, 6.2%, and 25%, respectively, versus 30%). A significant decrease in mean number of ossification sites in the hyoid body per fetus per litter was reported in the 3.75 mg/kg/day dose group (0.92) versus control (1); no change in mean number of hyoid ossification sites was reported in other dose groups (mean of 1 for the 0.1, 1, and 2.5 mg/kg/day dose groups). A significant increase in fetal incidence of a hole in the parietal bone was observed in the 3.75 mg/kg/day dose group versus control (6.5% versus 0%); no holes were detected in the 0.1, 1, and 2.5 mg/kg/day dose groups. Litter incidence of a hole in the parietal was 1 (8.3%) in the 3.75 mg/kg/day dose group and 0 (0%) in the 0, 0.1, 1, and 2.5 mg/kg/day dose groups. Fetal incidence of unossified pubis was also significantly increased in the 3.75 mg/kg/day group versus control (3.7% versus 0%). No other dose groups exhibited unossified pubis. A significant increase in litter incidence of unossified pubis was observed in the 3.75 mg/kg/day group versus control (16.7% vs 0%). The rest of the dose groups exhibited 0% litter incidence of unossified pubis. However, fetal alterations were observed in a similar percentage of litters across all dose groups (70%, 61.1%, 47.4%, 25%, and 66.7% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively). No significant difference was seen in the mean percentage of fetuses per litter with any alteration (14.1%, 17%, 9.5%, 3.6%, and 17.4% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively).

3.3.1.2.4 Fetal or Pup Body Weight

Four studies in different species reported data on fetal body weight (Figure 42). In a study in CD-1 mice with gestational PFOS exposure from GD11 to GD16, Lee et al. (2015, 2851075) reported mean fetal body weights on GD17 of 1.72, 1.54, 1.3, and 1.12 g in the 0, 0.5, 2, and 8 mg/kg/day dose groups, respectively. The mean fetal weights reported for the 2 and 8 mg/kg/day groups were significantly lower than those reported for the control dose group. Similarly, Li et al. (2016, 3981495) reported mean GD18.5 fetal body weights of 2.73, 2.68, and 2.48 g in the 0, 5, and 20 mg/kg/day dose groups (sexes combined) following exposure of Sprague-Dawley rat to PFOS from GD12 to GD18. Mean fetal body weight for the 20 mg/kg/day dose group was significantly different from that of the control group. Mean fetal body weight in males alone was also significantly decreased at 20 mg/kg/day (2.79, 2.74, and 2.43 g for the 0, 5, and 20 mg/kg/day dose groups, respectively). Thibodeaux et al. (2003, 757855) similarly observed a decrease in rat fetal weight following gestational exposure to 10 mg/kg/day PFOS. In a one-generation reproductive study in Sprague Dawley rats, Luebker et al. (2005, 757857) reported no effect on pooled fetal body weights with PFOS doses up to 2 mg/kg/day. In a study in New Zealand white rabbits, Argus (2000, 5080012) reported mean live fetal body weights of 44.15, 41.67, 42.37, 39.89, and 33.41 g/litter in 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively. Fetal body weights for the 2.5 and 3.75 mg/kg/day dose groups were significantly lower than fetal body weight reported in the control group.

Several other studies measured body weights of pups after birth (Figure 42). The most sensitive endpoint in the one- and two-generation reproductive studies in Sprague Dawley rats (dams treated with PFOS pre-conception through gestation for 63 or 84 days, respectively) was decreased pup body weight {Luebker, 2005, 757857; Luebker, 2005, 1276160}. The NOAEL and LOAEL for pup body weight effects was 0.1 and 0.4 mg/kg/day, respectively, in the two-generation study {Luebker, 2005, 1276160}; the lowest dose of 0.1 mg/kg/day was not tested (NT) in the one-generation study {Luebker, 2005, 757857} where the LOAEL was 0.4

mg/kg/day for decreased pup body weight, decreased maternal body weight, and decreased gestation length. Lau et al. (2003, 757857) also reported significant weight deficits in Sprague Dawley rat pups on PND0 after gestational PFOS exposures of 2, 3, or 5 mg/kg/day, but not 1 mg/kg/day.

For this endpoint, rats appear to be more sensitive than mice. Yahia et al. (2008, 2919381) reported significant decreases in ICR mouse neonatal weight at relatively high doses of 10 and 20 mg/kg/day. Lau et al. (2003, 757857) did not report statistically significant reductions in pup body weights of CD-1 mice gestationally exposed to PFOS doses up to 20 mg/kg/day. Zhong et al. (2016, 3748828) measured body weights of C57BL/6 mouse pups that had been exposed to 0, 0.1, 1, or 5 mg/kg/day PFOS in utero from GD1 to GD17. They did not see significant differences in body weight measurements of male or female mice at 4 and 8 weeks of age. Mshaty et al. (2020, 6833692) also reported no effects on C57BL/6J mouse pup body weight at PND21 following lactational exposure to 1 mg/kg/day PFOS from PND1 to PND14.



Figure 42. Offspring Weight in Mice, Rats, and Rabbits Following Exposure to PFOS, (logarithmic scale, sorted by observation time)

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; LD = lactational day; F1 = first generation; F2 = second generation; d = day.

3.3.1.2.5 Placenta

Placental endpoints were reported in three studies with rats, mice, or rabbits. Li et al. (2016, 3981495) reported a significant decrease in mean placental weight in Sprague-Dawley rat dams exposed to 20 mg/kg/day PFOS from GD12 to GD18 relative to control (442.8 mg vs. 480.4 mg). No significant difference in placental weights was detected in dams exposed to 5 mg/kg/day

PFOS relative to control (455.1 mg vs 480.4 mg). At ≥ 0.5 mg/kg/day, Lee et al. (2015, 2851075) observed significant decreases in mean absolute placental weight (185.63, 177.32, 163.22, and 151.54 mg at 0, 0.5, 2, and 8 mg/kg/day, respectively) and placental capacity (ratio of fetal weight/placental weight; 9.3, 8.68, 7.96, and 7.39 at 0, 0.5, 2, and 8 mg/kg/day, respectively) in mice exposed to PFOS from GD11 to GD16 and sacrificed at GD17. In the same study, microscopic evaluation revealed dose-dependent decreases in the frequency of glycogen trophoblast cells and sinusoidal trophoblast cells at dose levels ≥ 2.0 and ≥ 0.5 mg/kg/day, respectively {Lee, 2015, 2851075}. Argus (2000, 5080012) did not observe any placental effects in rabbits.

3.3.1.2.6 Postnatal Development

Gestational PFOS exposure is associated with effects on postnatal development. Lau et al. (2003, 757854) observed delayed eye opening in rats and mice following developmental exposure to PFOS. following gestational exposure to PFOS. A significant, treatment-related delay in eye opening was reported in mice following gestational exposure to PFOS (eye opening at PND14.8 in control versus eye opening at PND15.1, PND15.5, and PND15.6 at 1, 5, and 10 mg/kg/day, respectively). The NOAEL for delays in eye opening in rats was 1 mg/kg/day PFOS.

3.3.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse developmental outcomes is discussed in Section 3.3.4 of the 2016 PFOS HESD (EPA, 2016, 3603365). There are 31 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to developmental effects. A summary of these studies is shown in Figure 43. Additional analysis on the mechanistic actions of PFOS on developmental health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	5	6	4	14
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	11	16
Cell Signaling Or Signal Transduction	5	2	4	10
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	3	1	1	5
Hormone Function	2	0	1	2
Inflammation And Immune Response	0	1	1	2
Oxidative Stress	1	1	3	5
Xenobiotic Metabolism	1	0	1	2
Not Specified (Review Article)	1	0	0	1
Grand Total	14	8	12	31

Figure 43. Summary of Mechanistic Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.1.4 Evidence Integration

As noted in the epidemiological fetal growth restriction summary, there is *suggestive* evidence that PFOS may impact fetal growth restriction in humans. Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catch-up periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT. Overall, there was inconsistent evidence of PFOS impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available for other endpoints such as fetal loss and no evidence of associations in recent studies of PFOS and birth defects such as cryptorchidism or hypospadias. In contrast, there was fairly consistent evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as the majority of studies showed some adverse associations.

Collectively across the various endpoints outlined in the human epidemiological sections, there is *moderate* evidence of developmental effects related to PFOS based on the more recent epidemiological literature. As noted previously there is some uncertainty as to what degree the available evidence may be impacted by pregnancy hemodynamic and sample timing differences across studies, as this may result in either confounding or reverse causality {Steenland, 2018, 5079861}. Additional uncertainty exists due to the potential for confounding by other PFAS. Very few of the existing studies performed multipollutant modeling in comparison with single

pollutant estimates of PFOS associations. The results were often mixed from those that did this with some estimates increasing and some decreasing although PFOS was rarely chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants. There is some concern that controlling for other highly correlated co-exposures in the same model may amplify the potential confounding bias of another co-exposure rather than removing it {Weisskopf, 2018, 7325521}. Given these interpretation difficulties and potential for this co-exposure amplification bias, it remains unclear whether certain mutually adjusted models give a more accurate representation of the independent effect of specific pollutants for complex PFAS mixture scenarios. Overall, these sources of uncertainty in epidemiological studies are indicative of complex patterns of influence due to potential sources of biases that are not completely understood.

Collectively, the majority of SGA and LBW studies were supportive of an increased risk with increasing PFOS exposures. Four *medium* or *high* confidence studies on LBW showed increased risks with increased PFOS levels. The endpoint of LBW from Chu et al. (2020, 6315711), Sagiv et al. (2018, 4238410), Starling et al. (2017, 3858473), and Wikström et al. (2020, 6311677) was considered for the derivation of PODs.

In addition to the human epidemiological data, the available animal data outlined above provides considerable evidence in support of an association between PFOS exposure and developmental toxicity. Dose-dependent maternal and offspring effects were reported in mice, rats, and rabbits. The studies evaluated demonstrate that PFOS exposure is associated with various developmental toxicity endpoints including increased mortality (pup mortality, fetal death, stillbirth, abortion), decreased body weight or body weight change (fetal, pup, and maternal), skeletal and soft tissue effects, and delayed eye opening. The most consistent effects observed across studies were decreased maternal body weight (encompassing decreases in maternal body weight and maternal body weight change), decreased offspring weight during the perinatal developmental period (encompassing fetal weight and pup weight prior to weaning), and increased mortality (encompassing abortion, stillbirth, fetal death, and pup mortality).

Reductions in litter size or fetal/pup weight may be the driver of reductions seen in maternal weight. For all but one study, decreased maternal weight was observed at the same doses as the potential confounding effects of reduced fetal weight, increased incidence of abortion, increased pup mortality/stillbirth, and others. However, Argus (2000, 5080012) reported reduced maternal body weight change in the absence of statistically significant effects on pups that could influence maternal weight. In this case, maternal body weight may be an influential precursor to or sensitive indicator of potential offspring mortality.

Similarly, Luebker et al. (2005, 757857; 2005, 1276160) observed decreased pup weights as an average per litter at lower dose levels than effects on viability endpoints including decreases in implantations, increased number of dams with all pups dying, and decreased number of live pups per litter. These two studies indicate that pup weight may also be an influential precursor to or sensitive indicator of potential offspring mortality or viability. This is supported by results from Lau et al. (2003, 757854) showing significant decreases in rat pup body weight at birth and increases in pup mortality in the first 24–48 hours after birth. Significant reductions in both endpoints occurred at the same dose of 2 mg/kg/day. A final study {Lee, 2015, 2851075} also observed increased fetal death and decreased fetal weight. However, in this study, increased incidence of fetal death was statistically significant at all dose levels, while fetal weight was not

significant at the lowest dose of 0.5 mg/kg/day, indicating that decreased fetal weight may not be a primary factor in fetal death.

Overall, the available animal toxicity literature supports the evidence of LBW seen in the epidemiological literature. Pup body weight (PND0 and LD5 reported by Luebker et al., 2005, 757857; PND1 reported by Luebker et al., 2005, 1276160; PND0 (rat) reported by Lau et al., 2003, 7578854), and fetal death and weight (reported by Lee et al., 2015, 2851075) were considered for derivations of PODs given the consistency of these observed effects across studies and species and potential for confounding. Maternal weight (reported by Argus, 2000, 5080012) could not be considered for derivation of a POD because the toxicokinetic model to predict internal dose in the animal models is parameterized for mice, rats and monkeys.

3.3.2 Reproductive

3.3.2.1 Human Evidence

3.3.2.1.1 Male

3.3.2.1.1.1 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 Study (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 studies⁶ (24 publications) report on the association between PFOS and endocrine effects since the 2016 document. Eleven of the studies were in children and 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

⁶ Zhou, 2016, 3856472 and Zhou, 2017, 3858488 analyze participants from the same population using the same outcome.

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}.

3.3.2.1.1.2 Study Quality

Of the 23 studies (24 publications) identified since the 2016 assessment, two studies were classified as *high* confidence, fifteen studies as *medium* confidence, five studies as *low* confidence, and one study {Song, 2018, 4220306} was determined to be *uninformative* (Figure 44). Anand-Ivell, 2018, 4728675 was considered *low* confidence for cryptorchidism and *uninformative* for amniotic fluid hormones. Publications from the GBGA {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*.

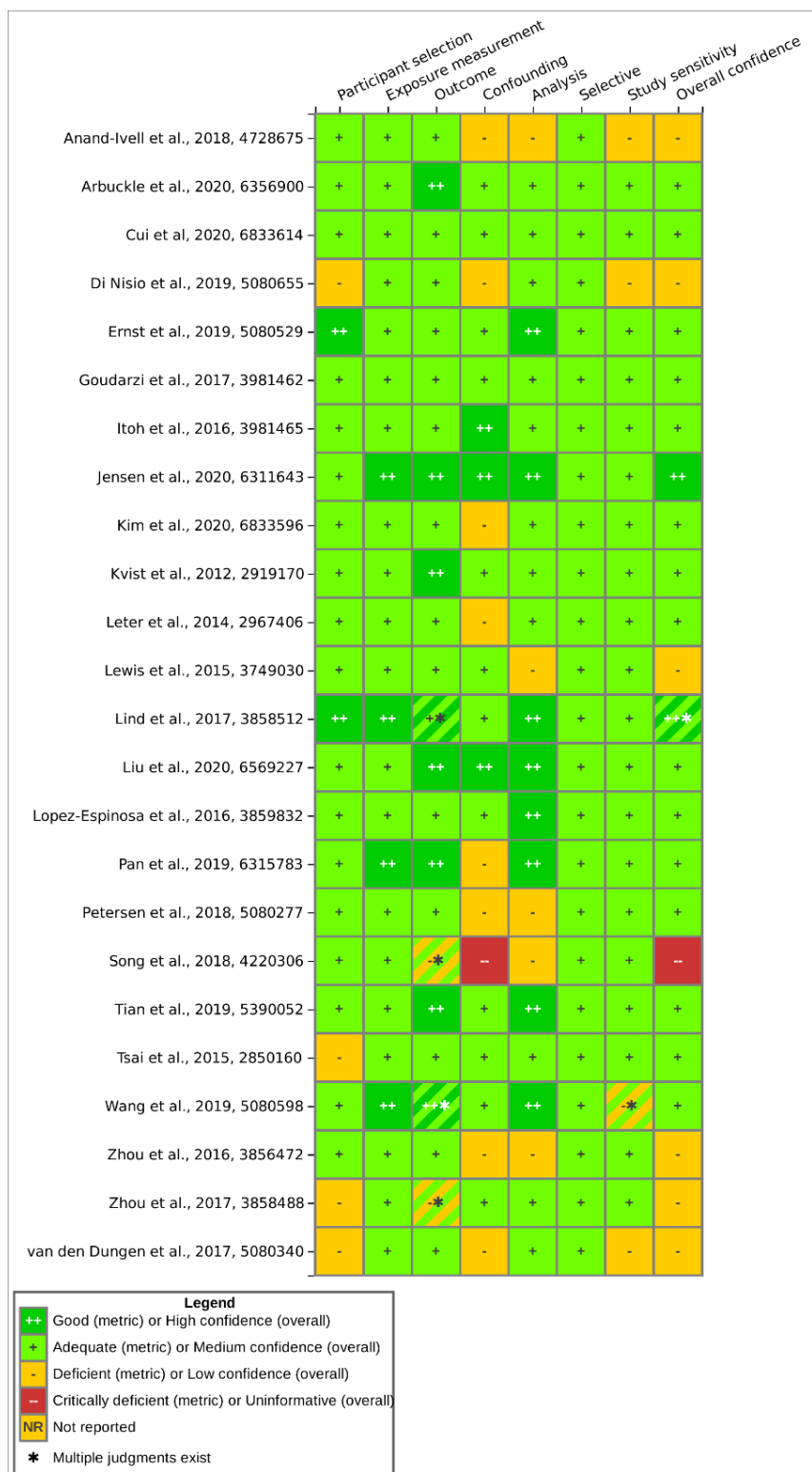


Figure 44. Summary of Study Evaluation for Epidemiology Studies of PFOS and Male Reproductive Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.2.1.1.3 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 (Table C-2). 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), etc.) 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 ($p\text{-trend} = 0.027$), T/E2 ($p\text{-trend} = 0.015$), and inhibin B ($p\text{-trend} < 0.001$) but did not support a significant trend for progesterone ($p\text{-trend} = 0.231$). 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 (Table C-2). 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 (Table C-2). 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 ($p = 0.064$).

3.3.2.1.1.4 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 (Table C-2). 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 (Table C-2). 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 Sata 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.

3.3.2.1.2 Female

3.3.2.1.2.1 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.

3.3.2.1.2.2 Study Quality

Among the 50 publications available for review, 5 were classified as *high* confidence, 25 as *medium* confidence, 18 as *low* confidence, and two were considered *uninformative* (Figure 45,

Figure 46). 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.

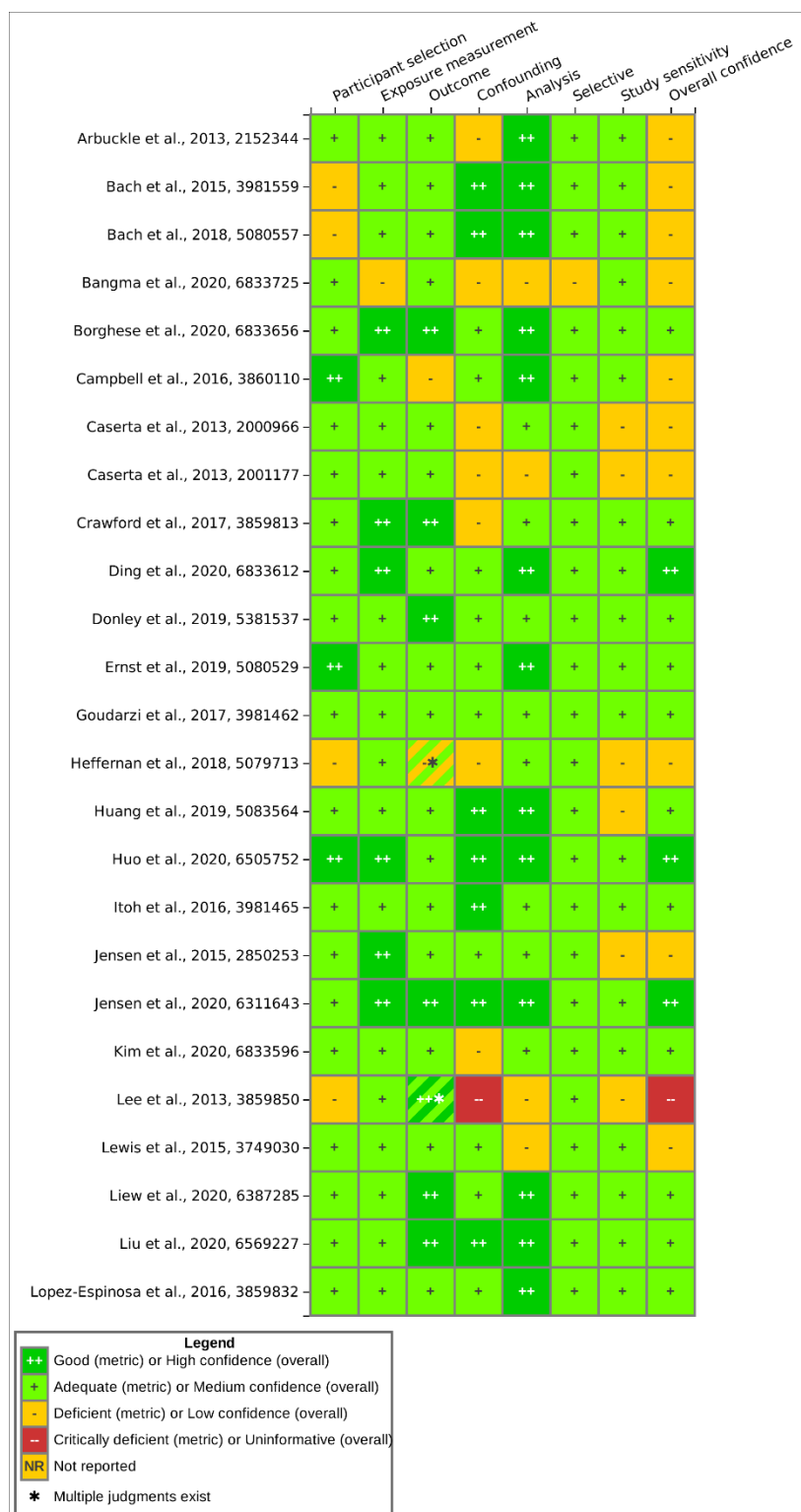


Figure 45. Summary of Study Evaluation for Epidemiology Studies of PFOS and Female Reproductive Effects

Interactive figure and additional study details available on [HAWC](#).

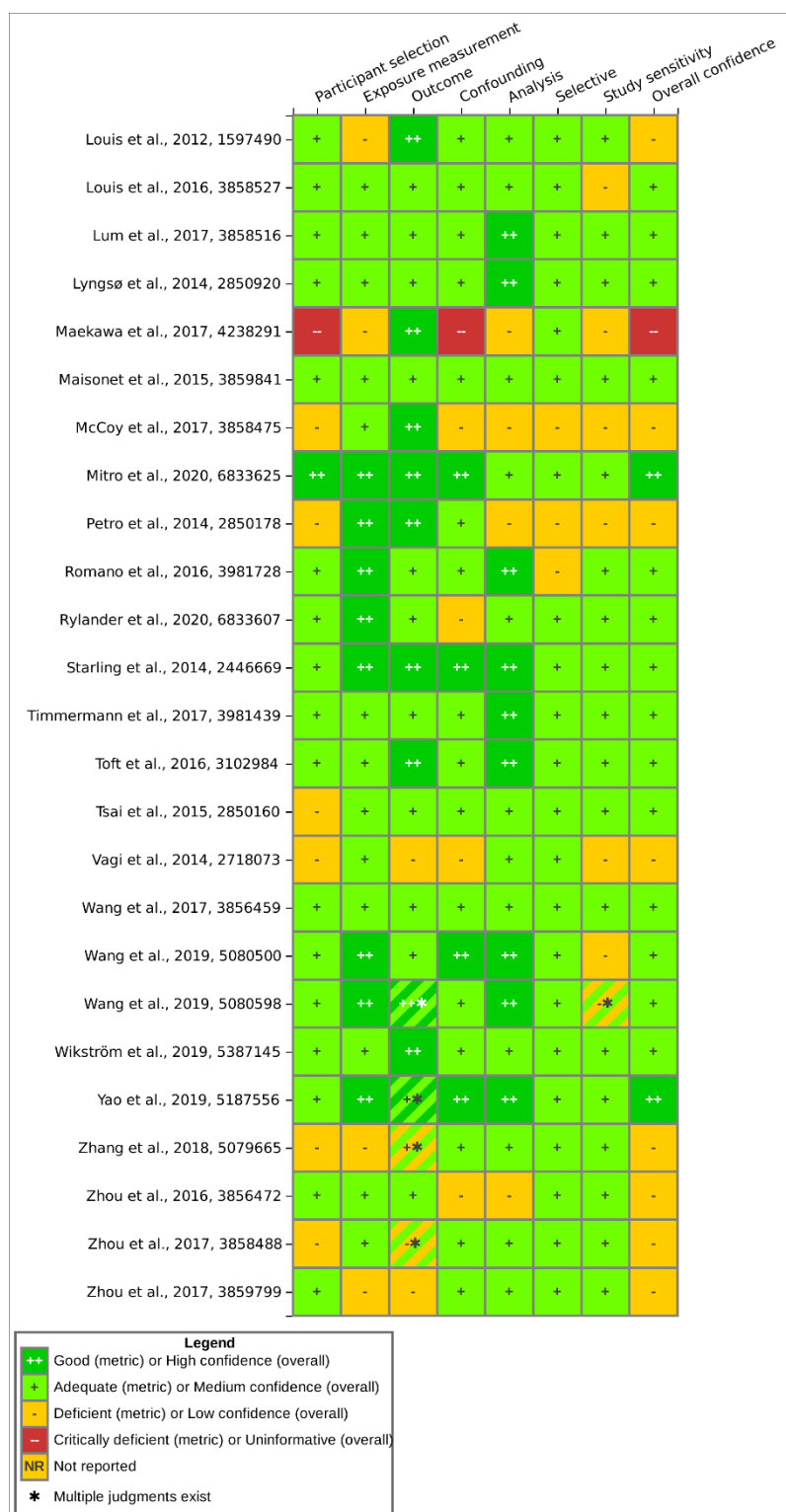


Figure 46. Summary of Study Evaluation for Epidemiology Studies of PFOS and Female Reproductive Effects (Continued)

Interactive figure and additional study details available on [HAWC](#).

3.3.2.1.2.3 Findings from Children and Adolescents

Two *high* confidence, eight *medium* confidence, and three *low* confidence studies assessed relationships between PFOA exposure and female reproductive outcomes in children and adolescents (Table C-3). 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 versus quartile 1 = -1.1 ; 95% CI: $-8.6, 7.1$; percent difference for quartile 3 versus quartile 1: -7.8 ; 95% CI: $-15, -0.1$; percent difference for quartile 4 versus 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.

3.3.2.1.2.4 Findings from Pregnant Women

One *high* confidence, five *medium* confidence studies, and one *low* confidence study examined the relationship between PFOS exposure and preeclampsia (Table C-4). 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 versus 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 log₂-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 versus 1 = 1.32; 95% CI: 0.97, 1.79; relative risk for PFOS quartile 3 versus quartile 1 = 1.39; 95% CI: 1.04, 1.88; relative risk for PFOS quartile 4 versus 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 versus 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 versus 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 versus 1 = 11; 95% CI: 0, 23; percent difference for PFOS tertile 3 versus 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 versus tertile 1 = 9; 95% CI: -2, 20; percent difference for PFOS tertile 3 versus 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 sex-hormone binding globulin (SHBG) levels three years postpartum.

One *medium* confidence study {Lyngsø, 2014, 2850920} examined the effects of serum PFOS levels on pre-pregnancy menstruation. The study did not report any significant associations for PFOA exposure and menstrual cycle length or irregularity. While evidence of increased odds of menstrual cycle irregularity was reported, the association was not significant.

3.3.2.1.2.5 Findings from the General Adult Population

Five *medium* confidence {Crawford, 2017, 3859813; Donley, 2019, 5381537; Kim, 2020, 6833596; Lum, 2017, 3858516; Wang, 2017, 3856459} and four *low* confidence studies {Arbuckle, 2013, 2152344; Bach, 2018, 5080557; McCoy, 2017, 3858475; Zhang, 2018, 5079665} examined implications of PFOS exposure on female fertility, reporting mixed results (Table C-5). 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}, gravida {Arbuckle, 2013, 2152344}, 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 versus tertile 1 (HR = 1.27; 95% CI: 1.01, 1.59) and between serum n-PFOS and risk of natural menopause for women in n-PFOS tertile 3 versus tertile 1 (HR = 1.26; 95% CI: 1.02, 1.57). Non-significant positive associations were observed for both Sm-PFOS and n-PFOS when analyzed as a continuous variable and for women in tertile 2 versus tertile 1.

One *medium* confidence {Tsai, 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}.

3.3.2.2 *Animal Evidence*

There are 9 studies from the most recent literature search conducted in 2020 and 9 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and reproductive effects. Study quality evaluations for these 18 studies are shown in Figure 47.

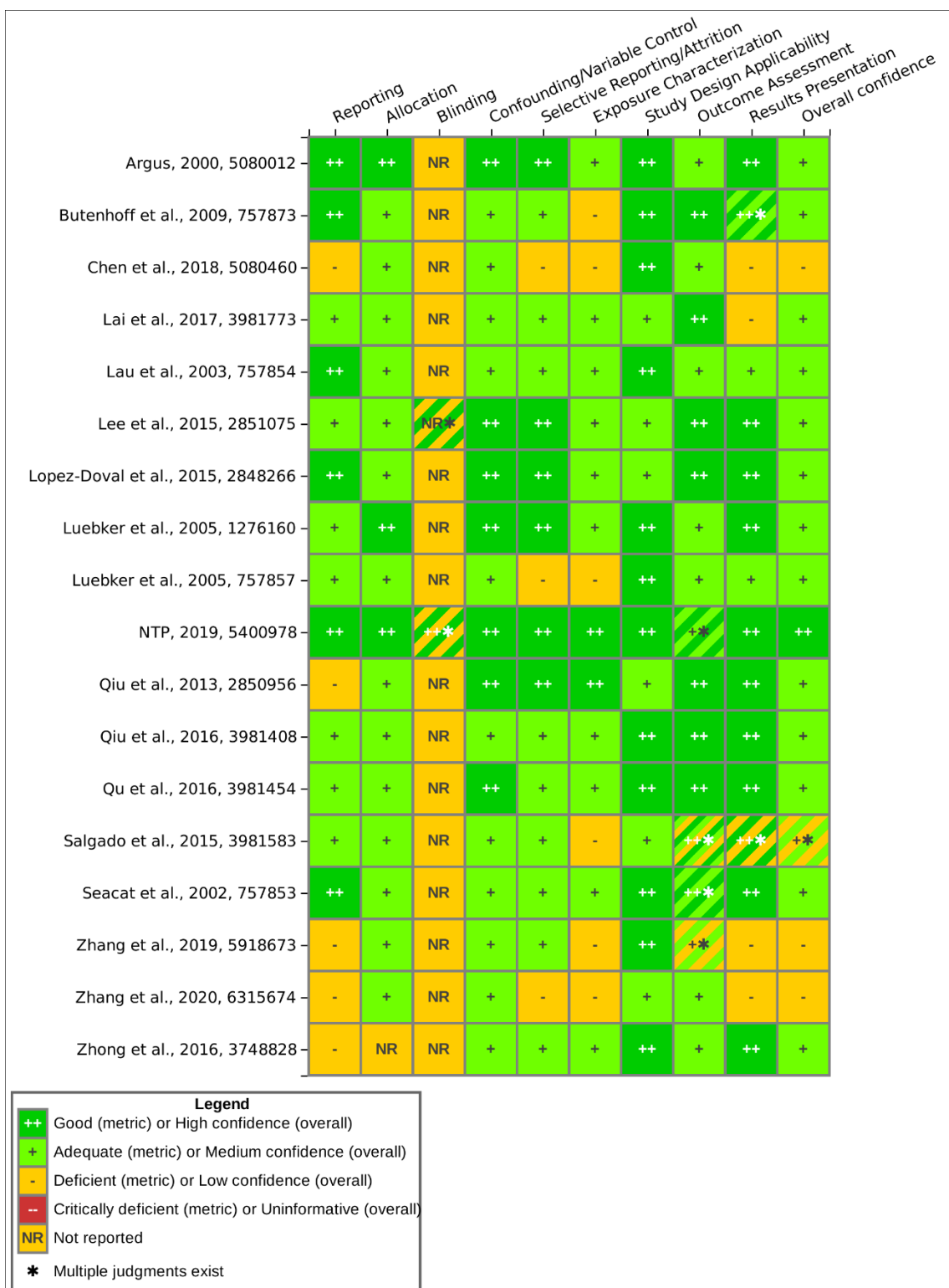


Figure 47. Summary of Study Evaluation for Toxicology Studies of PFOS and Reproductive Effects

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 testes weights, and decreased sperm count. In female rodents exposed to PFOS, effects on prolactin family hormones were observed. Although effects were predominately seen in rodent species there were inconsistencies among rats and mice. In cynomolgus monkeys no effects were noted in reproductive organ weights and histopathology, although a decrease in male E2 levels was observed (Seacat et al. 2002, 757853).

3.3.2.2.1 Female Fertility Parameters and Pregnancy Outcomes

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, were unaffected by PFOS doses as high as 3.2 mg/kg/day in a two-generation reproduction study as well as in rats exposed to up to 1 mg/kg/day PFOS from GD0 to PND20 (Luebker et al. 2005, 1276160; Butenhoff et al. 2009, 757873). Gestation and fertility indices were unaffected in one- and two-generation rat reproduction studies (Luebker et al. 2005, 757857; Luebker et al., 2005, 1276160); however, gestation length was significantly decreased in a dose-dependent manner in dams exposed to ≥ 0.8 mg/kg/day (Figure 48). A decrease in gestation length was also observed in a cross-fostering study in dams treated at 1.6 mg/kg/day (Luebker et al. 2005, 1276160). Decreases in maternal bodyweight change were noted in both the one- and two-generation studies and are detailed in Section 3.3.1.2 (Luebker et al. 2005a, 757857; Luebker et al. 2005, 1276160). 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 GD0 to PND20. Although F₁ pups exposed to PFOS had decreased BWTs, survival, and delayed sexual maturation (see Section 3.3.1.2), no effects were observed on reproductive performance or fertility in F₁ females as adults (Butenhoff et al. 2009, 757873).

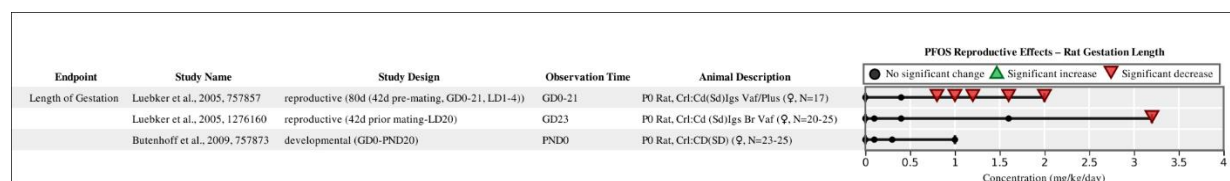


Figure 48. Gestation Length in Rats Following Exposure to PFOS

LD = lactation day; GD = gestation day; P₀ = parental generation.
Interactive figure and additional study details available on [HAWC](#).

In a single study in New Zealand white rabbits, dams were administered 0, 0.1, 1.0, 2.5, or 3.75 mg/kg/day PFOS via intubation from GD7 to GD20 (Argus, 2000, 5080012). The number of rabbits pregnant at the time of sacrifice (GD29) decreased with increasing dose due to an increased incidence of abortion with higher PFOS doses (see Section 3.3.1.2). Only 12/21 (57%) of dams that became pregnant in the study from the 3.75 mg/kg/day dose group were pregnant on GD29 compared to 100% pregnancy maintained in the 0, 0.1, 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, 2.5, and 3.75 mg/kg/day groups (Argus 2000, 5080012).

3.3.2.2.2 Male Sperm Parameters

Sperm parameters were evaluated in studies of male rats and mice, with conflicting results (Figure 49). 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 to 5.0 mg/kg/day (NTP 2019, 5400978). In contrast, a general reduction in epididymal sperm count was observed in mice among studies of varying durations including two 4-week studies in ICR mice exposed to 2.5 or 5 mg/kg/day, a 5-week study in C57 mice exposed to 10 mg/kg/day, and CD-1 pups on PND63 exposed to 3 mg/kg/day during gestation (Qiu et al. 2013, 2850956; Qiu et al. 2016, 3981408; Qu et al., 2016, 3981454; Lai et al. 2017, 3981773). Qiu et al. (2016, 3981408) did not observe alterations in epididymis weight that may have influenced epididymal sperm counts.

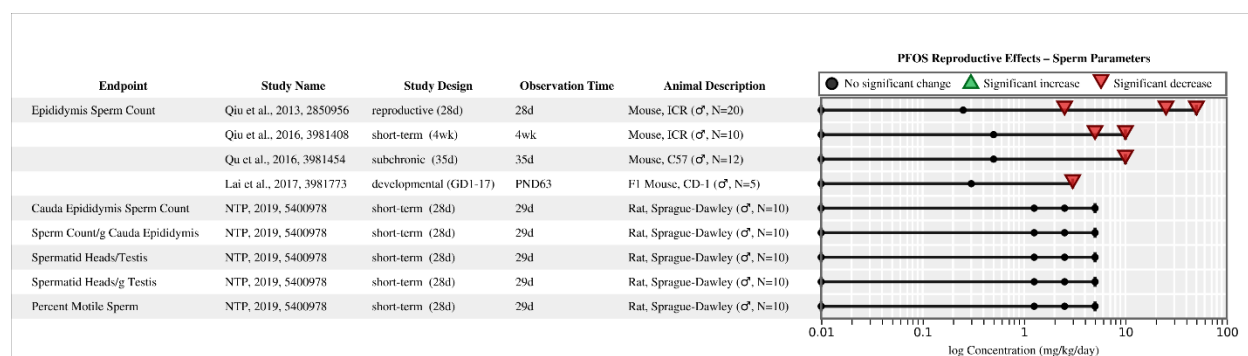


Figure 49. Sperm Parameters in Male Rodents Following Exposure to PFOS

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; F1 = first generation; d = day; wk = week.

3.3.2.2.3 Reproductive Hormones

3.3.2.2.3.1 Males

Reductions in testosterone levels in males were inconsistent across studies and species (Figure 50). Lopez-Doval et al. (2015, 2848266; 2014, 2850091) observed decreases of 40, 39, 32, and 37% at 0.5, 1, 3, and 5 mg/kg/day, respectively, in male rats treated by gavage for 28 days. Conversely, 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 et al. 2016, 3981454). Developmental studies in mice showed a 31% decrease in testosterone at PND63 in CD-1 mice exposed to 3 mg/kg/day throughout gestation (Lai et al. 2017, 3981773). C57BL/6 mouse pups treated with 1 and 5 mg/kg/day showed 35% and 52% decreases, respectively, at postnatal week 4 (PNW4) after maternal oral exposure from GD1 to GD17 (significantly different in the 5 mg/kg/day group) (Zhong et al., 2016, 3748828). In the same study, 38% and 34% decreases were observed in the 1 and 5 mg/kg/day groups, respectively, at PNW8, though only the response in the 1 mg/kg/day group was statistically different from controls. Cynomolgus

monkeys treated up to 0.75 mg/kg/day for 182 days showed no statistically significant effects on testosterone levels (Seacat et al. 2002, 757853).

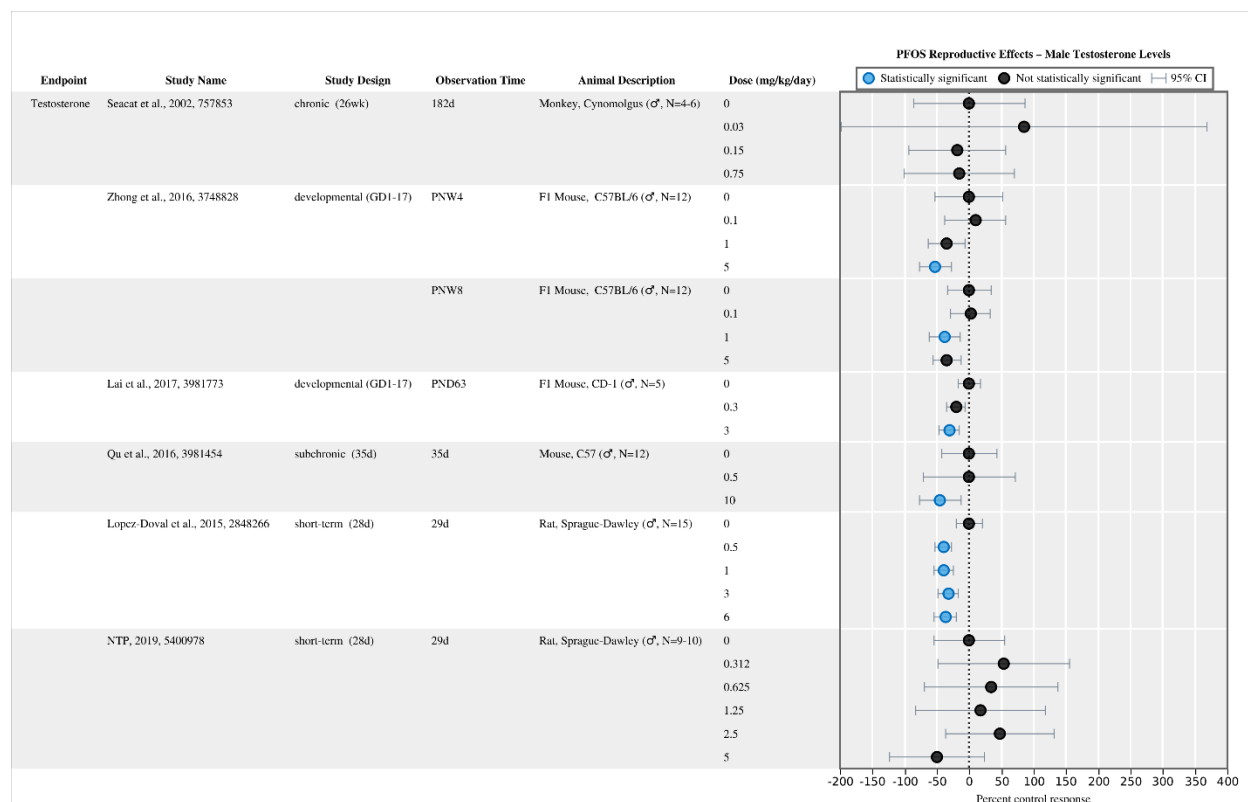


Figure 50. Percent Change in Testosterone Levels Relative to Controls in Male Rodents and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

GD = gestation day; PND = postnatal day; PNW = post-natal week; F1 = first generation

Changes in E2 levels in males were noted in rats, mice, and cynomolgus monkeys across studies of varying durations (Figure 51); 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 and 10 mg/kg/day showed no statistically significant effect on E2 levels, but there was a general increasing trend with increasing dose (5% and 10% increase, respectively) (Qu et al. 2016, 3981454). Male mouse pups exposed to 5.0 mg/kg/day from GD1 to GD17 exhibited a 42% increase in serum E2 levels at PNW4 (Zhong et al. 2016, 3748828). By PNW8 the increase was no longer statistically significant but remained 28% higher than the control group (Zhong et al. 2016, 3748828). There was an apparent dose-dependent increase in serum E2 at both PNW4 and 8. Seacat et al. (2002, 757853) observed a 97% decrease in cynomolgus monkeys treated at 0.75 mg/kg/day for 182 days (Seacat et al. 2002, 757853).

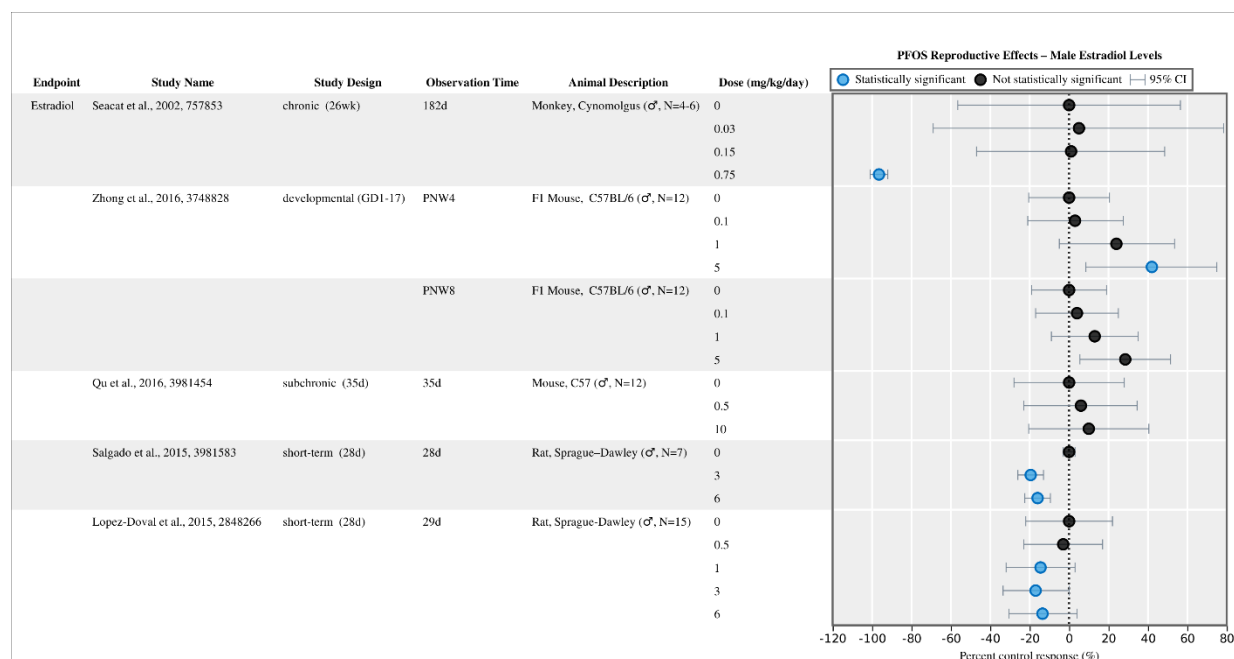


Figure 51. Percent Change in Estradiol Levels Relative to Controls in Male Rodent and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).
GD = gestation day; PND = postnatal day; PNW = post-natal week; F₁ = first generation

Short-term exposure studies examining the effect of PFOS exposure on LH, FSH, and prolactin levels in male rats were available (Figure 52). Groups treated for 28 days with doses ≥ 0.5 mg/kg/day as well as 3.0 and 6.0 mg/kg/day exhibited decreases in LH (15–30%) and prolactin (54–78%), respectively (Lopez-Doval et al. 2014, 2850091; Lopez-Doval et al., 2015, 2848266; Salgado et al. 2015, 3981583). Additionally, increases ranging from 88–133% in serum FSH levels were observed in all treated groups (0.5–6 mg/kg/day) when compared to controls (Lopez-Doval et al. 2014, 2850091).

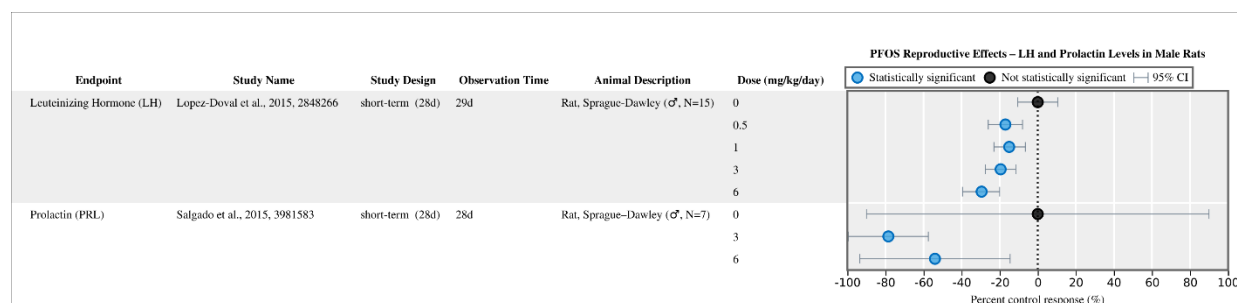


Figure 52. Percent Change in LH and Prolactin Levels Relative to Controls in Male Rats Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

3.3.2.2.3.2 Females

Evidence that oral exposure to PFOS results in changes to levels of hormones in the prolactin family in female mice was noted in an investigation by Lee et al. (2015, 2851075) (Figure 53). In this study, the authors reported dose-dependent reductions in prolactin-family hormones, including mouse placental lactogen (mPL-II) (46–71%), prolactin-like protein (mPLP)-C α (20–53%), and mPLP-K (30–57%), in pregnant CD-1 mice treated with 0, 0.5, 2.0 and 8 mg/kg/day PFOS from GD11 to GD16. Concurrent dose-dependent decreases in bodyweight of 2, 6, and 21%, respectively, were also observed in these mice (Lee et al. 2015, 2851075).

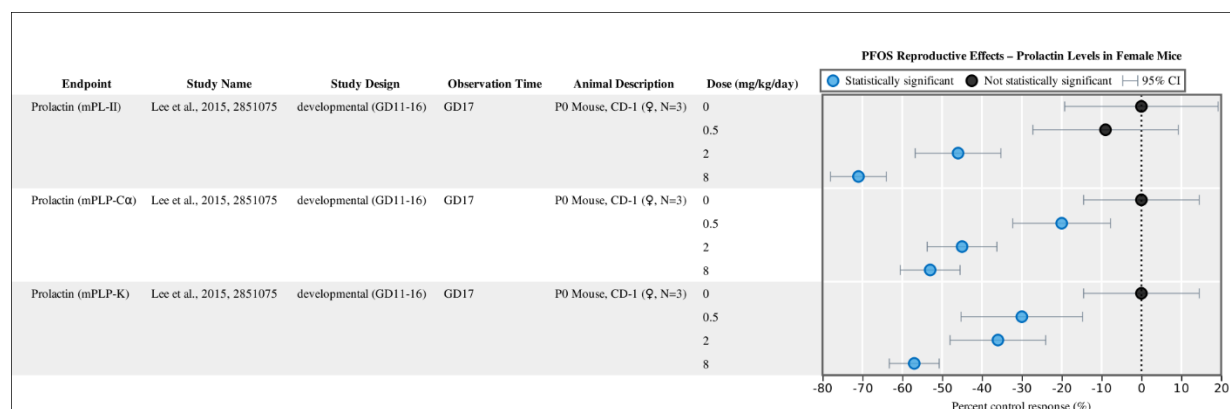


Figure 53. Percent Change in Prolactin Levels Relative to Controls in Female Mice Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).
GD = gestation day; P₀ = 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, 0.15, and 0.75 mg/kg/day dose groups, respectively) (Seacat et al. 2002, 757853) (Figure 54). In the same study, testosterone levels were not affected in females in a dose-dependent or statistically significant manner, though a decrease of 72% was observed in the 0.15 mg/kg/day dose group (Seacat et al. 2002, 757853). In contrast to female monkeys, evaluation of F₁ female mouse pups treated 0.1, 1.0, or 5.0 mg/kg/day from GD1 to GD17 showed increases in E2 levels relative to the control at PNW4 (increases of 10%, 17%, and 8%, respectively) and PNW8 (increases of 11%, 19%, and 12%, respectively), although statistical significance was not achieved (Zhong et al. 2016, 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, 1, and 5 mg/kg/day groups, respectively), but was not statistically significant (Zhong et al. 2016, 3748828). In female rats exposed to PFOS for 28 days, testosterone levels were significantly decreased with 1.25 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).

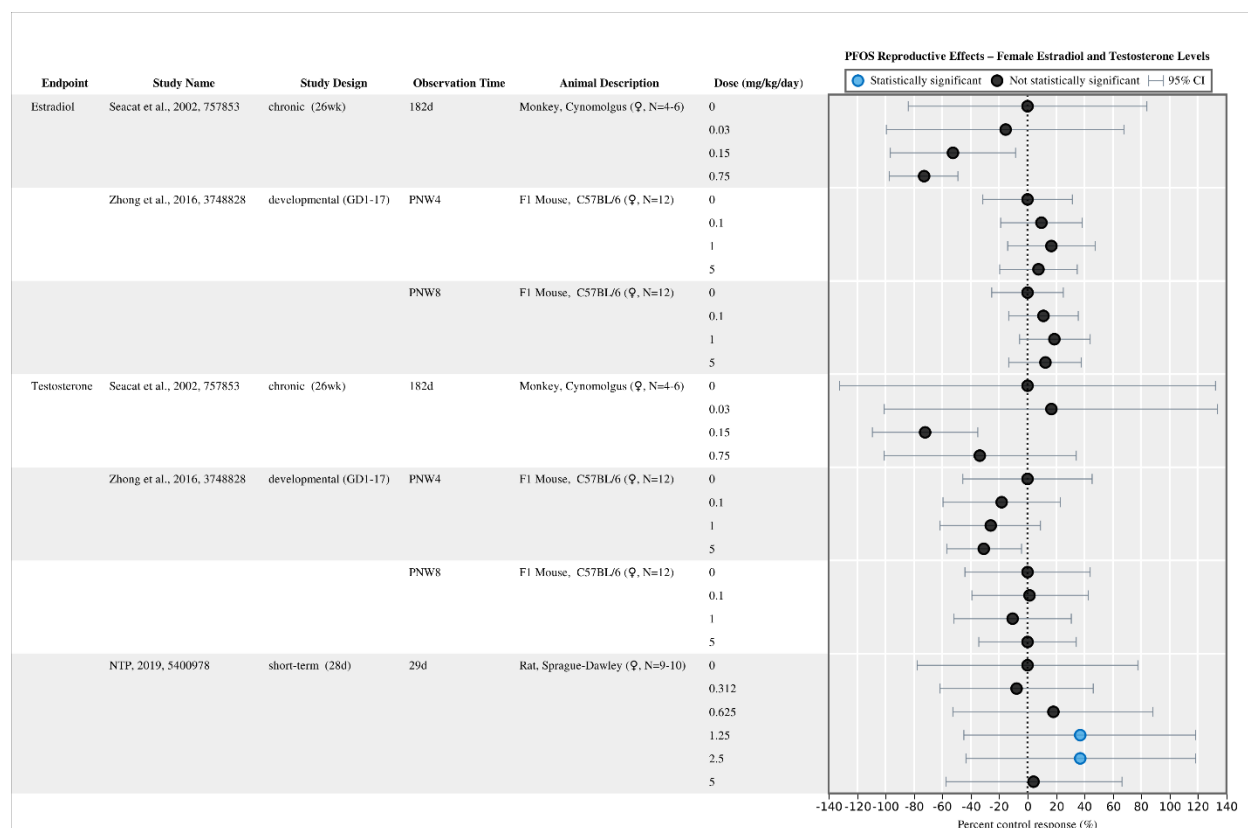


Figure 54. Percent Change in Estradiol and Testosterone Levels Relative to Controls in Female Rodents and Non-Human Primates Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

GD = gestation day; P₀ = parental generation; F₁ = first generation; PNW = postnatal week; d = day; wk = week.

3.3.2.2.4 Reproductive System Development, Including Markers of Sexual Differentiation and Maturation (Female and Male)

In females, a dose dependent increase in estrous cycle length was observed in rats treated with 0.625 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 extended 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 P₀ females treated with up to 3.2 mg/kg/day for 6 weeks prior to mating (Luebker et al. 2005, 1276160).

No significant changes in the number or distribution of corpora lutea were noted in P₀ rats exposed prior to mating and during gestation in the one- and two-generation reproductive toxicity studies (Luebker et al. 2005, 757857; Luebker et al. 2005, 1276160). Likewise, no changes were seen in P₀ female rabbits exposed during gestation (Argus 2000, 5080012). Reproductive and developmental studies additionally reported no impact of gestational PFOS

exposure on preputial and balanopreputial separation in rats (Luebker et al., 2005, 1276160; Lau et al., 2003, 757854; Butenhoff et al., 2009, 757873).

3.3.2.2.5 Reproductive Organ Weights and Histopathology

3.3.2.2.5.1 Male

Several studies investigated the effect of PFOS exposure on male reproductive organ weights. No effects were noted in the absolute and/or relative epididymal and/or testes weights in rats treated up to 5.0 mg/kg/day for 28 days (NTP, 2019, 5400978). Effects in mice exposed to PFOS were observed in a subchronic study in which significant decreases in absolute and relative testis weights were noted in mice exposed to 10 mg/kg/day for 35 days (Qu et al., 2016, 3981454). No effects were seen in relative epididymal weights of mice treated up to 10 mg/kg/day for four weeks, nor were any effects noted in the relative testis weight of mouse pups treated from GD1 to GD17 (Qiu et al. 2016, 3981408 and Lai et al., 2017, 3981773). Male cynomolgus monkeys treated with up to 0.75 mg/kg/day for 182 days showed no changes in absolute or relative testes weights (Seacat et al. 2002, 757853).

Histopathological examination of the aforementioned NTP study (2019, 5400978) revealed no treatment related changes in the testes, epididymis, seminal vesicle, or prostate (NTP 2019, 5400978). However, Lopez-Doval et al. (2014, 2850091; 2015, 2848266) noted edema around seminiferous tubules and malformed spermatids in male rats treated at ≥ 1 mg/kg/day with marked edema and loss and degeneration of the spermatozooids observed at 6 mg/kg/day following PFOS exposure up to 6 mg/kg/day for 28 days. The specific incidences of histopathological findings were not reported in this study, and statistical analysis was not conducted. In another study, subchronic exposure in rats revealed lesions including vacuolations in spermatogonia, spermatocytes, and Leydig cells, as well as exaggerated intracellular space and disturbed germ cells in rats treated at 10 mg/kg/day; however, incidences of specific findings were not reported, and statistical analyses were not conducted (Qu et al. 2016, 3981454).

Relevant histopathological findings in two 28-day studies in mice included Sertoli cell vacuolization and derangement of the cell layers at 2.5, 25, and 50 mg/kg/day and dislocated immature germ cells in seminiferous tubules at 50 mg/kg/day (Qiu et al. 2013, 2850956; Qiu et al., 2016, 3981408); 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 induce the functional disassemble of Sertoli cells junction barrier in vitro and disruption to Sertoli cell blood testis barrier function both in vitro and in vivo (Qiu et al., 2013, 2850956; Qiu et al., 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, cell proliferation in the testis was examined following exposure to PFOS for 182 days, however no difference was noted when compared to controls (Seacat et al. 2002, 757853).

3.3.2.2.5.2 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 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). 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 and are discussed further in Section 3.3.1.2.

3.3.2.3 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 50 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to reproductive effects. A summary of these studies is shown in Figure 55. Additional analysis on the mechanistic actions of PFOS on reproductive health outcomes is pending and is expected to be completed after the EPA Scientific Advisory Board review.

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	22	28
Cell Signaling Or Signal Transduction	7	1	14	22
Extracellular Matrix Or Molecules	2	0	2	4
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	1	1	5
Hormone Function	9	1	10	19
Oxidative Stress	2	0	5	7
Xenobiotic Metabolism	2	0	4	6
Other	2	0	1	3
Not Specified (Review Article)	1	0	0	1
Grand Total	18	3	33	50

Figure 55. Summary of Mechanistic Studies of PFOS and Reproductive Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.2.4 Evidence Integration

In summary, human epidemiological studies evaluating children observed inconsistent evidence for effects of PFOS on testosterone and E2, and evidence was limited in adults. Negative

associations with testosterone were observed in two *medium* confidence studies on children, and one study reported an inverse association for E2. Among *low* confidence studies, there was contrasting evidence for the relationship between PFOS and testosterone in cross-sectional studies {Di Nisio, 2019, 5080655; Zhou, 2016, 3856472; Zhou, 2017, 3858488} on children and adolescents. However, these contrasting relationships were observed in populations at different stages of pubertal development. Results showed decreasing testosterone with increasing serum PFOS in children, but increased testosterone was observed in adolescents in with higher PFOS exposure levels. Studies on adolescents did not identify effects on pubertal development, but associations were observed for penile measurements, testicular measurements, and sperm parameters {Di Nisio, 2019, 5080655}. 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, etc.) in sperm were inconsistent.

As in the 2016 Health Assessment, there is suggestive evidence available from human epidemiological studies of an association between PFOS and preeclampsia and gestational hypertension, with most studies observing positive non-significant associations. This has been observed both in populations with high exposure levels (2016 Health Assessment) and at levels typical in the general population (2016 Health Assessment and this updated review).

Human 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, 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, 2017, 3981439 observed negative associations between PFOS exposure and exclusive and total breastfeeding duration, while Romano, 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 (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, 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.

Three human epidemiological studies reported on the relationship between PFOS exposure and pregnancy loss since the 2016 Health Assessment, reporting mixed results. Louis, 2016, 3858527 reported a non-significant, negative association between serum PFOS levels and pregnancy loss in the first 7 weeks following conception, while Liew, 2016, 6387285 observed a non-significant positive association between plasma PFOS levels for odds of miscarriage.

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, 2016, 3981465 observed significant negative associations for maternal serum PFOS and cord blood prolactin and progesterone levels and Wang, 2019, 5080598 observed significant positive associations for cord blood PFOS and cord blood estrone and E3. In pregnant women, Yao, 2019, 5187556 observed significant, positive associations for cord blood PFOS and testosterone and testosterone to E2 ratio and Toft, 2016, 3102984 observed significant, positive trends in 17-OHP, androstenedione, progesterone, and testosterone across amniotic fluid PFOS tertiles.

Similar to the findings from the 2016 Health Effects Support Document, the recent human epidemiological evidence is suggestive of some male reproductive toxicity of PFOS, especially for semen parameters. The associations are inconsistent across various parameters, and it is difficult to assess the adversity of these alterations. Overall, the effects of PFOS on male reproductive outcomes were not consistent. 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.

In animal models, studies of varying durations suggest that oral exposure to PFOS can affect the male and female reproductive systems of animal species. However, many of the observed reproductive effects (e.g., decreased gestation length in female rats, decreased testosterone in male monkeys, prolactin levels in female mice) occurred at doses that also resulted in reduced body weight or decreased gestational weight gain which can be confounding effects for reproductive endpoints. Additionally, several of the observed effects were not consistent across species (e.g., sperm parameters, testis weight, E2 levels in males) which increases uncertainty about the relevance of these effects to humans or potential differences in the MOA between species.

Several studies reported effects of PFOS exposure on male mouse and rat reproductive organ histopathology (Qiu et al. 2013, 2850956; Qiu et al., 2016, 3981408; Qu et al. 2016, 3981454;

Lopez-Doval et al., 2014, 2850091; Lopez-Doval et al., 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 et al. 2013, 2850956; Qiu et al., 2016, 3981408; Qu et al. 2016, 3981454; Lai et al., 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 et al., 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. NTP (2019, 5400978) reported modest increases in E2 concentrations (37% increase) with PFOS doses of 1.25 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 E2 were accompanied by dose-dependent increases in estrous cycle length, though this increase was not statistically significant and alterations in the estrous cycle were not observed in a second study in female rats (Luebker et al. 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.

Overall, there is some mixed and suggestive evidence of reproductive toxicity available in the epidemiological literature. However, it is difficult to quantify these effects due to inconsistencies and limitations of the available data. 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. Therefore, no studies or endpoints from the available epidemiological and animal studies were considered for the derivation of PODs.

3.3.3 Hepatic

3.3.3.1 Human Evidence

3.3.3.1.1 Introduction

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive {Boone, 2005, 782862}. Bilirubin and γ -glutamyltransferase (GGT) are also routinely used to evaluate potential hepatobiliary toxicity {Boone, 2005, 782862; EMEA, 2008, 3056793; Hall, 2012, 2718645}. Elevation of liver serum biomarkers is frequently an indication of liver injury, though they are not as specific as functional tests, such as histology findings and liver disease.

In the 2016 Health Assessment for PFOS, two available cross-sectional studies reported positive associations with ALT in adults of the general population. Null or less consistent associations were reported with GGT and bilirubin. No evidence of functional hepatic endpoints was

available. The assessment concluded that the evidence was not strong enough to support an association because the participants were exposed to a mixture of PFAS.

3.3.3.1.2 Study Quality

For this updated review, 17 new epidemiology studies (19 publications)⁷ report on the relationship between PFOS exposure and liver effects. Of these, nine were classified as *medium* confidence, three as *low* confidence, and four were considered *uninformative* due to potential confounding {Jiang, 2014, 2850910; Predieri, 3889874} or use of PFAS as the dependent variable {Jain, 2020, 6833623; Fan, 2014, 2967086} (Figure 56). Of the twelve informative studies, four cross-sectional {Jain, 2019, 5381541; Nian, 2019, 5080307; van den Dungen, 2017, 5080340; Liu, 2018, 4238514}, one prospective cohort in elderly adults {Salihovic, 2018, 5083555}, and one occupational cohort of fluorochemical plant workers {Olsen, 2012, 2919185} examined liver enzymes in adults. In addition, two of the cross-sectional studies {Rantakokko, 2015, 3351439, Liu, 2018, 4238396} examined functional liver endpoints in adults. In children and adolescents, four studies were available including one cohort {Mora, 2018, 4239224} and three cross-sectional studies {Khalil, 2018, 4238547; Jin, 2020, 6315720; Attanasio, 2019, 5412069}, with one examining function liver endpoints {Jin, 2020, 6315720}. All of the studies measured PFOS exposure using biomarkers in blood.

⁷ Multiple publications of the same data: Jain et al. (2019, 5381566); Jain et al. (2019, 5080621); Jain et al. (2019, 5381541); Jain et al. (2020, 6833623).

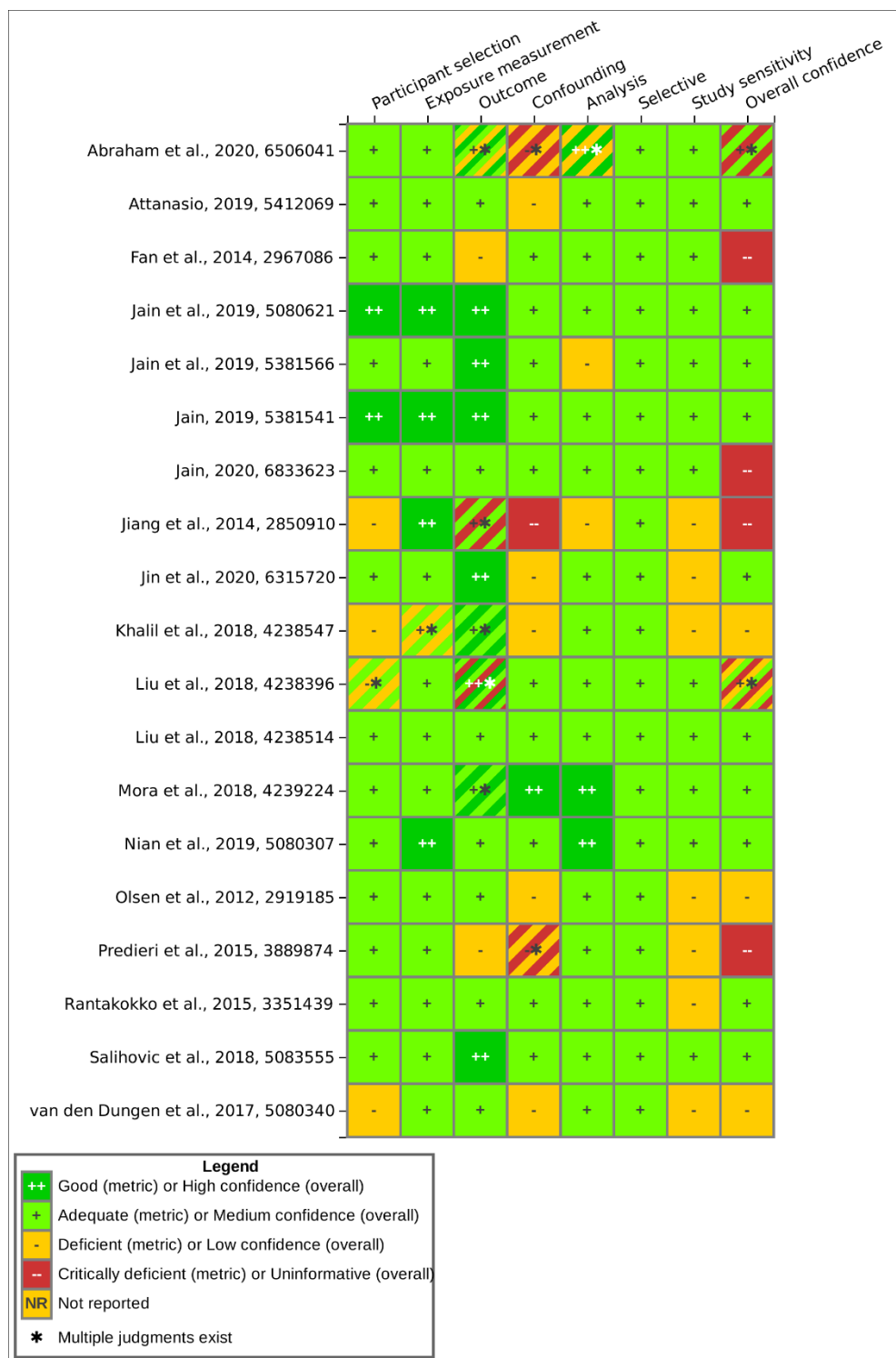


Figure 56. Summary of Study Evaluation for Epidemiology Studies of PFOS and Hepatic Effects^a

Interactive figure and additional study details available on [HAWC](#).

^aMultiple publications of the same data: Jain et al., 2019, 5381566; Jain et al., 2019, 5080621; and Jain, 2019, 5381541. Attanasio et al., 2019, 5412069 also includes Attanasio et al., 2019, 5918605.

3.3.3.1.3 Findings

Results for the eight studies that examined ALT are presented in Table C-6. In adults, statistically significant positive associations between ALT and PFOS (i.e., increased in ALT as a continuous measure with higher PFOS exposure levels) were observed in three of five studies {Salihovic, 2018, 5083555; Nian, 2019, 5080307; Jain, 2019, 5381541}, including all the *medium* confidence studies. However, the positive associations in Jain et al. (2019, 5381541) were observed only in obese participants. In non-obese participants, associations were generally null, with an inverse association in non-obese participants with glomerular filtration (GF) stage of 3B/4. Among *low* confidence studies in adults, an inverse association was reported ($p < 0.05$) in Olsen et al. (2012, 2919185). However, this analysis differed from the other studies in that the exposure measure used was change in PFOS levels during the study period. In van den Dungen et al. (2017, 5080340), no association was observed. In children and adolescents, positive associations were observed in girls in the fourth quartile in Attanasio et al. (2019, 5412069) and in the *low* confidence study in obese children {Khalil, 2018, 4238547}. However, inverse associations were observed in Mora et al. (2018, 4239224), so associations in this age group are less consistent than in adults.

Six studies examined AST and are presented in Table C-6. In adults, statistically significant positive associations were observed in the two *medium* confidence studies (Jain, 2019, 5381541; Nian, 2019, 5080307). van den Dungen et al. (2017, 5080340) reported a non-significant positive association. No association was observed in Olsen et al. (2012, 2919185). In children and adolescents, the *medium* confidence study {Attanasio, 2019, 5412069} also observed a positive association in girls but not boys, while the *low* confidence study {Khalil, 2018, 4238547} reported an inverse association, both not statistically significant. For the other liver enzymes (bilirubin, GGT), results were generally consistent with ALT and AST {van den Dungen, 2017, 5080340; Nian, 2019, 5080307; Attanasio, 2019, 5912069} with the exception of inverse associations (not statistically significant) for GGT in Jain (2019, 5381541) and bilirubin in {Salihovic, 2018, 5083555}.

For functional measures of liver injury, two *medium* confidence studies, one in adults and one in children and adolescents, examined histology endpoints. Both studies examined lobular inflammation. Rantakokko et al. (2015, 3351439) reported higher PFOS exposure levels were associated with reduced odds of lobular inflammation, while Jin et al. (2020, 6315720) reported the opposite, with OR of 2.9 for 2–4 foci vs none, though the results in the latter study were non-monotonic and both were not statistically significant. Jin et al. (2020, 6315720) additionally reported higher odds (not statistically significant) of nonalcoholic steatosis ($p < 0.05$), ballooning, fibrosis, and portal inflammation).

3.3.3.2 Animal Evidence

There are 10 studies from the most recent literature search conducted in 2020 and 5 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 15 studies are shown in Figure 57.

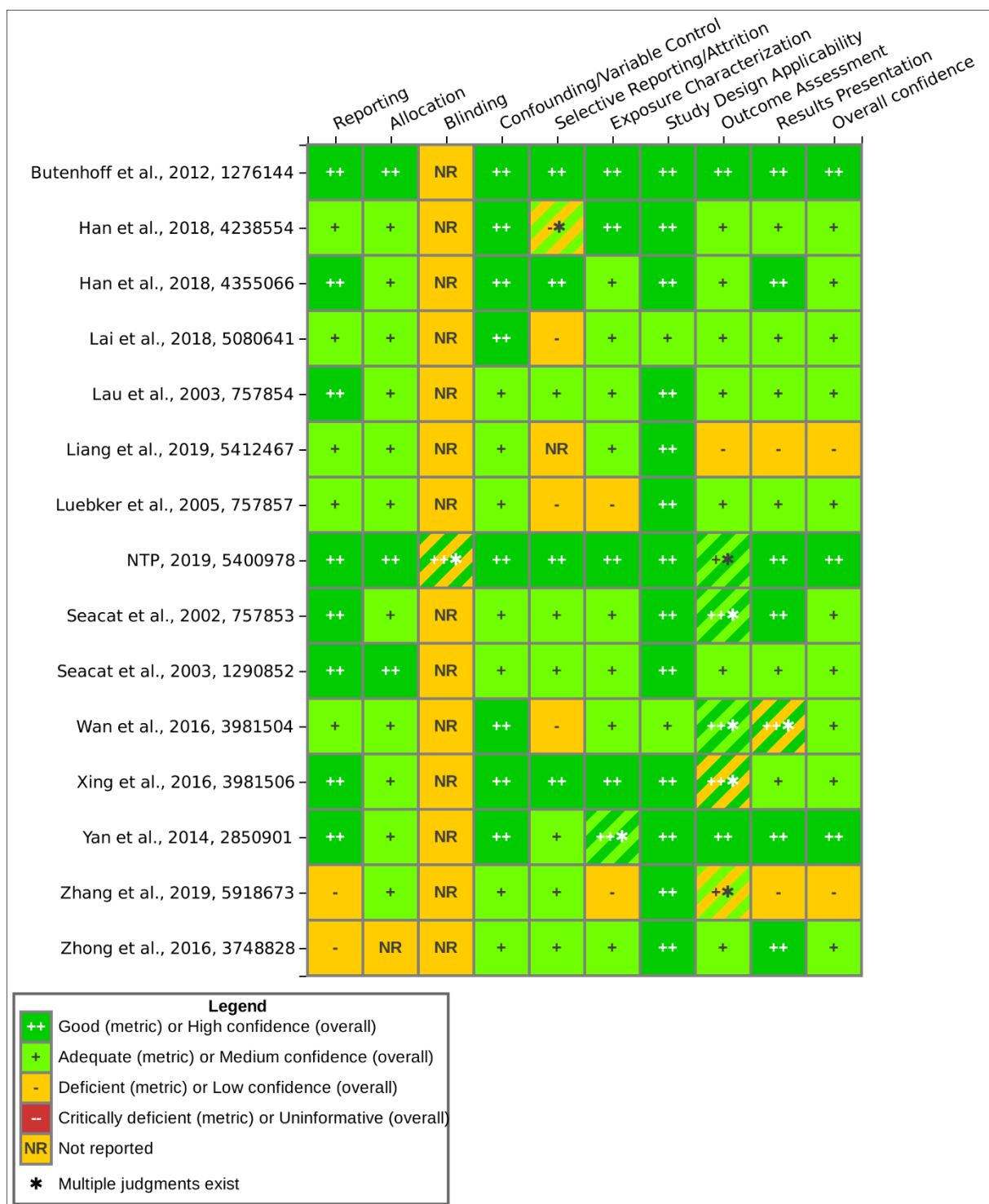


Figure 57. Summary of Study Evaluation for Toxicology Studies of PFOS and Hepatic Effects

Interactive figure and additional study details available on [HAWC](#).

Hepatic effects were observed in male and female mice, rats, and monkeys after varying oral exposure durations and PFOS doses. This includes effects such as increased absolute and relative liver weight, altered clinical parameters indicating potential liver injury, and histopathological alterations of liver tissue. Data from numerous studies provide evidence confirming the liver as a target of PFOS toxicity.

3.3.3.2.1 Liver Weight

Significant increases in liver weight relative to body weight and absolute liver weights were observed in several strains of male and female mice exposed to 1.25–10 mg/kg/day of PFOS for short-term, subchronic, and gestational durations {Lai, 2018, 5080641; Xing, 2016, 3981506; Yan, 2014, 2850901; Lau, 2003, 757854; Zhong et al., 2016, 3748828}. In male BALB/c mice, significant increases in both relative and absolute liver weights were observed after 28-day exposure to PFOS doses of 1.25 and 5 mg/kg/day {Yan, 2014, 2850901}. Xing et al. (2016, 3981506) similarly observed increased relative liver weight in male C57BL/6J mice dosed with 2.5–10 mg/kg/day PFOS for 30 days. In a 7-week gavage study in female CD-1 mice, Lai et al. (2018, 5080641) reported significant increases in absolute and relative liver weights with 3 mg/kg/day but not 0.3 mg/kg/day. Two available developmental studies in mice, indicate that relative liver weight of pups exposed to PFOS during gestation may increase and subsequently return to control levels after prolonged cessation of exposure during postnatal development {Zhong, 2016, 3748828; Lau, 2003, 757854}. Zhong et al. (2016, 3748828) dosed C57BL/6J mouse dams with 0, 0.1, 1, or 5 mg/kg/day PFOS from GD1–GD17. Relative liver weights of the male and female pups in the 5 mg/kg/day group were significantly increased at PNW4; however, liver weights of both sexes returned to levels statistically indistinguishable from controls by PNW 8. Similarly, Lau et al. (2003, 757854) exposed pregnant CD-1 mice to 0, 1, 5, or 10 mg/kg/day PFOA from GD1–GD17 and found significant increases in offspring liver weights from the 5 and 10 mg/kg/day dose groups at PND0 and PND7 but not PND35.

Significant increases in liver weight relative to body weight and absolute liver weights were also observed in male and female rats exposed to 0.15–20 mg/kg/day PFOS for short-term, chronic, and gestational durations {NTP, 2019, 5400978; Curran, 2008, 757871; Seacat, 2003, 1290852; Lau, 2003, 757854; Cui, 2009, 757868; Wan, 2012, 1332470; Wan, 2016, 3981504; Han, 2018, 4355066}. An increase in relative liver weight was observed with exposure as low as 0.15 mg/kg/day PFOS administered to female Sprague Dawley rats for 28 days {Curran, 2008, 757871}. In males from the same study, relative liver weight was significantly increased at a higher concentration of 1.33 mg/kg/day. However, a similar study in Sprague Dawley rats found that relative and absolute liver weights were increased in both males and females dosed with ≥ 0.312 mg/kg/day PFOS for 28 days, the lowest exposure included in the study design {NTP, 2019, 5400978}. In a 14-week feeding study, Seacat et al. (2003, 1290852) also observed similar responses in male and female Sprague Dawley rats, with significant increases in relative liver weight at the highest dose tested in each sex (1.33 and 1.56 mg/kg/day, respectively). Absolute liver weight was only increased in males from the 1.33 mg/kg/day dose group. In a developmental study, Lau et al. (2003, 757854) observed inconsistent alterations in liver weight across time points in offspring exposed to 0, 1, 2, or 3 mg/kg/day PFOS from GD2–GD21. Significant increases in relative liver weight were observed in the 2 and 3 mg/kg/day dose groups at PND5, but not PND0 or PND35.

In a subchronic study in cynomolgus monkeys, relative and absolute liver weights were increased in males and females dosed with 0.75 mg/kg/day PFOS for 182 days (26 weeks) {Seacat, 2002, 757853}.

3.3.3.2.2 Clinical Chemistry

Increases in enzymes including ALT, alkaline phosphatase (ALP), AST, and GGT following PFOS exposures were observed across multiple species, sexes, and exposure paradigms (Figure 58 (mice), Figure 59 (male rats), Figure 60 (female rats)). These enzymes are often useful indicators of hepatic enzyme induction, hepatocellular damage, or hepatobiliary damage as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood {USEPA, 2002, 625713}. Alterations in serum enzymes are generally considered to reach biological significance and indicate potential adversity at levels ≥ 2 -fold compared to controls (i.e., $\geq 100\%$ change relative to control response) {U.S. EPA, 2002, 625713; Hall, 2012, 2718645}.

Two studies in male mice showed statistically and biologically significant increases in serum enzymes indicative of hepatic or hepatobiliary damage due to oral PFOS exposure (Figure 58) {Yan, 2014, 2850901; Xing, 2016, 3981506}. Xing et al. (2016, 3981506) observed a dose-dependent increase in ALT of male C57BL/6J mice after 30 days of 0, 2.5, 5, or 10 mg/kg/day PFOS exposure, though ALT levels only reached a 50% or 88% increase compared to control levels in the 5 and 10 mg/kg/day groups, respectively. In comparison, after a 28-day exposure to 0, 1.25, or 5 mg/kg/day PFOS in male BALB/c mice, Yan et al. (2014, 2850901) observed much larger increases in ALT in the 5 mg/kg/day group ($> 700\%$ change), though there was not an apparent linear dose-response relationship observed across all dose levels tested. Both Yan et al. (2014, 2850901) and Xing et al. (2016, 3981506) observed statistically but not biologically significant responses in AST with increasing PFOS doses (responses did not exceed 50% change with any dose). Xing et al. (2016, 3981506) observed a similar statistically but not biologically significant response in ALP levels (53% change in the 10 mg/kg/day group). Similar to the response in ALT levels, Yan et al. (2014, 2850901) also reported relatively large increases in ALP of 321% change in the 5 mg/kg/day dose group. Interestingly, a significant dose-dependent increase in GGT was observed by Xing et al. (2016, 3981506), with an increase of approximately 140% in the lowest dose group (2.5 mg/kg/day) and 535% in the highest dose group (10 mg/kg/day), indicating potential damage to the biliary system {U.S. EPA, 2002, 625713}.

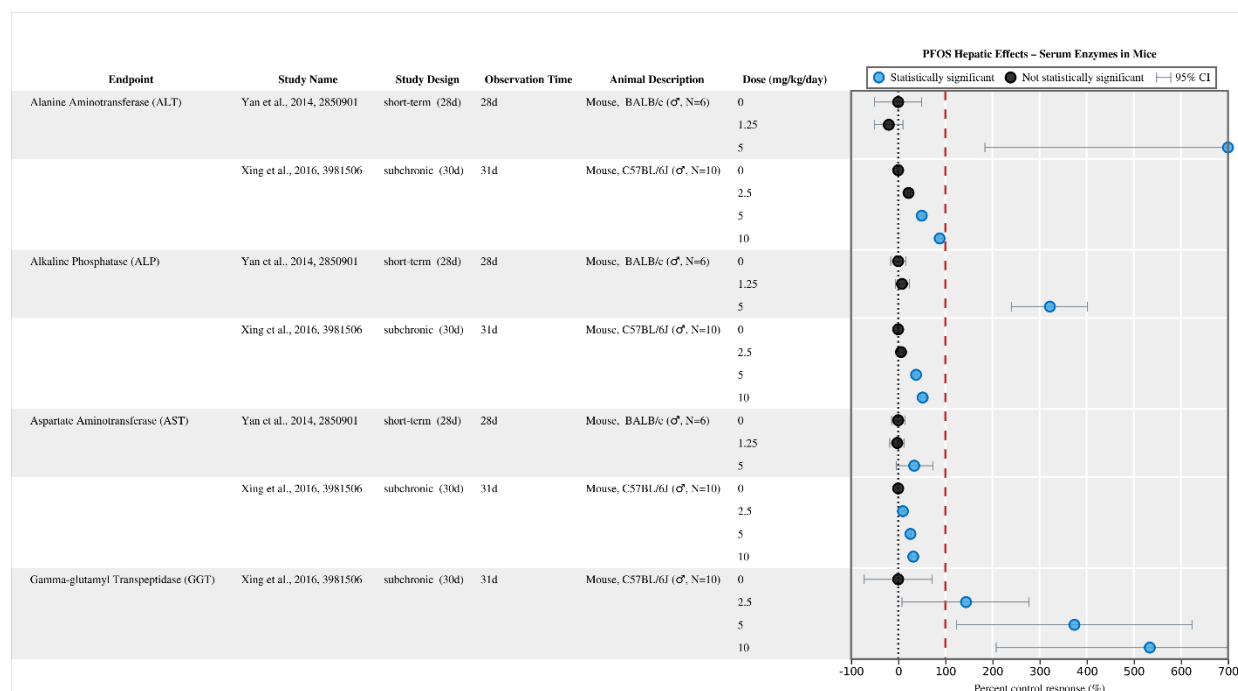


Figure 58. Percent Change in Serum Enzyme Levels Relative to Controls in Mice Following Exposure to PFOS^a

Interactive figure and additional study details available on [HAWC](#) and [Tableau](#).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase; d = day; CI = confidence interval.

^aResults for Yan et al., 2014, 2850901 are presented for 3 doses (0, 1.25, and 5 mg/kg-day), and a statistically significant response of 756% occurs at the highest dose for the endpoint alanine aminotransferase (ALT). The axis has been truncated at 700% to allow results at lower doses for other studies and endpoints to be legible.

The red dashed line indicates a 100% increase from the control response.

Multiple studies are available that assessed serum enzymes in male and female Sprague Dawley rats exposed to PFOS for short-term and chronic exposure durations (Figure 59, Figure 60) {Han, 2018, 4238554/Han, 2018, 4355066/Wan, 2016, 3981504; NTP, 2019, 5400978; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144; Curran, 2008, 757871}. NTP (2019, 5400978), Han et al. (2018, 4355066), and Curran et al. (2008, 757871) reported statistically significant increases in ALT levels in male rats exposed to PFOS for 28 days. However, these increases did not exceed 75% change at even the highest doses tested in each study (5, 10, and 6.34 mg/kg/day, respectively). Seacat et al. (2003, 1290852) similarly observed statistically but not biologically significant increases in ALT in male rats from the highest dose group (1.33 mg/kg/day) in a 14-week dietary PFOS study. Butenhoff et al. (2012, 1276144) did not observe consistent dose-related changes in ALT levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks, though this study used a relatively low dose range of approximately 0.02–1 mg/kg/day.

Similar to ALT levels, AST levels in male Sprague Dawley rats exposed to PFOS for varying durations did not exceed two-fold changes compared to controls. Han et al. (2018, 4355066) reported dose-dependent increases in AST levels in male rats dosed with 0, 1, or 10 mg/kg/day PFOS for 28 days, though the increase did not exceed 20% change in the 10 mg/kg/day dose group. Three other 28-day studies assessing AST levels in male rats either reported changes in

AST that were not dose-dependent {NTP, 2019, 5400978} or reported no statistical differences between PFOS treated and control groups {Seacat, 2003, 1290852; Curran, 2008, 757871}. Butenhoff et al. (2012, 1276144) also did not observe statistically significant changes in AST levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks with doses up to 0.984 mg/kg/day.

In male rats, NTP (2019, 5400978) reported statistically significant increases in ALP with 28-day PFOS exposure as low as 0.625 mg/kg/day. However, these increases only ranged from approximately 15–35% change across all doses with statistically significant responses. Similarly, Curran et al. (2008, 757871) did not observe consistent effects of 28-day dietary consumption of PFOS concentrations up to ~6.34 mg/kg/day in male rats.

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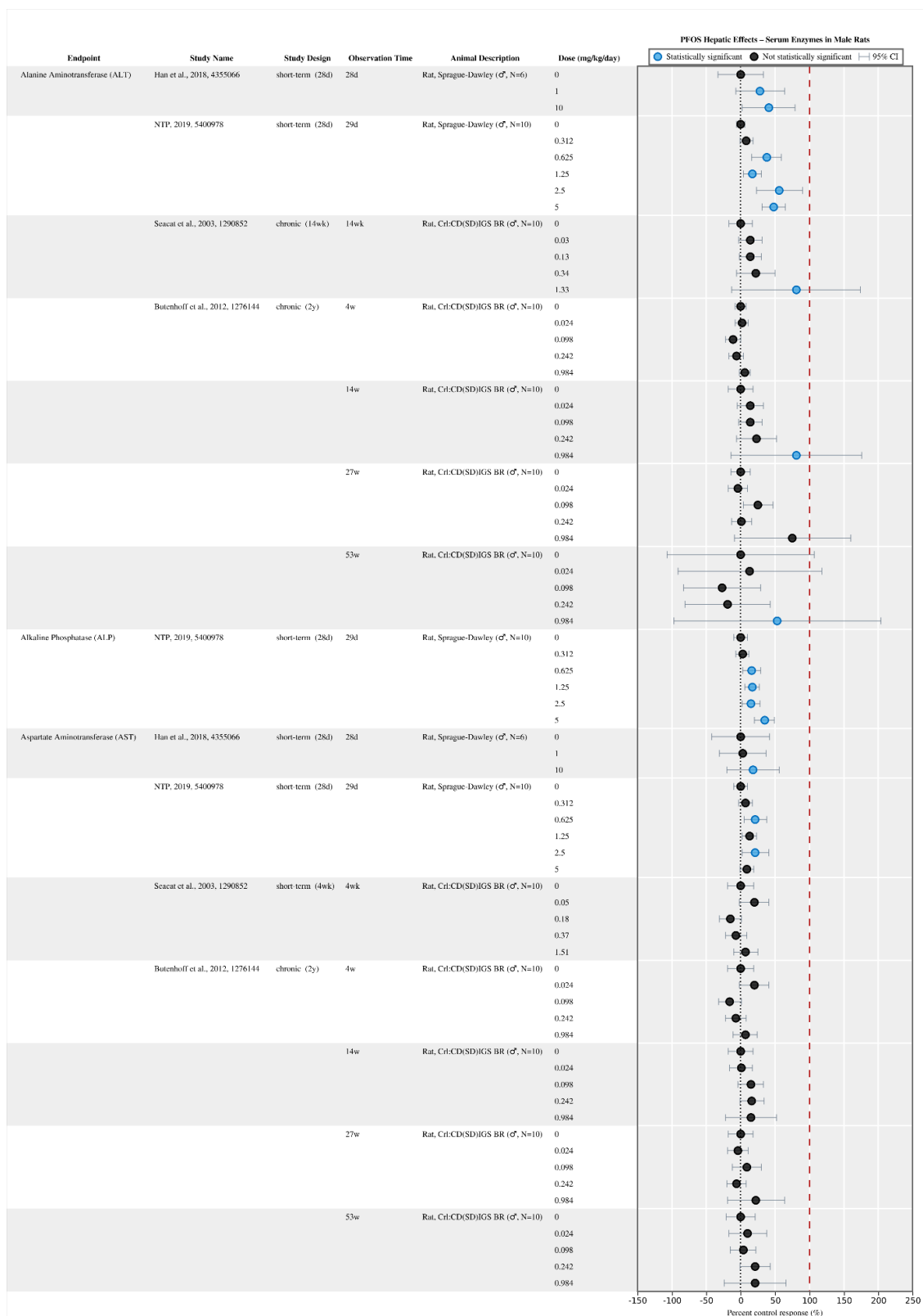


Figure 59. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOS^a

Interactive figure and additional study details available on [HAWC](#) and [Tableau](#).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = yr;
CI = confidence interval.

^aTwo publications (Han et al., 2018, 4238554 and Wan et al., 2016, 3981504) reported on the same data as Han et al. 2018, 4355066 and are not shown in the figure.

The red dashed line indicates a 100% increase from the control response.

As generally observed in male Sprague Dawley rats, there were also statistically significant but not biologically significant alterations in serum enzyme levels observed in female Sprague Dawley rats exposed to PFOS for 4–53 weeks {NTP, 2019, 5400978; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144; Curran, 2008, 757871}. In a 28-day study in female rats, NTP (2019, 5400978) reported dose-dependent increases in ALT, though these increases reached only approximately 62% change with the highest dose tested (10 mg/kg/day). A second dietary 28-day study in female rats reported no statistical difference between the control group or groups treated with up to ~7.58 mg/kg/day PFOS (Curran et al., 2008, 757871). Similarly, Seacat et al. (2003, 1290852) observed no significant differences in ALT levels of female rats exposed to dietary concentrations of PFOS up to ~1.56 mg/kg/day for 14 weeks. Butenhoff et al. (2012, 1276144) also did not observe significant changes in ALT levels in female rats exposed to dietary concentrations of PFOS for 4, 14, 27, or 53 weeks with doses up to ~1.25 mg/kg/day.

Both Curran et al. (2008, 757871) and Butenhoff et al. (2012, 1276144) observed statistically significant decreases in AST levels of female rats exposed to PFOS for 28 days at the highest dose tested in each study (7.58 and 1.251 mg/kg/day, respectively). These alterations were approximately 25–26% decreases from control levels in both studies. In contrast, two other 28-day studies in female rats did not observe significant changes in AST levels compared to controls {NTP, 2019, 5400978; Seacat, 2003, 1290852} and the significant decreases observed by Butenhoff et al. (2012, 1276144) at the 4-week time point were not observed at the 14-, 27-, or 53-week time points.

NTP (2019, 5400978) reported statistically but not biologically significant increases in ALP at doses of 2.5 and 5 mg/kg/day in female rats exposed to PFOS for 28 days (increases did not exceed 35% change with either dose). In another 28-day study, ALP levels in female rats administered up to 7.58 mg/kg/day PFOS were not significantly different from control levels {Curran, 2008, 757871}.

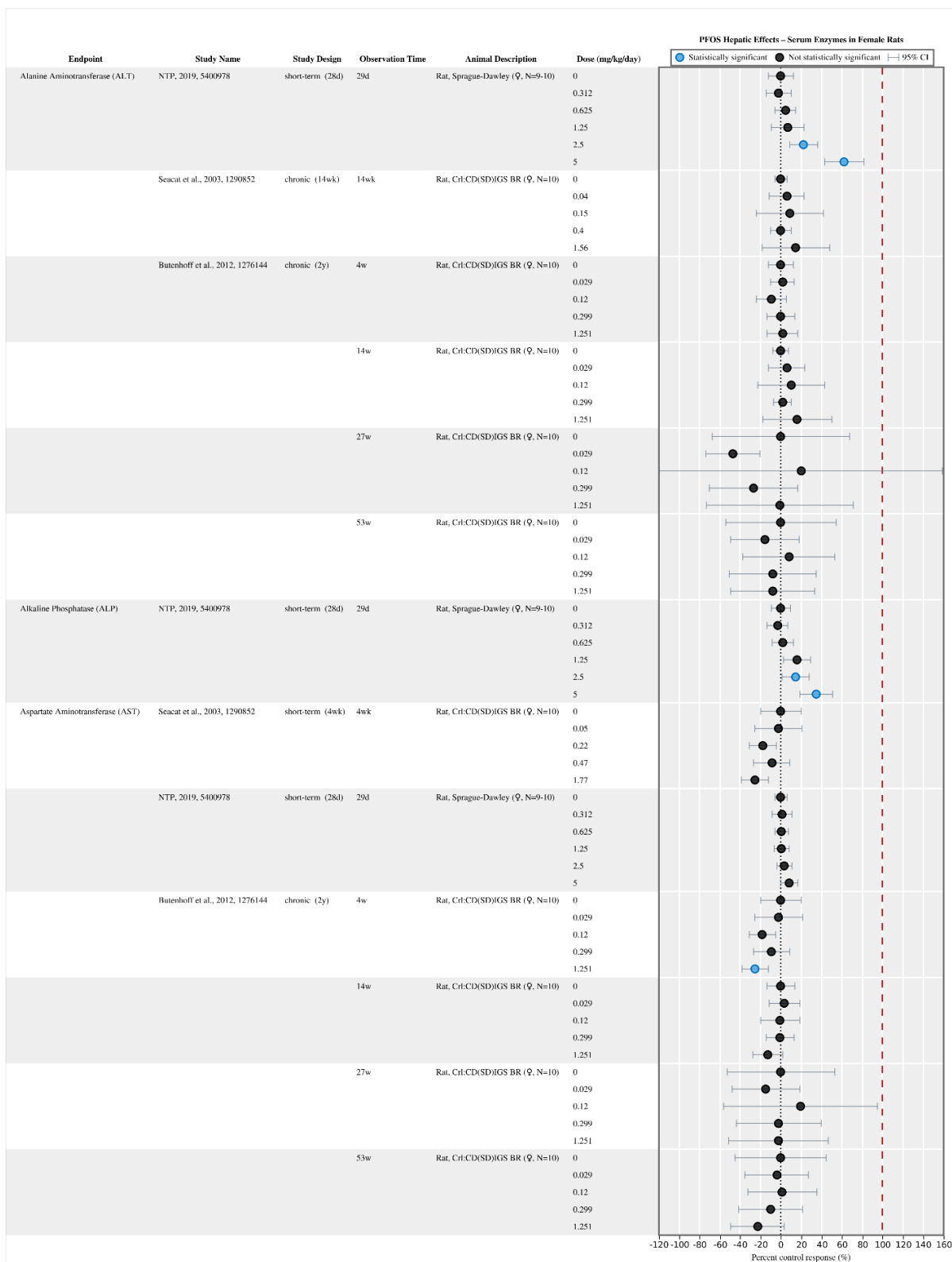


Figure 60. Percent Change in Serum Enzyme Levels Relative to Controls in Female Rats Following Exposure to PFOS^a

Interactive figure and additional study details available on [HAWC](#) and [Tableau](#).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = yr;
CI = confidence interval.

^aTwo publications (Han et al., 2018, 4238554 and Wan et al., 2016, 3981504) reported on the same data as Han et al. 2018, 4355066 and are not shown in the figure.

The red dashed line indicates a 100% increase from the control response.

Neither ALT nor ALP were significantly altered in male or female cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 26 weeks {Seacat, 2002, 757853}.

Levels of bilirubin, albumin, and bile salt/acids were also observed to be altered in several studies in mice, rats, and monkeys. However, these clinical chemistry measurements were generally altered at higher concentrations of PFOS than were serum enzymes and changes were inconsistent among studies. Bilirubin (direct, indirect, or total) was either unchanged or increased in male rats exposed to ≥ 5 mg/kg/day PFOS and in female rats exposed to ≥ 2.5 mg/kg/day PFOS {NTP, 2019, 5400978; Curran, 2008, 757871; Seacat, 2003, 1290852}. Total bilirubin was decreased in male monkeys exposed to 0.75 mg/kg/day from 91–182 days but did not show a statistically significant response in female monkeys {Seacat, 2002, 757853}. Five studies examined albumin levels, but only two studies found significant alterations due to PFOS treatment {Yan, 2014, 2850901; NTP, 2019, 5400978; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144; Curran, 2008, 757871}. In male mice dosed with 1.25 or 5 mg/kg/day of PFOS for 28 days, albumin was significantly increased above control levels at both doses {Yan, 2014, 2850901}. In rats dosed with PFOS for 28 days, albumin was significantly increased in females dosed with 1.25–5 mg/kg/day and in males dosed with 5 mg/kg/day {NTP, 2019, 5400978}. Bile salt/acids were significantly increased in male rats exposed to 5 mg/kg/day PFOS and in female rats exposed to 2.5 and 5 mg/kg/day PFOS {NTP, 2019, 5400978}. In monkeys, serum bile acids were significantly increased in males, but not in females, dosed with 0.75 mg/kg/day PFOS {Seacat, 2002, 757853}.

3.3.3.2.3 Histopathology

Liver lesions were confirmed microscopically in male mice and male and female rats in several short-term and subchronic studies {Wan, 2012, 1332470; Xing, 2016, 3981506; Curran, 2008, 757871; Cui, 2009, 757868; Han, 2018, 4238554/Han, 2018, 4355066/Wan, 2016, 3981504; NTP, 2019, 5400978} and in two chronic studies of male and female rats and monkeys {Seacat, 2002, 757853; Butenhoff, 2012, 1276144}. Only three of these studies provided quantitative incidence data {NTP, 2019, 5400978; Butenhoff, 2012, 1276144; Curran, 2008, 757871}.

Hepatocellular hypertrophy was shown to be significantly increased in male Sprague Dawley rats dosed with 2.5 and 5 mg/kg/day PFOS and in females dosed with 5 mg/kg/day PFOS for 28 days {NTP, 2019, 5400978} (Table 6). Cytoplasmic vacuolation and alterations were significantly increased in a dose-dependent manner in male and female rats, respectively, in the 2.5 (females only) and 5 mg/kg/day (males and females) exposure groups {NTP, 2019, 5400978}. Another 28-day study in Sprague Dawley rats observed higher incidence of hepatocellular hypertrophy in zone 3 of the liver in males exposed to 3.21 and 6.24 mg/kg/day PFOS, the two highest concentrations; no incidence was seen in females {Curran, 2008, 757871} (Table 7). A higher incidence of cytoplasmic homogeneity in zone 3 of the liver was also observed in both males and females exposed to 3.21 and 6.24 mg/kg/day PFOS {Curran, 2008, 757871}. In the chronic study in Sprague Dawley rats {Butenhoff, 2012, 1276144}, hepatocellular hypertrophy was significantly increased in males exposed to 0.098–0.984 mg/kg/day of PFOS and in females

exposed to 0.299–1.251 mg/kg/day for 14–103 weeks; a dose-response relationship was observed (Table 8).

Table 6. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by NTP (2019, 5400978)

	0 mg/kg/day	0.312 mg/kg/day	0.625 mg/kg/day	1.25 mg/kg/day	2.5 mg/kg/day	5 mg/kg/day
Males						
Hepatocyte, Hypertrophy	0/10	0/10	0/10	3/10	8/10**	10/10**
Hepatocyte, Vacuolization, Cytoplasmic	0/10	0/10	0/10	0/10	2/10	4/10*
Females						
Hepatocyte, Hypertrophy	0/10	0/10	0/10	2/10	3/10	10/10**
Hepatocyte, Cytoplasmic Alteration	0/10	0/10	0/10	3/10	5/10*	10/10**

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

Table 7. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Curran et al. (2008, 757871)

Males					
	0 mg/kg/day	0.14 mg/kg/day	1.33 mg/kg/day	3.21 mg/kg/day	6.34 mg/kg/day
Hepatocyte, Hypertrophy in Zone 3	0/4	0/4	0/4	1/4	3/4
Cytoplasmic Homogeneity in Zone 3	0/4	0/4	0/4	1/4	3/4
Females					
	0 mg/kg/day	0.15 mg/kg/day	1.43 mg/kg/day	3.73 mg/kg/day	7.58 mg/kg/day
Hepatocyte, Hypertrophy in Zone 3	0/4	0/4	0/4	0/4	0/4
Cytoplasmic Homogeneity in Zone 3	0/4	0/4	0/4	1/4	3/4

Table 8. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Butenhoff et al. (2012, 1276144)

	Males				
	0 mg/kg/day	0.024 mg/kg/day	0.098 mg/kg/day	0.242 mg/kg/day	0.984 mg/kg/day
Hypertrophy, Hepatocellular, Centrilobular	0/65**	2/55	4/55*	22/55**	42/65**
Necrosis, Individual Hepatocyte	5/65*	4/55	6/55	5/55	14/65*
Vacuolation, Hepatocellular Midzonal/Centrilobular	3/65**	3/55	6/55	13/55**	19/65**
Degeneration, Cystic	5/65**	15/55**	19/55**	17/55**	22/65**
	Females				
	0 mg/kg/day	0.029 mg/kg/day	0.120 mg/kg/day	0.299 mg/kg/day	1.251 mg/kg/day
Hypertrophy, Hepatocellular, Centrilobular	2/65**	1/55	4/55	16/55*	52/65**
Necrosis, Individual Hepatocyte	7/65*	6/55	6/55	6/55	15/65*
Infiltrate, Lymphohistiocytic	42/65**	42/55	38/55	41/55	56/65**
Infiltrate, Macrophage, Pigmented	2/65**	3/55	5/55	6/55	23/65**
Degeneration, Cystic	0/65*	1/55	1/55	2/55	4/65

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group.

Statistical significance for the vehicle control indicates a significant trend test

*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

Butenhoff et al. (2012, 1276144) also observed a statistically significant dose-dependent increase in cystic degeneration in males exposed to 0.024–0.984 mg/kg/day of PFOS (Table 8); this effect was not significant in any female exposure group, but there was a statistically significant positive trend across all exposure groups. Lymphohistiocytic and macrophage infiltrate were significantly increased in a dose-dependent manner in females exposed to 1.251 mg/kg/day. A dose-response relationship was also observed with hepatocellular single cell necrosis, which was significantly increased in males and females exposed to 0.984 and 1.251 mg/kg/day PFOS, respectively {Butenhoff, 2012, 1276144}.

The most consistently observed liver lesions following short-term, subchronic, and chronic exposure to PFOS were hepatocellular hypertrophy and vacuolization. Other liver lesions commonly observed include single-cell and/or focal necrosis, hepatocytic or cystic degeneration, and inflammatory cell infiltration. However, in many instances these are only qualitatively described as being observed by the study authors without incidence provided. A single study in male mice dosed with PFOS for 30 days observed hepatocellular hypertrophy and cytoplasmic vacuolation in all treatment groups (2.5, 5, and 10 mg/kg/day), but did not provide incidence data

to evaluate a dose response {Xing, 2016, 3981506}. Male rats were used in multiple studies and this effect was observed at a range of exposures. Three studies from the same lab observed hepatocellular hypertrophy in male Sprague Dawley rats dosed with 1 mg/kg/day of PFOS for 28 days {Han, 2018, 4238554/Han, 2018, 4355066/Wan, 2016, 3981504}; however, none of the studies provided incidence data. Hepatocellular hypertrophy was also observed in another 28-day rat study that was conducted with higher concentrations of PFOS (5 and 20 mg/kg/day) {Cui, 2009, 757868}. Hepatocellular hypertrophy was also observed in male and female cynomolgus monkeys exposed to 0.75 mg/kg/day PFOS for 182 days (incidence data not provided) {Seacat, 2002, 757853}.

Hepatocytic or cystic degeneration, inflammatory cell infiltration, and/or necrosis, were observed in several short-term and subchronic studies (28–30 days) in male mice and rats {Xing, 2016, 3981506; Cui, 2009, 757868; Han, 2018, 4238554/Han, 2018, 4355066/Wan, 2016, 3981504}. Livers of male C57BL/6J mice and Sprague Dawley rats dosed with PFOS concentrations ranging from 2.5–20 mg/kg/day for approximately 4 weeks showed focal or flakelike necrosis, hepatocytic degeneration, and/or inflammatory cell infiltration {Xing, 2016, 3981506; Cui, 2009, 757868}. Three publications from the same lab described hepatocyte degeneration and inflammatory infiltration in male Sprague Dawley rats dosed with lower concentrations of 1 mg/kg/day PFOS for 28 days {Han, 2018, 4238554/Han, 2018, 4355066/Wan, 2016, 3981504}. However, no quantification or statistical analyses were performed on these studies.

3.3.3.3 *Mechanistic Evidence*

Mechanistic evidence linking PFOS exposure to adverse hepatic outcomes is discussed in Sections 3.2.2, 3.2.3, 3.2.5, 3.3.4, 3.3.5, and 3.4.1.1 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 53 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to hepatic effects. A summary of these studies is shown in Figure 61. Additional analysis on the mechanistic actions of PFOS on hepatic health outcomes is pending and is expected to be completed after the EPA Scientific Advisory Board review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	7	0	4	11
Cell Growth, Differentiation, Proliferation, Or Viability	10	1	23	30
Cell Signaling Or Signal Transduction	8	1	15	20
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	12	0	10	19
Hormone Function	4	1	1	6
Inflammation And Immune Response	5	1	1	6
Oxidative Stress	5	0	6	10
Renal Dysfunction	1	0	0	1
Xenobiotic Metabolism	5	1	8	13
Other	3	0	0	3
Not Specified (Review Article)	1	0	0	1
Grand Total	27	2	31	53

Figure 61. Summary of Mechanistic Studies of PFOS and Hepatic Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.3.4 Evidence Integration

In summary, the updated human epidemiological evidence provides further support to the positive association between PFOS exposure and ALT in adults described in the 2016 Health Assessment. This has been observed consistently in *medium* confidence studies of general population adults. However, the associations were not large in magnitude and it is unclear whether the observed changes are clinically adverse. Evidence for other liver enzymes and in children and adolescents is less consistent. Results for functional measures of liver toxicity, specifically histology results, are mixed. There is some indication of higher risk of liver disease with higher exposure, coherent with the liver enzyme findings, but there is inconsistency for lobular inflammation among the two available studies, which decreases certainty. Additional uncertainty exists due to the potential for confounding across PFAS. None of the existing studies performed multipollutant modeling to assess this issue. Among the studies of ALT in adults, two presented correlations across PFAS {Nian, 2019, 5080307; Salihovic, 2018, 5083555}; PFOA and PFOS were moderately correlated in both studies ($r = 0.4$ – 0.5). Jin et al. (2020, 6315720), which reported positive associations with histology, reported fairly low correlations between PFOS/PFOA ($r = 0.14$), which reduces the concern for confounding in that population. It is not possible to rule out potential confounding across PFAS with this evidence, but there is also no evidence that confounding can explain the observed associations. Though there was a consistent

positive association between PFOS exposure and ALT in adults, this endpoint was not considered for derivation of a POD because the associations were not large in magnitude and it is unclear whether the observed changes are clinically adverse.

Much of the available literature in animal models also provides evidence of PFOS-induced hepatic alterations. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those that indicate functional impairment or lesions {U.S. EPA, 2002, 625713; FDA, 2009, 6987952; EMEA, 2010, 3056796; Hall, 2012, 2718645}. EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant increases in enzymes indicative of liver toxicity {U.S. EPA, 2002, 625713}.

Multiple studies in mice and rats report increases in relative liver weights accompanied by statistically significant increases in serum enzymes, though these increases were generally under two-fold (100% change relative to control) as compared to control {Seacat, 2003, 1290852; Curran, 2008, 757871; Butenhoff, 2012, 1276144; Xing, 2016, 3981506; Yan, 2014, 2850901; NTP, 2019, 5400978; Han, 2018, 4355066}. However, these changes in serum enzyme levels were accompanied by histopathological evidence of damage.

Of the three available animal studies with quantitative histopathological data, a chronic study in rats {Butenhoff et al., 2012, 1271644} was the only study that identified dose-dependent increases in hepatocellular hypertrophy, hepatocellular vacuolation, hepatocytic necrosis, and inflammatory cell infiltration, though these effects were qualitatively reported in other studies {Xing, 2016, 3981506; Han, 2018, 4355066; Cui, 2009, 757868}. A 28-day study in male and female rats also reported dose-dependent increases in hepatocellular hypertrophy and cytoplasmic alterations {NTP, 2019, 5400978}. A second short-term study in rats {Curran, 2008, 757871} only had a limited sample size of 4 rats/sex/treatment group, though there were apparent dose-dependent increases in hypertrophy and cytoplasmic alterations in PFOS-exposed rats. These two studies are supportive of the results observed by Butenhoff et al. (2012, 1271644).

Butenhoff et al. (2012, 12761440) is the only chronic animal study available that quantitatively examines hepatic histopathology after PFOS exposure. It utilizes a relatively low dose range (0.024–0.984 mg/kg/day in males and 0.029–1.251 mg/kg/day in females) and large sample size (n=55-65/sex/treatment group) in both male and female rats. From this study, the endpoint of individual hepatocyte necrosis in females was considered for the derivation of a POD.

3.3.4 Immune

3.3.4.1 Human Evidence

3.3.4.1.1 Immunosuppression

Immune function—specifically immune system suppression—can affect numerous health outcomes, including risk of common infectious diseases (e.g., colds, influenza, otitis media) and some types of cancer. The WHO guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications {WHO, 2012, 9522548}.

In the 2016 Health Assessment for PFOS, there was consistent evidence of an association between PFOS exposure and immunosuppression in children. Two studies reported decreases in response to one or more vaccines in relation to higher exposure to PFOS in children {Grandjean, 2012, 1248827; Granum, 2013, 1937228}. In one study of adults, no association was observed {Looker, 2014, 2850913}. For this updated review, associations between prenatal, childhood, or adult PFOS exposure and immunosuppression, specifically infectious disease incidence and antibody response to vaccination, were examined in 17 studies. A summary of the study evaluation of these studies is provided in Figure 62. One study from the 2016 assessment {Grandjean, 2012, 1248827} was updated during this period, and the update was included in the systematic review {Grandjean, 2017, 3858518}.

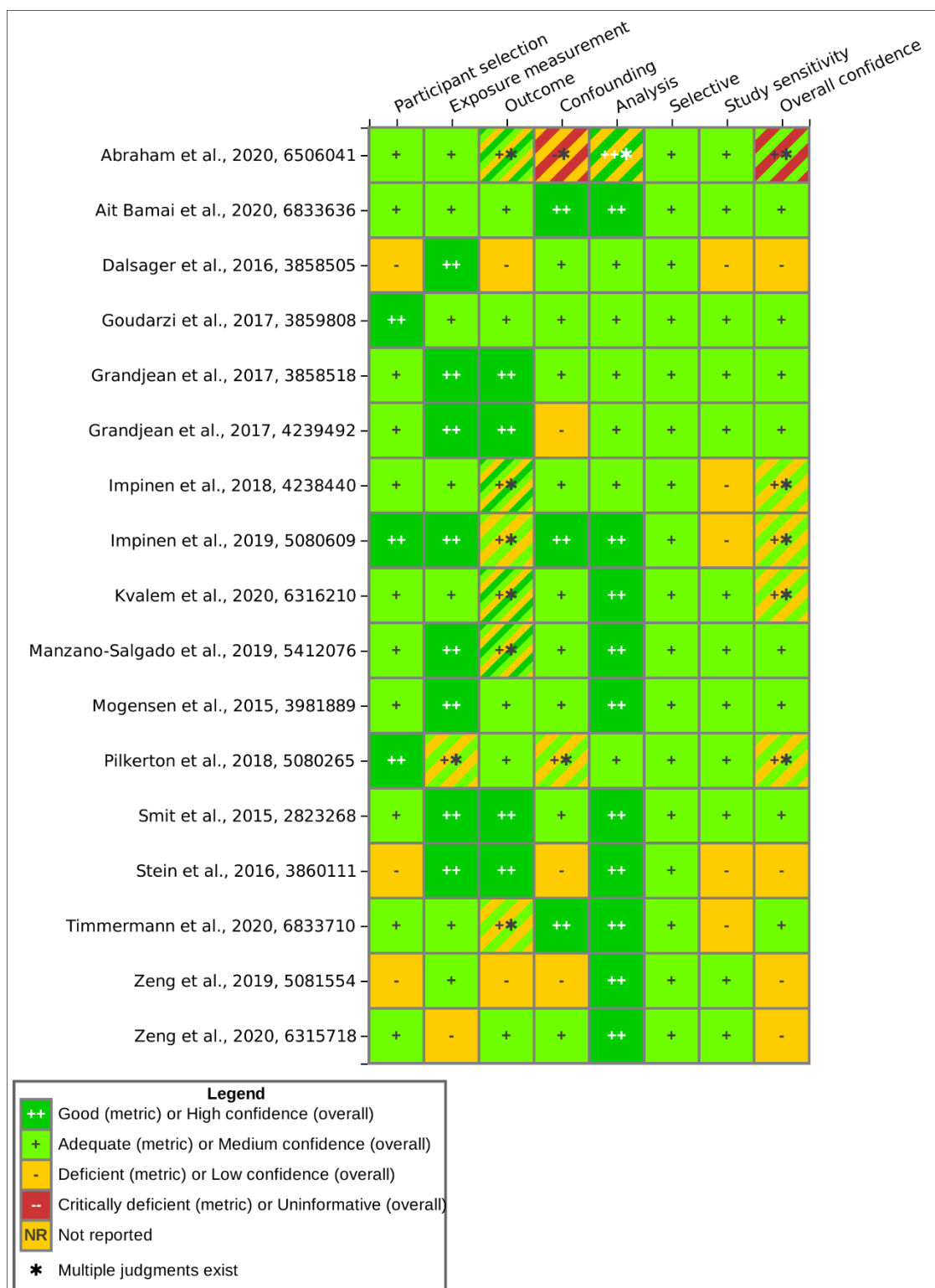


Figure 62. Summary of Study Evaluation for Epidemiology Studies of PFOS and Immunosuppression Effects

Interactive figure and additional study details available on [HAWC](#).

Seven studies studied the relationship between antibody response to vaccination and PFOS exposure. Five of these studies investigated antibody response to vaccination in children {Timmermann, 2020, 6833710; Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}, one study investigated adult flu vaccine response {Stein, 2016, 3860111}, and one study measured rubella antibodies in both adolescents (aged 12 and older) and adults {Pilkerton, 2018, 5080265}. In addition, one study {Zeng, 2019, 5081554} measured natural antibody exposure to hand, foot, and mouth disease (HFMD), and one study {Zeng, 2020, 6315718} measured hepatitis b antibodies in adults. Overall, five studies were *medium* confidence {Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Timmermann, 2020, 6833710; Mogensen, 2015, 3981889} and two were *low* confidence {Stein, 2016, 3860111; Zeng, 2019, 5081554}.

Of the studies that measured antibody response to vaccination in children, four studies were cohorts {Timmermann, 2020, 6833710; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}, and one was cross-sectional {Abraham, 2020, 6506041}. The Faroe Islands cohort participants had multiple exposure measurements, with a maternal sample taken at pregnancy or two weeks after the expected term date, and in samples from child serum at 18 months, 5 years, and 7 years old, depending on the length of follow-up {Grandjean, 2012, 1248827; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}. Mogensen (2015, 3981889) provided additional analyses to Grandjean (2012, 1248827) using serum PFOS concentrations at 7 years of age. Grandjean et al. (2017, 4239492) combined data with the cohort reported in Grandjean et al. (2012, 1248827) and included some comparisons to findings Grandjean et al. (2012, 1248827) and Grandjean et al. (2017, 3858518). Timmermann et al. (2020, 6833710) measured maternal exposure 4–7 months after birth, and Abraham et al. (2020, 6506041) measured child exposure to PFOS at study inclusion (when children were one year old).

Four studies measured antibody response to tetanus vaccination {Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}; four studies measured antibody response to diphtheria vaccination {Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}; one study measured antibody response to measles antibodies {Timmermann, 2020, 6833710}, and one study to *Haemophilus influenzae* type b (Hib) antibodies {Abraham, 2020, 6506041}.

The results for this set of studies are shown in Table C-7. The three studies that examined tetanus and diphtheria antibody levels were generally consistent in observed associations. The Faroe Islands studies {Grandjean, 2012, 1248827; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492} observed associations between elevated levels of PFOS and decreased adjusted levels against tetanus and diphtheria in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. These studies measured exposure levels in maternal blood during late pregnancy and at later time periods from children at age 5, 7, and 13 years. Timmermann et al. (2020, 6833710) also observed inverse associations between elevated levels of PFOS and decreased adjusted antibody levels against measles (statistically significant only in group with fewer measles vaccinations). In contrast, Abraham et al. (2020, 6506041) did not observe associations between adjusted tetanus, Hib, and diphtheria antibody levels and PFOS concentrations. Abraham et al. (2020, 6506041) utilized a single measurement of child blood at inclusion (at age one).

Of the two studies that measured vaccine response in adults or adolescents, one was a cohort {Stein, 2016, 3860111}, and one was a cross-sectional analysis {Pilkerton, 2018, 5080265}. Both studies measured PFOS concentrations from participants at study inclusion and were *low* confidence in adults. Stein et al. (2016, 3860111) utilized a convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding. The adult population in Pilkerton et al. (2018, 5080265) suffered from potential exposure misclassification due to concurrent exposure and outcome measurements. Pilkerton et al. (2018, 5080265) was rated as *medium* confidence for adolescence antibody response to vaccinations. Neither study reported associations with immunosuppression.

It is plausible that the observed associations with PFOS exposure could be explained by confounding across the PFAS. Exposure levels to PFOS were higher than PFOA (PFOS 17 ng/mL, PFOA 4 ng/mL), and there was a moderately high correlation between PFOS and PFOA, PFHxS, and PFNA (0.50, 0.57, 0.48, respectively). The authors assessed the possibility of confounding in a follow-up paper {Budtz-Jorgensen, 2018, 5083631} where estimates were adjusted for PFOA and there was no notable attenuation of the observed effects. The other available studies did not perform multipollutant modeling, so it is difficult to determine the potential for highly correlated PFAS to confound the effect estimates. Overall, although it is not possible to rule out confounding across PFAS, the available evidence suggests that it is unlikely to completely explain the observed effects.

In addition to these studies of antibody response to vaccination, there are two studies that examined antibody response to HFMD {Zeng, 2019, 5081554} and hepatitis B infection {Zeng, 2020, 6315718}. This birth cohort in China {Zeng, 2019, 5081554} measured antibody levels in infants at birth and age 3 months, which represent passive immunity from maternal antibodies. This study was rated *low* confidence because the clinical significance of the outcome is difficult to interpret in infants and there are concerns for confounding by timing of HFMD infection as well as other limitations. Statistically significant increased odds of HFMD antibody concentration below clinically protected levels per doubling of PFOS were observed. This is coherent with the vaccine antibody results, but there is uncertainty due to study deficiencies. Zeng et al. (2020, 6315718) observed negative associations between serum n-PFOS concentration and hepatitis B surface antibody; however, there are study limitations due to concurrent measurement of exposure and outcome and potential for reverse causality.

Overall, eight studies measured associations between PFOS exposure and infectious diseases (or disease symptoms) in children with follow-ups between one and 16 years. Infectious diseases measured included: common cold, lower respiratory tract infections, respiratory syncytial virus (RSV), otitis media, pneumonia, chickenpox, varicella, bronchitis, bronchiolitis, ear infections, gastric flu, urinary tract infections, and streptococcus. Of the studies measuring associations between infectious disease and PFOS exposure, six were cohorts {Ait Bamai, 2020, 6833636; Dalsager, 2016, 3858505; Kvaalem, 2020, 6316210; Manzano-Salgado, 2019, 5412076; Goudarzi, 2017, 3859808; Impinen, 2019, 5080609}, one was a case control study nested in a cohort (Impinen et al., 2018, 4238440), and one was a cross-sectional study {Abraham, 2020, 6506041}. Five studies measured PFOS concentrations from mothers during pregnancy {Ait Bamai, 2020, 6833636; Dalsager, 2016, 3858505; Manzano-Salgado, 2019, 5412076; Goudarzi, 2017, 3859808; Impinen, 2019, 5080609}. Impinen et al. (2018, 4238440) measured PFOS concentrations from cord blood at delivery. Two studies measured PFOS concentrations in

children's serum at age one year {Abraham, 2020, 6506041} and at age 10 years {Kvalem, 2020, 6316210}.

Most of the studies measured infectious disease incidences as parental self-report, which may have led to outcome misclassification {Kvalem, 2020, 6316210; Abraham, 2020, 6506041; Impinen, 2018, 4238440; Impinen, 2019, 5080609}. Three studies measured infections as the doctor-diagnosed incidence of disease over a particular period {Goudarzi, 2017, 3859808; Manzano-Salgado, 2019, 5412076; Ait Bamai, 2020, 6833636}. Overall, four studies were *medium* confidence {Abraham, 2020, 6506041; Ait Bamai, 2020, 6833636; Goudarzi, 2017, 3859808; Manzano-Salgado, 2019, 5412076} and four were *low* confidence {Dalsager, 2016, 3858505; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Kvalem, 2020, 6316210}.

Increased incidence of some infectious diseases in relation to PFOS exposure was observed, although results were not consistent across studies. Results from infectious disease literature can be seen in Table C-8. One *medium* confidence study {Goudarzi, 2017, 3859808} reported higher odds of total infectious diseases. Two studies, one *medium* and one *low* confidence, observed associations between elevated PFOS concentration and increased risk of developing pneumonia in 0 to 3-year-old children {Impinen, 2019, 5080609} and 7-year-old children {Ait Bamai, 2020, 6833636}, with statistical significance in Impinen et al. (2019, 5080609); however, one other *medium* confidence study observed a null association {Abraham, 2020, 6506041}. For lower respiratory infections, one *low* confidence study {Kvalem, 2020, 6316210} reported a positive association ($p < 0.05$), but two studies (one *medium* and one *low* confidence) reported no association {Manzano-Salgado, 2019, 5412076; Impinen, 2019, 5080609}. There were also non statistically significant positive associations seen for PFOS in relation to chickenpox {Ait Bamai, 2020, 6833636} and cough {Dalsager, 2016, 3858505}, but statistically significant inverse associations were observed for RSV {Ait Bamai, 2020, 6833636}, common cold, ear infection, and urinary tract infection {Impinen, 2018, 4238440}.

3.3.4.1.2 Immune Hypersensitivity

Another major category of immune response is the evaluation of sensitization-related or allergic responses resulting from exaggerated immune reactions (e.g., allergies or allergic asthma) to foreign agents {IPCS, 2012, 1249755}. A chemical may be either a direct sensitizer (i.e., promote a specific immunoglobulin E (IgE)-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. For example, chemical exposure could promote a physiological response resulting in a propensity for sensitization to other allergens (pet fur, dust, pollen etc.). Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. Although these responses are mediated by circulating factors such as T-cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of health effects such as allergies or asthma and skin prick tests.

In the 2016 Health Assessment for PFOS, one of two studies reported higher odds of asthma with higher PFOS exposure in children. For this updated review, associations between PFOS

exposure and immune hypersensitivity, specifically asthma, allergy, and eczema were examined in 21 studies. Results of the risk of bias to studies measuring immunosuppression can be seen in Figure 63.

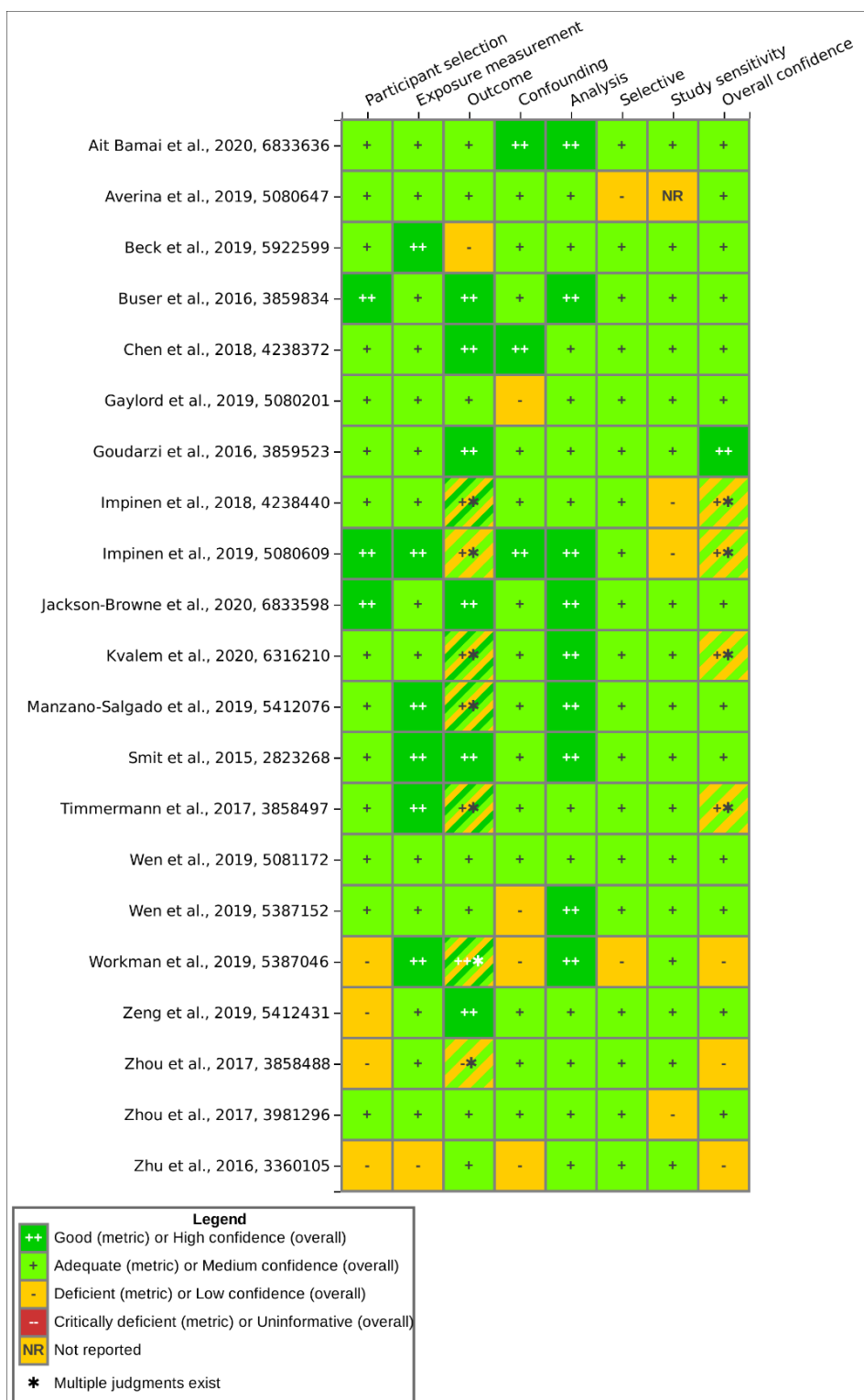


Figure 63. Summary of Study Evaluation for Epidemiology Studies of PFOS and Immune

Hypersensitivity Effects

Interactive figure and additional study details available on [HAWC](#).

Thirteen studies (fifteen publications) examined asthma (or asthma symptoms) and PFOS exposure. Nine of these studies were cohorts {Averina, 2019, 5080647; Beck, 2019, 5922599; Kvalheim, 2020, 6316210; Manzano-Salgado, 2019, 5412076; Zeng, 2019, 5412431; Impinen, 2019, 5080609; Smit, 2015, 2823268; Timmermann, 2017, 3858497; Workman, 2019, 5387046}; three studies (five publications) were case-control investigations {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105}, including one nested case-control, {Gaylord, 2019, 5080201; Impinen, 2018, 4238440}; and one was a cross-sectional analysis {Jackson-Browne, 2020, 6833598}. Seven studies measured the prevalence of “current” asthma for at least one time point {Averina, 2019, 5080647; Beck, 2019, 5922599; Manzano-Salgado, 2019, 5412076; Kvalheim, 2020, 6316210; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Zeng, 2019, 5412431}. Eight studies measured “ever” asthma for at least one time point {Averina, 2019, 5080647; Manzano-Salgado, 2019, 5412076; Jackson-Browne, 2020, 6833598; Gaylord, 2019, 5080201; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Smit, 2015, 2823268; Timmermann, 2017, 3858497}. Incident or recurrent wheeze was examined in one study {Workman, 2019, 5387046}. Overall, nine studies were rated *medium* confidence, and six studies were *low* confidence for asthma (Figure 63). Timmermann et al. (2017, 3858497) was *low* confidence for asthma because the questionnaire used to ascertain status was not validated. Averina et al. (2019, 5080647) was considered *low* confidence because results were not provided quantitatively. Studies from the GBCA {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105} were considered *low* confidence based on participant selection. Cases and controls were recruited from different catchment areas, and the resulting differences between cases and controls indicated potential for residual confounding by age. Additionally, the timing of exposure assessment in relation to outcome assessment was unclear, and it was not reported whether outcome status was confirmed in controls.

Results across these studies were inconsistent (Table C-9). Several studies observed positive associations with ORs greater than 1.2 between PFOS concentration levels and increased “current” or “ever” asthma {Beck, 2019, 5922599; Timmermann, 2017, 3858497; Jackson-Browne, 2020, 6833598; Zeng, 2019, 5412431; Impinen, 2018, 4238440; Averina, 2019, 5080647}, but often only within population subgroups. Averina et al. (2019, 5080647) observed statistically significant increased odds of self-reported doctor diagnosed asthma among adolescents in their first year of high school. Jackson-Browne et al. (2020, 6833598) reported statistically significant increased odds of “ever” asthma from increased PFOS concentrations in children aged 3 to 5 years. No association was observed at ages 6–11 years, and the overall association was small (OR: 1.1). Beck et al. (2019, 5922599) observed increased odds of self-reported asthma per PFOS increase in boys ($p > 0.05$), but this was not observed in girls. For doctor diagnosed asthma in the same study, an inverse association ($p > 0.05$) was observed in boys and a positive association ($p > 0.05$) was observed in girls. Zeng et al. (2019, 5412431) observed a positive association in boys and an inverse association in girls (both $p > 0.05$). Impinen et al. (2018, 4238440) reported higher odds of ever asthma. The *low* confidence study, Timmermann et al. (2017, 3858497), observed positive associations ($p > 0.05$) between increased asthma odds and elevated PFOS concentrations in small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella (MMR) vaccination before age 5. However, in

children of the same ages who had received their MMR vaccination before age 5, no association was observed. *Low* confidence studies from the GBCA study {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105} observed elevated PFOS levels ($p = 0.002$) in children with asthma compared to those without {Zhou, 2016, 3981296}, and the odds of current asthma was also found to be elevated among boys and girls with increasing PFOS exposure {Zhu, 2016, 3360105}. One other study {Impinen, 2019, 5080609} observed a small positive association (OR: 1.1) with current asthma in boys only. Two studies reported non-significant inverse associations with asthma {Manzano-Salgado, 2019, 5412076; Smit, 2015, 2823268}, and in one study, all results were non-significant {Gaylord, 2019, 5080201}. One *low* confidence study did not observe a significant effect for recurrent wheeze {Workman, 2019, 5387046}.

Seven studies observed associations between PFOS exposure and allergies, specifically allergic rhinitis or rhinoconjunctivitis, skin prick test, and food or inhaled allergies. Five of these studies were cohorts {Goudarzi, 2016, 3859523; Ait Bamai, 2020, 6833636; Kvalheim, 2020, 6316210; Impinen, 2019, 5080609; Timmermann, 2017, 3858497}, one study was a case-control analysis {Impinen, 2018, 4238440}, and one study was a cross-sectional study using data from NHANES 2005–2006 and 2007–2010 {Buser, 2016, 3859834}. All studies were considered *medium* confidence for allergy outcomes. Results for these outcomes are presented in Table C-10.

Three studies conducted skin prick tests on participants to determine allergy sensitization at age 10 years {Kvalheim, 2020, 6316210; Impinen, 2018, 4238440}, at age 13 years {Timmermann, 2017, 3858497}, and at age 16 years {Kvalheim, 2020, 6316210}. Skin prick tests were conducted to test sensitization to dust mites, pets, grass, trees and mugwort pollens and molds, cow's milk, wheat, peanuts, and cod. Results were inconsistent across studies. Kvalheim et al. (2020, 6316210) reported a statistically significant but small association (OR: 1.09) with a positive skin prick test at age 16 years (results were similar at age 10 years but $p > 0.05$). Timmermann et al. (2017, 3858497) also reported a positive association ($p > 0.05$) in children who had received an MMR before age 5 years, but an inverse association in those who had not received an MMR, and Impinen et al. (2018, 4238440) reported an inverse association ($p > 0.05$). Five studies measured symptoms of “current” or “ever” allergic rhinitis or rhinoconjunctivitis {Goudarzi, 2016, 3859523; Ait Bamai, 2020, 6833636; Impinen, 2018, 4238440; Kvalheim, 2020, 6316210; Timmermann, 2017, 3858497}, and 16 years old {Kvalheim, 2020, 6316210}. Rhinitis was defined as at least one symptom of runny or blocked nose or sneezing. Rhinoconjunctivitis was defined as having symptoms of rhinitis, in addition to itchy and watery eyes. Results were null for these outcomes in all five studies. Impinen et al. (2019, 5080609) measured parent-reported, doctor-diagnosed “current” or “ever” allergy symptoms at 7 years old, in addition to known food and inhaled allergies and reported higher odds of “ever” inhaled allergies ($p > 0.05$) but no associations with food allergies or “current” inhaled allergies. Buser et al. (2016, 3859834) measured food sensitization (defined as having at least 1 food-specific serum IgE ≥ 0.35 kU/L) and self-reported food allergies and reported statistically significant positive associations with self-reported food allergies in NHANES 2007–2010 but not in NHANES 2005–2006.

Seven studies measured the association between PFOS concentration and eczema (described by some authors as atopic dermatitis). Six of these studies were cohorts {Goudarzi, 2016, 3859523; Wen, 2019, 5387152; Wen, 2019, 5081172; Manzano-Salgado, 2019, 5412076; Chen, 2018, 4238372; Timmermann, 2017, 3858497}, and one was a case-control analysis {Impinen, 2018, 4238440}. Four studies measured PFOS concentrations in cord blood at delivery {Wen, 2019,

5387152; Wen, 2019, 5081172; Chen, 2018, 4238372; Impinen, 2018, 4238440 }, three studies measured PFOS concentrations in pregnancy {Goudarzi, 2016, 3859523; Manzano-Salgado, 2019, 5412076; Timmermann, 2017, 3858497 }, and one study measured child blood at age 5 and 13 years {Timmermann, 2017, 3858497 }. All the studies were considered *medium* confidence for eczema. Results are presented in Table C-11.

Positive associations ($p > 0.05$) with eczema were observed in two studies {Wen, 2019, 5387152; Wen, 2019, 5081172; Chen, 2018, 4238372 }, as well as a small positive association at age 0–2 years in Impinen et al. (2018, 4238440). However, inverse associations ($p > 0.05$) were reported in Manzano-Salgado et al. (2019, 5412076), Timmermann et al. (2017, 3858497), Goudarzi et al. (2016, 3859523), and at age 10 years in Impinen et al. (2018, 4238440).

3.3.4.1.3 Autoimmune Disease

Autoimmunity and autoimmune disease arise from immune responses against endogenously produced molecules. The mechanisms of autoimmune response rely on the same innate and adaptive immune functions responding to foreign antigens: inflammatory mediators, activation of T lymphocytes, or the production of antibodies for self-antigens (IPCS, 2012, 1249755). Chemical exposures that induce immune response or immunosuppression may initiate or exacerbate autoimmune conditions through the same functions. Autoimmune conditions can affect specific systems in the body, such as the nervous system (e.g., multiple sclerosis (MS)), or the effects can be diffuse, resulting in inflammatory responses throughout the body (e.g., lupus).

The 2016 Health Assessment for PFOS did not identify epidemiological evidence examining the association between PFOS exposure and autoimmune conditions.

Three case-control studies examined PFOS exposure and autoimmune diseases (Figure 64). Two studies examined MS {Ammitzbøll, 2019, 5080379} and ulcerative colitis {Steenland, 2018, 5079806} in adults, and one study examined celiac disease in children and young adults {Gaylord, 2020, 6833754}. PFOS was measured in blood components (i.e., blood, plasma, or serum) for all studies (Table C-12). One study was *medium* confidence {Gaylord, 2020, 6833754} with minimal deficiencies, and two studies were considered *low* confidence {Ammitzbøll, 2019, 5080379; Steenland, 2018, 5079806}. Information on participant selection, particularly control selection, was not reported in Ammitzbøll (2019, 5080379). Additionally, PFOS was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOS exposure and risk of MS, and contributed to a *low* confidence rating. Steenland (2018, 5079806) examined exposure concentrations one to two years after diagnosis of celiac disease, resulting in some concern for reverse causation. Additionally, there was potential for residual confounding by SES which was not considered in the analysis. These factors together contributed to a *low* confidence rating (Figure 64).

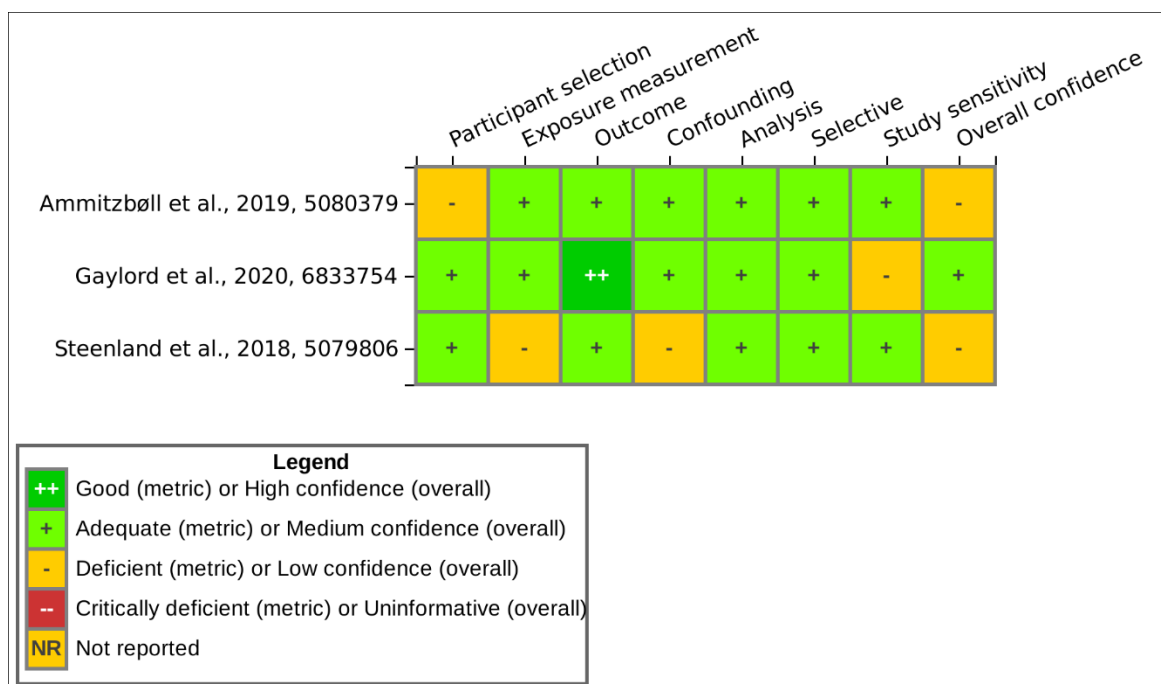


Figure 64. Summary of Study Evaluation for Epidemiology Studies of PFOS and Autoimmune Effects

Interactive figure and additional study details available on [HAWC](#).

Ammitzbøll, 2019, 5080379 observed lower PFOS concentrations among healthy controls compared to those with MS. Serum PFOS concentrations were 17% lower (95% CI: -27%, -6%; $p = 0.004$) in healthy controls compared to cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOS levels were 28% lower (95% CI: -32%, -3%; $p = 0.023$) in healthy controls compared to cases. The result was similar among women but did not reach significance ($p = 0.093$). In children and young adults, the odds of celiac disease were elevated but not significantly {Gaylord, 2020, 6833754}. However, the effect was much stronger in females only (OR: 12.8; 95% CI: 1.17, 141; $p < 0.05$). A marginally significant ($p = 0.06$) decrease in serum PFOS was observed among adult cases of ulcerative colitis compared to healthy controls {Steenland, 2018, 5079806}.

Overall, the associations between PFOS exposure and autoimmune disease were very limited and mostly null, with one study with evidence of elevated odds of celiac disease. Two studies observed that PFOS levels in healthy controls were either higher than UC cases {Steenland, 2018, 5079806} or lower than in MS cases {Ammitzbøll, 2019, 5080379}.

3.3.4.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 3 key studies from the 2016 PFOS HESD {EPA, 2016, 3603365} that investigated the association between PFOS and immune effects. Study quality evaluations for these 7 studies are shown in Figure 65.

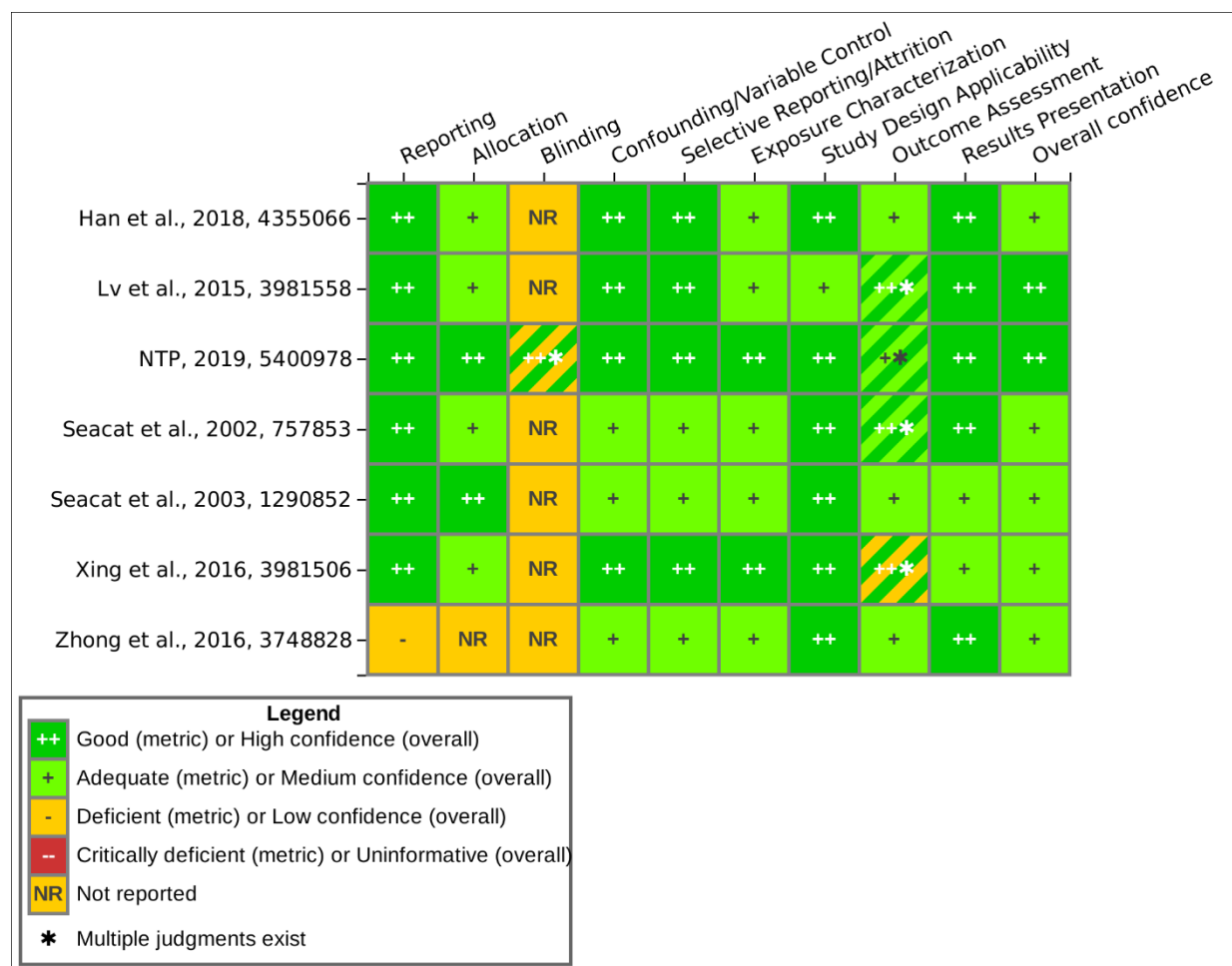


Figure 65. Summary of Study Evaluation for Toxicology Studies of PFOS and Immune Effects

Interactive figure and additional study details available on [HAWC](#).

The immune system could be a target of PFOS toxicity as effects have been observed across animal studies of varying durations of oral exposure to PFOS. Effects include changes in spleen and/or thymus weights, extramedullary hematopoiesis, perturbations in activity level or composition of various immune cell populations, and diminished ability to generate an immune response. Studies indicate that PFOS exposure may result in dose- and sex-specific immunomodulatory effects.

3.3.4.2.1 Organ Weight

Several rodent studies have reported changes in thymus and/or spleen weights following oral exposure to PFOS.

3.3.4.2.1.1 Spleen

Absolute and relative spleen weights were reported in a study of male and female Sprague-Dawley rats administered oral PFOS (0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day) for 28 days {NTP, 2019, 5400978}. Dose dependent reductions in absolute spleen weights were observed at 1.25

mg/kg/day and higher in males only; no effects were observed in females. Spleen weights relative to body weight were not significantly reduced in either sex. While body weights were not significantly different throughout treatment, the high-dose group tended to have lower body weight with a significant, but < 10%, difference from the control. Therefore, differences in body weight cannot explain the decreased absolute weight.

In three separate studies, male C57BL/6 mice were administered 0, 5, 20, or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, fed chow with 0.001 or 0.02% PFOS (equivalent to ~40 mg/kg/day) for 10 days {Qazi, 2009, 1937260}, or 0.008–2.083 mg/kg/day PFOS for 60 days {Dong, 2009 1424951}. Decreased absolute and relative splenic weights tended to be observed only at the highest doses for each study. Female mice were not assessed. These findings are complimented by Xing et al. (2016, 3981506), where reductions in spleen weight were observed in male C57BL/6J mice following exposure to 300 mg/kg/day PFOS for 30 days via gavage. No effects were observed at other doses (75 and 150 mg/kg/day) {Xing, 2016, 3981506}.

In a developmental study, spleens were weighed in four and eight week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD1–17 via gavage. Relative spleen weights were reduced in male pups from the 5 mg/kg/day exposure group at four-weeks. No effects were observed in lower dose groups or in females {Zhong, 2016, 3748828}.

Not all studies observed effects on spleen weight. Spleen weights were not altered in male and female B6C3F1 mice administered 0.00017–0.166 mg/kg/day PFOS for 28 days {Peden-Adams, 2008, 1424797}, nor in male C57BL/6 (H-2^b) mice administered 0.005% PFOS in the diet for 10 days {Qazi, 2010, 1276154}. Similarly, relative spleen weight in male BALB/c mice was not affected at the end of a three-week exposure to 2.5–5 mg/kg/day PFOS (Lv et al., 2015, 3981558). Although Qazi et al.(2009, 1276154), observed that relative spleen weight was slightly reduced in C57BL/6 mice following 10-day exposure to 0.02 or 0.05% PFOS, the effects did not reach significance.

3.3.4.2.1.2 Thymus

Reductions in thymus weight have been reported across studies of varying durations (7–60 days) and species (mice or rats). It is unclear whether sex has an influence on toxicity, as a number of studies did not include females in their investigations.

The aforementioned 28-day study by NTP (2019, 5400978) also reported absolute and relative thymus weights in male and female Sprague-Dawley rats administered oral PFOS (0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day). In males, reductions in thymus weight (absolute and relative) were observed only at the highest dose (Figure 66). In contrast, females exhibited reduced thymus weights at doses as low as 1.25 mg/kg/day, suggesting a higher sensitivity in females {NTP, 2019, 5400978} (Figure 66). Similarly, reduced thymic weights were observed in male C57BL/6 mice administered 0, 5, 20, or 40 mg/kg/day PFOS for 7 days (Zheng et al., 1429960 1429960), 0.001 or 0.02% PFOS for 10 days {Qazi, 2009, 1937260}, or 0.008–2.083 mg/kg/day PFOS for 60 days {Dong, 2009, 1424951}. Female mice were not assessed in that study.

In a developmental exposure study, the thymus was weighed in 4 and 8 week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD1–GD17 via gavage. In male pups from the 5 mg/kg/day exposure group, relative thymus weights were reduced at 4 and

8 weeks of age. However, no effects were observed in lower dose groups or in females {Zhong, 2016, 3748828} (Figure 66).

In contrast to the several studies that reported reductions in thymus weight, Qazi et al. (2010, 1276154) and Peden-Adams et al. (2008, 1424797) did not observe any changes in thymus weight. Qazi et al. (2010, 1276154) exposed male C57BL/6 (H-2^b) mice administered 0.005% PFOS in the diet for 10 days, while Peden-Adams et al. (2008, 1424797) exposed male and female B6C3F1 mice to 0.00017–0.166 mg/kg/day PFOS for 28 days. The contrasting results of the 28-day study by Peden-Adams et al. and NTP (2019, 5400978), may underscore species differences, however the dose levels in these studies were not comparable so it is not possible to make a direct comparison of the endpoints.

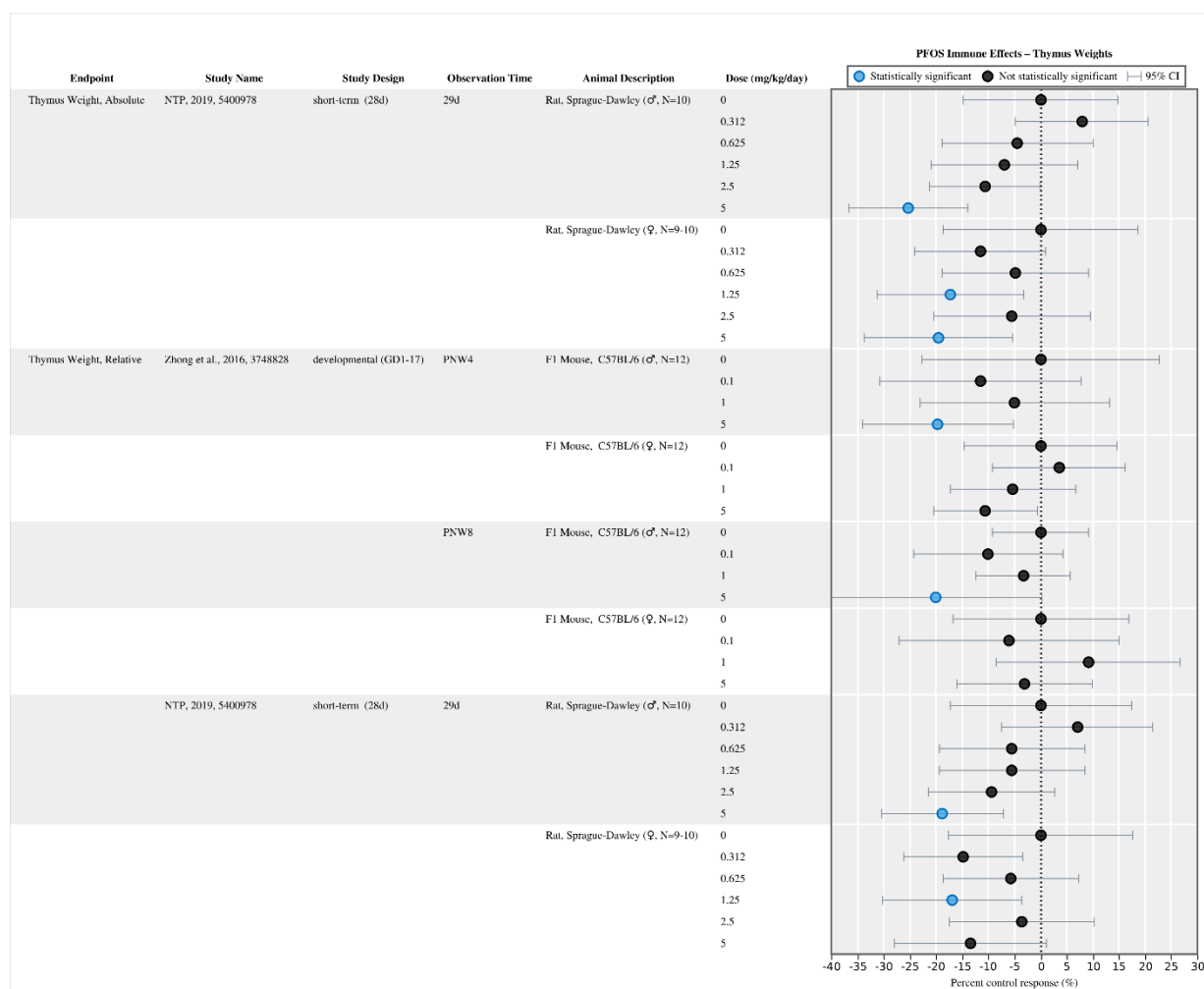


Figure 66. Percent Change in Thymus Weights Relative to Controls in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).
GD = gestation day; PNW = postnatal week; F1 = first generation

3.3.4.2.2 Histopathology

Histopathology of the spleen, thymus, and/or lymph nodes has been evaluated following oral exposure to PFOS across studies of varying durations in rodents (Figure 67). In general, short-term and subchronic studies have observed histopathology such as extramedullary hematopoiesis {NTP 2019, 5400978}, bone marrow hypocellularity {NTP, 2019, 5400978}, and other aberrations in the immune organs {Qazi, 2009, 1937260; Lv, 2015, 3981558}.

One study included in the 2016 HESD {U.S. EPA, 2016, 3603365} by Qazi et al. (2009, 1937260) described perturbations in the thymus of male C57BL/6 (H-2^b) mice exposed to 0.02% (equivalent to ~40 mg/kg/day) PFOS in feed for 10 days; the thymic cortex was smaller and devoid of cells and the cortical/medullary junction was indistinguishable. These observations may coincide with the reduction in thymus weight described above {Qazi, 2009, 1937260; NTP, 2019, 5400978}.

No significant increases in non-neoplastic lesions were observed in spleens of BALB/c mice given 2.5, 5, or 10 mg/kg/day PFOS for three weeks. However, the authors {Lv, 2015, 3981558} state that alterations in spleen architecture were observed at the end of the exposure in the 5 and 10 mg/kg/day groups. Moreover, splenic sinusoids, which drain into pulp veins, were dilated and hyperemic. Peripheral splenic pulp structure and splenic cords (also known as red pulp cords or cords of Billroth) were destroyed, the marginal zone disappeared, and megakaryocytes (myeloid cell precursors) were abundant.

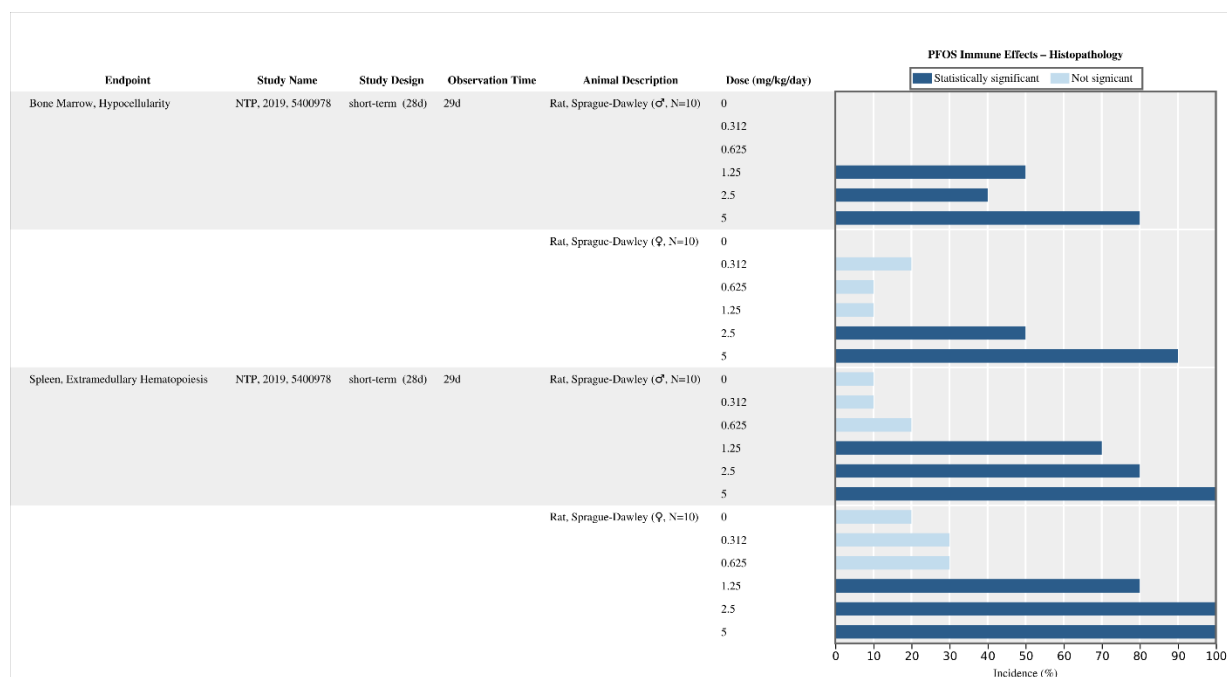


Figure 67. Incidences of Immune Cell Histopathology in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

Xing et al. (2016, 3981506) examined spleens of male C57BL/6J mice for histopathology; no distinguishable morphological differences were observed between any exposure group (2.5, 5, or 10 mg/kg/day for 30 days) and control.

One study reported histology for the lymphatic system, but no histopathology was observed in the lymph nodes (mandibular and mesenteric) following PFOS exposure {NTP, 2019, 5400978}.

3.3.4.2.3 Circulating Immune Cells

Effects of PFOS exposure on circulating immune cells have been reported in rodents and non-human primates.

Qazi et al. (2009, 1937259) performed a study to see if exposure to PFOS influenced circulating immune cells. Male C57BL/6 mice were fed chow laden with 0.02% PFOS for 10 consecutive days, after which levels of white blood cells (WBCs) were evaluated in blood collected from retroorbital puncture. The absolute WBC count was significantly reduced and was mainly a reflection of decreased lymphocytes, as no change in neutrophils was seen. A significant reduction of the relative proportion and absolute number of macrophages in the bone marrow was also reported {Qazi, 2009, 1937259}. In a study by Seacat et al. (2003, 1290852), male and female Sprague-Dawley rats were exposed to 0, 0.5, 2, 5, or 20 parts per million (ppm) PFOS for 14 weeks and WBC counts were determined. The only statistically significant change was an increase in neutrophils in the 20 ppm exposure group (130 mg/kg cumulative dose equivalent) in the males only. No effects were observed at lower exposure groups (0.5, 2.0, 5.0 ppm) nor in females {Seacat, 2003, 1290852}.

Evidence from one paper {Seacat, 2002, 757853} suggests that the effects of PFOS on WBCs in rodent studies do not extend to non-human primates. Male and female cynomolgus monkeys, orally administered 0.3–0.75 mg/kg/day PFOS for 26 weeks, exhibited no change in WBC counts, including neutrophils and total lymphocytes {Seacat, 2003, 757853}. However, reduced numbers of neutrophils were observed in male rats, but not females, in an NTP (2019, 5400978) study. In that report, NTP also reported that male rats, and not females, exhibited significantly reduced WBC counts {NTP, 2019, 5400978} (Figure 68).

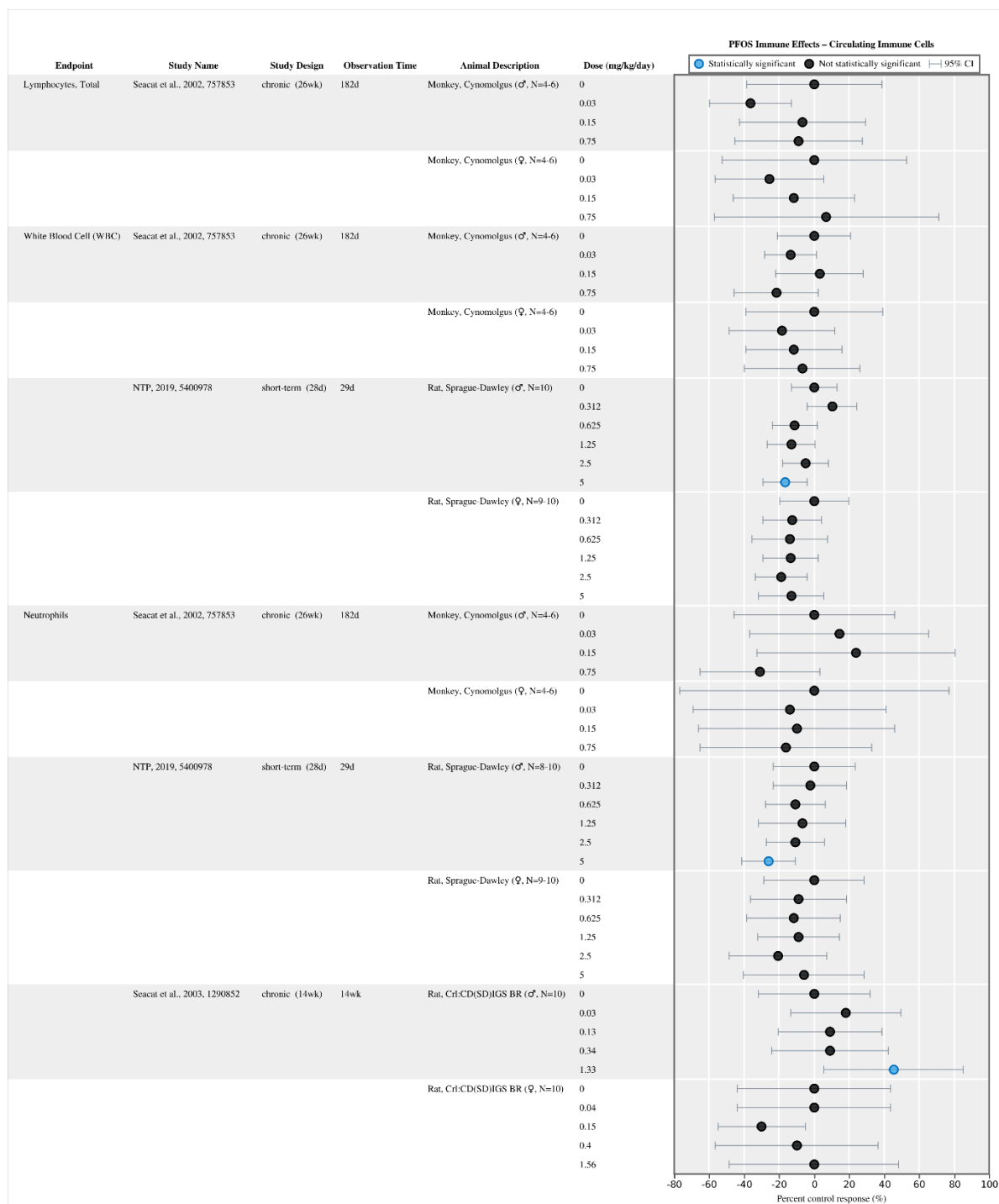


Figure 68. Percent Change in Circulating Immune Cells Relative to Controls in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on [HAWC](#).

3.3.4.2.4 Natural Killer Cell Activity

The available data on the effect of PFOS exposure on Natural Killer (NK) cell activity indicate that there may be different effects in NK cell activity based on dose, but there are too few studies

to make any determination and no single study assesses the continuum of doses to see if there is an opposing effect at different areas of the dose response curve. Oral administration of 0.00017–0.166 mg/kg/day PFOS to male and female B6C3F1 mice for 28 days resulted in increased NK cell activity in males only exposed to 0.017, 0.033, and 0.166 mg/kg/day {Peden-Adams, 2008, 1424797}. Male C57BL/6 mice exposed to 0.083 mg/kg/day PFOS daily for 60 days displayed significantly increased NK cell activity by 38%, but treatment with 0.833 and 2.083 mg/kg/day resulted in decreased NK cell activity {Dong, 2009, 1424951}. Female mice were not assessed in this study. In another assessment of male C57BL/6 mice administered 0–40 mg/kg/day for 7 days, natural killer (NK) cell activity was reduced following exposure to 20 and 40 mg/kg/day {Zheng, 2009, 1429960}. Similarly, Zhong et al. (2016, 3748828) reported that NK cell activity was decreased in 4 week-old male offspring from the 5 mg/kg/day group and also reduced in 8 week old offspring from the 1 or 5 mg/kg/day group. The latter result was recapitulated in the study by Keil et al. (2008, 1332422) where the female C57BL/6 mice were mated with C3H to derive B6C3F1 offspring. Female offspring from both studies were less sensitive to the PFOS-induced reduction in NK cell activity (Keil, 2008, 1332422; Zhong, 2016, 3748828) as indicated by statistical significance of each sex in each study. Moreover, at 8 weeks, NK cell activity was suppressed by 42.5% and 32.1% in males at the 1 and 5 mg/kg/day treatments, respectively, and was suppressed by 35.1% in females at the 5 mg/kg/day treatment {Keil, 2008, 1332422}. These studies indicate that male mice may be more susceptible to PFOS-induced altered NK cell activity, and that NK cell activity can be increased or decreased following low or high PFOS exposure, respectively (Table 9).

Table 9. Associations Between PFOS Exposure and Natural Killer Cell Activity in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008, 1424797)	28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, 0.166	M	↓ 0.017–0.166 mg/kg/day
			F	n.s.
Dong et al. (2009, 1424951)	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↑ (0.083 mg/kg/day)
				↓ (0.833–2.083 mg/kg/day)
Zheng et al. (2009, 1429960)	7 days	0, 5, 20, 40	M	↓ (20–40 mg/kg/day)
Zhong et al. (2016, 3748828)	GD1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 5 mg/kg/day
			F	n.s.
	GD1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			F	↓ 5 mg/kg/day
	GD1–17 4-week assessment	0, 0.1, 1, 5	M	n.s.
			F	n.s.
Keil et al. (2008, 1332422)	GD1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			F	↓ 5 mg/kg/day
	GD1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			F	↓ 5 mg/kg/day

M = male; F = female; n.s. = nonsignificant; GD = gestation day.

3.3.4.2.5 Spleen Cellularity

Splenocyte sub-classes were quantified in several rodent studies (Figure 69). Splenic T-cell immunophenotypes were slightly affected in male and female B6C3F1 mice exposed to oral administration of 0.00017–0.166 mg/kg/day PFOS for 28 days {Peden-Adams, 2008, 1424797}. In males, CD4⁺/CD8⁺ and CD4⁺/CD8⁻ cells were increased, whereas numbers of CD4⁺/CD8⁻ and CD4⁺/CD8⁺ cells were decreased beginning at 0.0033 mg/kg/day. In females, splenic CD4⁺/CD8⁺ and CD4⁺/CD8⁻ cells were decreased beginning at 0.0033 mg/kg/day. Significantly decreased splenocyte populations were also observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days {Qazi, 2009, 1937260}, 20 or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, and 0.417–2.083 mg/kg/day for 60 days {Dong, 2009, 1424951}. Female mice were not evaluated in these studies.

Altered splenic cellular composition was observed in a study by Lv et al. (2015, 3981558) where male BALB/c mice were exposed to 0, 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks {Lv, 2015, 3981558}, and spleens harvested for lymphocyte counting and phenotyping. Fluctuations in lymphocyte counts and T-cell proliferation were apparent at the 3-week timepoint. A dose-dependent increase in the abundance of splenic T-cells (CD3⁺) relative to controls was observed at the end of three weeks, reaching significance in the 2.5 and 10 mg/kg/day exposure groups. This coincided with a non-significant increase in T-helper (CD3⁺CD4⁺) and T-cytotoxic (CD3⁺CD8⁺) lymphocytes in the 5 and 10 mg/kg/day groups, all relative to controls. The absolute numbers of (CD3⁺) T cell subpopulations were not altered at any dose. Absolute numbers of T-helper (CD3⁺CD4⁺) and T-cytotoxic (CD3⁺CD8⁺) lymphocytes were increased in the 10 mg/kg/day groups {Lv, 2015, 3981558}.

Further effects of PFOS on immune cell composition in the spleen have also been reported following developmental exposure by Keil et al. and Zhong et al. (2016, 3748828). Zhong et al. exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD1–GD17, and then quantified various immune cell populations in male and female pups. Decreased splenic cell subpopulations (CD4⁺ and CD8⁺ cell counts) were observed in the 4-week old male pups from the 5 mg/kg/day exposure group. At 8-weeks, reductions in CD8⁺ cells in the spleen were observed in the 5 mg/kg/day exposure group {Zhong, 2016, 3748828}.



Figure 69. Splenocyte Cellularity in Rodents 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; F1 = first generation.

3.3.4.2.6 Thymus Cellularity

Thymus cell populations were less sensitive to the effects of PFOS compared to the effects observed in the spleen, as determined by the dose where the change occurred and the number of endpoints that changed following PFOS exposure (Figure 70). Indeed, while all splenic T-cell CD4/CD8 subpopulations were altered in one study of male B6C3F1 mice beginning at 0.1 mg/kg/day exposures, none of the thymic T-cell subpopulations were affected. Furthermore, the effects appeared to also have a female-bias; although thymic CD4⁺/CD8⁺ cells were increased in female B6C3F1 mice exposed to 0.033 or 0.166 mg/kg/day, no effects were observed in males {Peden-Adams, 2008, 1424797}. In contrast, significantly decreased thymocyte populations were observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days {Qazi, 2009, 1937260}, 20 or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, and 0.417–2.083 mg/kg/day for 60 days (Dong, 2009, 1424951). Female mice were not evaluated in these studies.

Effects of PFOS on immune cell composition in the thymus have also been reported following developmental exposure. Pregnant female C57BL/6 mice were dosed with 0.1–5 mg/kg/day PFOS from GD1–GD17, and immune cell populations were quantified in male and female pups at 4 and 8 weeks after birth. Decreased thymic lymphocyte sub-populations (CD4⁺, and CD4⁺/CD8⁺ cell counts) and decreased thymic cellularity were observed in the 4-week-old male pups from the 5 mg/kg/day exposure group, and no effects were observed in females {Zhong, 2016, 3748828}. At 8-weeks, no effects were observed in females and reductions in thymic CD4⁺ cells were observed in males from the 5 mg/kg/day exposure group. These findings were complimented by Keil et al. (2008, 1332422), who observed a reduction in CD3⁺ and CD4⁺ thymocytes in 8-week C57BL/6N male mice following exposure to 0.1–5 mg/kg/day from GD1–GD17 {Keil, 2008, 1332422}.

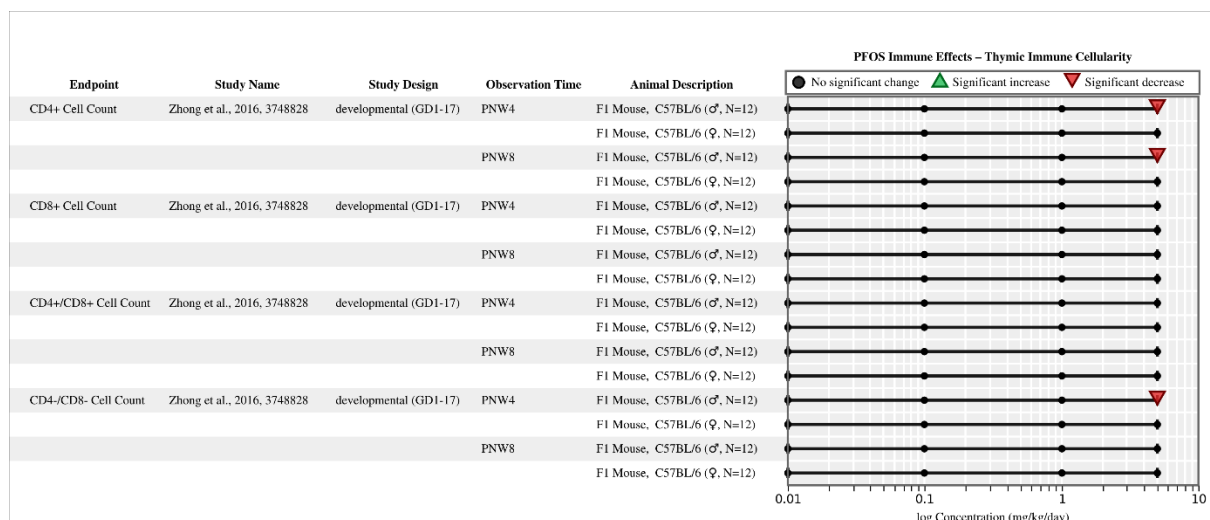


Figure 70. Thymocyte Cellularity in Rodents Following Exposure to PFOS (logarithmic scale)

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; F1 = first generation.

3.3.4.2.7 Ability to Generate an Immune Response

Many studies have investigated the effect of PFOS on the ability of rodents to generate an immune response to various antigens.

Several have found evidence indicative of immunosuppression, including reduced immunoglobulin M (IgM) titers. Peden-Adams et al. (2008, 1424797) found that the sheep red blood cell (SRBC) plaque-forming cell (PFC) response, which measures IgM-producing cells, was reduced in male and female B6C3F1 mice administered 0.0017–0.166 mg/kg/day PFOS daily for 28 days. The response was suppressed at lower PFOS doses in male mice (effect first observed at 0.0017 mg/kg/day) than the female mice (effect first observed at 0.017 mg/kg). Because IgM suppression can result from effects on both T- and B-cells, antibody production was also measured in response to a bacteria-like challenge, trinitrophenyl (TNP)-lipopolysaccharide (LPS), which would induce a T-independent response. Following the TNP-LPS challenge, a decrease in IgM titers was observed in female B6C3F1 mice that had been exposed to 0.334 mg/kg/day PFOS for 21 days. Male animals were not assessed in this study {Peden-Adams, 2008, 1424797}. Similarly, Dong et al. (2009, 1424951) observed a dose-dependent reduction in the SRBC-specific IgM PFC response in male C57BL/6 mice exposed to PFOS daily for 60 days. Female mice were not assessed in this study. A third study also found that the PFC response to a SRBC challenge was suppressed in male C57BL/6 mice given 5, 20, or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}. These rodent studies provide evidence of a PFOS-induced suppression of the immune response to a SRBC challenge that may be more sensitive in male mice (Table 10).

Table 10. Associations Between PFOS Exposure and Plaque Forming Cell Response in Mice^a

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008, 1424797)	28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, 0.166	M	↓ 0.0017–0.166 mg/kg/day
			F	↓ 0.017–0.166 mg/kg/day
Dong et al. (2009, 1424951)	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↓ 0.083–2.083
Zheng et al. (2009, 1429960)	7 days	0, 5, 20, 40	M	↓ 5–40 mg/kg/day
Zhong et al. (2016, 3748828)	GD1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 1–5mg/kg/day
			F	↓ 5 mg/kg/day
	GD1–17 8-week assessment	0, 0.1, 1, 5	M	n.s.
			F	n.s.
Keil et al. (2008, 1332422)	GD1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 5 mg/kg/day
			F	n.s.

M = male; F = female; GD = gestation day; n.s. = nonsignificant.

^aSheep red blood cell-specific IgM production.

Similar observations were reported in two developmental PFOS exposure studies. Keil et al. (2008, 1332422) and Zhong et al. (2016, 3748828), each exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD1–GD17 and then tested the immune responses in offspring at 4 and 8 weeks of age. Four days before sacrifice, mice were injected with SRBC to induce an immune response. In males from the 5 mg/kg/day exposure group, the primary IgM response to SRBC was significantly suppressed by 53% at 8-weeks. In females, the primary IgM response was not altered Keil et al. (2008, 1332422). Similarly, Zhong et al. (2016, 3748828) observed that SRBC-specific IgM production by B-lymphocytes in the spleens of 4-week-old mouse pups exposed to 1 or 5 mg/kg/day PFOS in utero was reduced by 15% or 28%, respectively. In females, the SRBC-specific IgM response was only significantly suppressed in the 5 mg/kg/day group. Cytokine secretion patterns involved in directing the type of immune response (e.g., TH1 or TH2) were also reported in this study. Increased spontaneous production of interleukin 4 (IL-4) and reduced interleukin 2 (IL-2) was observed in splenocytes harvested from 4-week old male pups in the 5 mg/kg/day exposure group. IL-4 was elevated in their female counterparts. At 8 weeks, the SRBC-response had recovered in all treatment groups, and only cells obtained from male pups had increased IL-4 production {Zhong, 2016, 3748828}.

Alterations in the serum levels of globulin can be associated with decreases in antibody production {FDA, 2002, 88170}. In a 28-day study with male and female Sprague-Dawley rats orally administered 0.312–5 mg/kg/day PFOS to, male rats exhibited significantly decreased globulin while female globulin did not significantly differ from control values {NTP, 2019, 5400978}.

One study by Lee et al. (2018, 5085013) found evidence that PFOS exposure can exacerbate an allergic immune response. Lee et al. sensitized male ICR mice with ovalbumin on day 0 and day 7 and exposed them to 50–150 mg/kg/day PFOS on study day 9, 11, and 13. Serum histamine, TNF- α , IgE, and IgG levels were increased following exposure, suggesting that PFOS exacerbates mast cell-mediated allergic inflammation.

3.3.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse immune outcomes is discussed in Sections 3.1.1.6, 3.3.2, 3.3.4, and 3.3.6 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 21 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to immune effects. A summary of these studies is shown in Figure 71. Additional analysis on the mechanistic actions of PFOS on immune health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	4	0	8	11
Cell Signaling Or Signal Transduction	1	0	3	4
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	0	2	2
Hormone Function	1	0	0	1
Inflammation And Immune Response	4	5	11	17
Oxidative Stress	1	0	3	4
Other	1	0	0	1
Grand Total	6	5	13	21

Figure 71. Summary of Mechanistic Studies of PFOS and Immune Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.4.4 Evidence Integration

The findings from human epidemiological studies are generally consistent with an association between PFOS exposure and immunosuppression in children. Changes in antibody levels of 10–20% per doubling of exposure were observed in the Faroe Islands. The variability in the results, including null and positive associations, could be related to differences in sample sizes, individual variation, vaccine type, and differences in timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available data. Overall, the evidence indicates an association between increased serum levels of PFOS and decreased antibody production

following routine vaccinations in children. Multiple *medium* confidence epidemiological studies reporting a positive association for this effect are available. Evidence in adults does not indicate an association with immunosuppression, but high-quality studies are not available.

Results are most consistent for antibody response to vaccination in children, and multiple *medium* confidence studies reporting a positive association for this outcome are available. Grandjean et al. (2012, 1248827), Grandjean et al. (2017, 3858518), and Grandjean et al. (2017, 4239492) were considered for POD derivation. These studies have exposure levels typical in the general population and low risk of bias. Two publications with BMD modeling of these data are available {Budtz-Jorgensen, 2018, 5083631; Grandjean, 2013, 1937222}.

Associations in human epidemiological studies measuring PFOS exposure and hypersensitivity outcomes were mixed. Additionally, there is some evidence from human epidemiological studies of an association between PFOS exposure and asthma, but there is considerable uncertainty due to inconsistency across studies and sub-groups. Sex-specific differences were reported in multiple studies, but there was inconsistency in the direction of association within each sex. There is not an obvious pattern of results by analysis of “ever” vs “current” asthma, and no studies beyond the Dong et al. (2013, 1937230) described in the 2016 Health Assessment examined asthma incidence. For allergy and eczema outcomes, results were inconsistent across studies.

Due to inconsistency across studies and outcomes, there is limited evidence of an association between PFOS exposure and infectious diseases. While one medium confidence study reported higher odds of total infectious diseases, results from studies examining individual diseases including respiratory infections, chickenpox, cough, RSV, common cold, ear infections, and urinary tract infections were inconsistent.

Human epidemiological evidence was limited to three studies reporting on different autoimmune conditions. Similar to the 2016 Health Assessment, there was insufficient information to draw conclusions on the effect of PFOS exposure on autoimmune disease.

Available evidence from rodent studies supports epidemiological evidence that PFOS exposure can have immunosuppressive effects, as reflected in decreased PFC responses and NK cell activities. Additionally, fluctuations in splenic and thymic cell populations, increased bone marrow hypocellularity in conjunction with extramedullary hematopoiesis were observed. Extramedullary hematopoiesis, blood cell production outside of the bone marrow, occurs when normal cell production is impaired. Bone marrow hypocellularity in parallel with extramedullary hematopoiesis suggest that PFOS impedes hematopoiesis in the bone marrow. As such, EPA concluded that elevated extramedullary hematopoiesis and bone marrow hypocellularity, as well as reduced ability to generate an immune response to a bacteria-like challenge and reduced NK cell activity indicate toxicity of relevance to humans exposed to PFOS. EPA identified extramedullary hematopoiesis in male and female rats (NTP, 2019, 5400978) for POD derivation. This is a well-powered study that reports dose-responsive effects that are consistent between each sex and apparent at low-doses. Several studies (Dong et al., 2009, 1424951; Peden-Adams et al., 2008, 1424797; Zhong et al., 2016, 3748828) reported non-monotonic dose-response curves for NK cell activity. Therefore, EPA did not consider NK cell activity for POD derivation. EPA identified PFC Response in male PNW4 mice in Zhong et al. (2016, 3748828)

as an additional endpoint for dose-responsive modeling. This study is well-powered, includes low-doses, and exhibits dose-dependent responses to PFOS exposure.

3.3.5 Cardiovascular

3.3.5.1 Human Evidence

3.3.5.1.1 Cardiovascular Endpoints

3.3.5.1.1.1 Introduction

Cardiovascular disease (CVD) is the primary cause of death in the United States with approximately 12% of adults reporting a diagnosis of heart disease {Schiller, 2012, 1798736}. Studied health effects include ischemic heart diseases (IHD), coronary artery disease (CAD), coronary heart disease (CHD), hypertension, cerebrovascular disease, atherosclerosis (plaque build-up inside arteries and hardening and narrowing of their walls), microvascular disease, markers of inflammation (e.g., C-reactive protein), and mortality. These health outcomes are interrelated—IHD is caused by decreased blood flow through coronary arteries due to atherosclerosis resulting in myocardial ischemia.

The 2016 Health Advisory {U.S. EPA, 2016, 3982043} and HESD {U.S. EPA, 2016, 3603365} assessments did not assess evidence for associations between CVD diseases and PFOS, besides the review of its effects on serum lipids. The developmental section in the 2016 Health Advisory describes results from Geiger et al. (2014, 2851286) which reported no association with hypertension in 1,655 children aged 12–18 years from the NHANES (1999–2000 and 2003–2008 cycles).

For this updated review, 30 new epidemiological studies report on the association between PFOS and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 13 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed in Section 3.3.2.1.2. All studies were conducted on the general population with four {Honda-Kohmo, 2019, 5080551; Hutcheson, 2020, 6320195; Bao, 2017, 3860099; Mi, 2020, 6833736} in a high-exposure community (i.e., C8 Health Project and “Isomers of C8 Health Project” populations). Different study designs were also used including two controlled trial studies {Cardenas, 2019, 5381549; Liu, 2018, 4238396}, seven cohort studies {Fry, 2017, 4181820; Donat-Vargas, 2019, 5080588; Lin, 2020, 6311641; Manzano-Salgado, 2017, 4238509; Matilla-Santander, 2017, 4238432; Mitro, 2020, 6833625; Warmenbourg, 2019, 5881345}, one case-control study {Mattsson, 2015, 3859607}, and 21 cross-sectional studies {Bao, 2017, 3860099; Chen, 2019, 5387400; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Graber, 2019, 5080653; Honda-Kohmo, 2019, 5080551; Huang, 2018, 5024212; Hutcheson, 2020, 6320195; Jain, 2020, 6311650; Jain, 2020, 6833623; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Liao, 2020, 6356903; Lin, 2013, 2850967; Lin, 2016, 3981457; Lind, 2017, 3858504; Liu, 2018, 4238514; Ma, 2019, 5413104; Mi, 2020, 6833736; Mobacke, 2018, 4354163; Yang, 2018, 4238462}. The two controlled trial studies {Cardenas, 2019, 5381549; Liu, 2018, 4238396} were not controlled trials of PFAS exposures, but rather health interventions: prevention of type 2 diabetes in Diabetes Prevention Program and Outcomes Study (DPPOS) {Cardenas, 2019, 5381549} and weight loss in Prevention of Obesity Using Novel Dietary Strategies (POUNDS) Lost Study (Liu, 2018, 4238396). Thus, these studies could be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The studies were conducted in different study populations with the majority of studies conducted in the United States {Cardenas, 2019, 5381549; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Fry, 2017, 4181820; Graber, 2019, 5080653; Honda-Kohmo, 2019, 5080551; Huang, 2018, 5024212; Hutcheson, 2020, 6320195; Jain, 2020, 6311650; Jain, 2020, 6833623; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Liao, 2020, 6356903; Lin, 2020, 6311641; Liu, 2018, 4238396; Liu, 2018, 4238514; Ma, 2019, 5413104; Mi, 2020, 6833736; Mitro, 2020, 6833625}. The remaining studies were conducted in China {Bao, 2017, 3860099; Yang, 2018, 4238462}, Taiwan {Lin, 2013, 2850967; Lin, 2016, 3981457}, Spain {Manzano-Salgado, 2017, 4238509; Matilla-Santander, 2017, 4238432}, Croatia {Chen, 2019, 5387400}, Sweden {Donat-Vargas, 2019, 5080588; Lind, 2017, 3858504; Mattsson, 2015, 3859607; Mobacke, 2018, 4354163}, Denmark {Jensen, 2020, 6833719}, and a single study conducted in several European countries {Warembourg, 2019, 5881345}. All the studies measured PFOS in blood components (i.e., serum or plasma) with one study measuring levels in maternal serum {Warembourg, 2019, 5881345}, and three studies measuring levels in maternal plasma {Warembourg, 2019, 5881345; Manzano-Salgado, 2017, 4238509; Mitro, 2020, 6833625}.

3.3.5.1.1.2 Study Quality

Of the 30 studies identified since the 2016 assessment (Figure 72, Figure 73), four studies were *high* confidence, 15 were *medium* confidence, nine were *low* confidence, and three studies included an outcome considered *uninformative* {Jain, 2020, 6833623; Jain, 2020, 6311650; Seo, 2018, 4238334}. The main concerns with the *low* confidence studies included the possibility of outcome misclassification (e.g., reliance on self-reporting) in addition to the potential for residual confounding or selection bias (e.g., unequal recruitment and participation among subjects with outcome of interest, lack of consideration and potential exclusion due to medication usage). Residual confounding was possible due to socioeconomic status (SES), which can be associated with both exposure and the cardiovascular outcome. Although PFOS has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further, temporality of PFOS exposure could not be established for several low confidence studies due to their cross-sectional design. Several of the *low* confidence studies also had sensitivity issues due to limited sample sizes. Two studies were considered *uninformative* {Jain, 2020, 6833623; Jain, 2020, 6311650} because PFOS levels were compared by cardiovascular disease and other characteristics (e.g., kidney function) with PFOS levels considered as the dependent variable in analysis, and there were concerns for residual confounding.

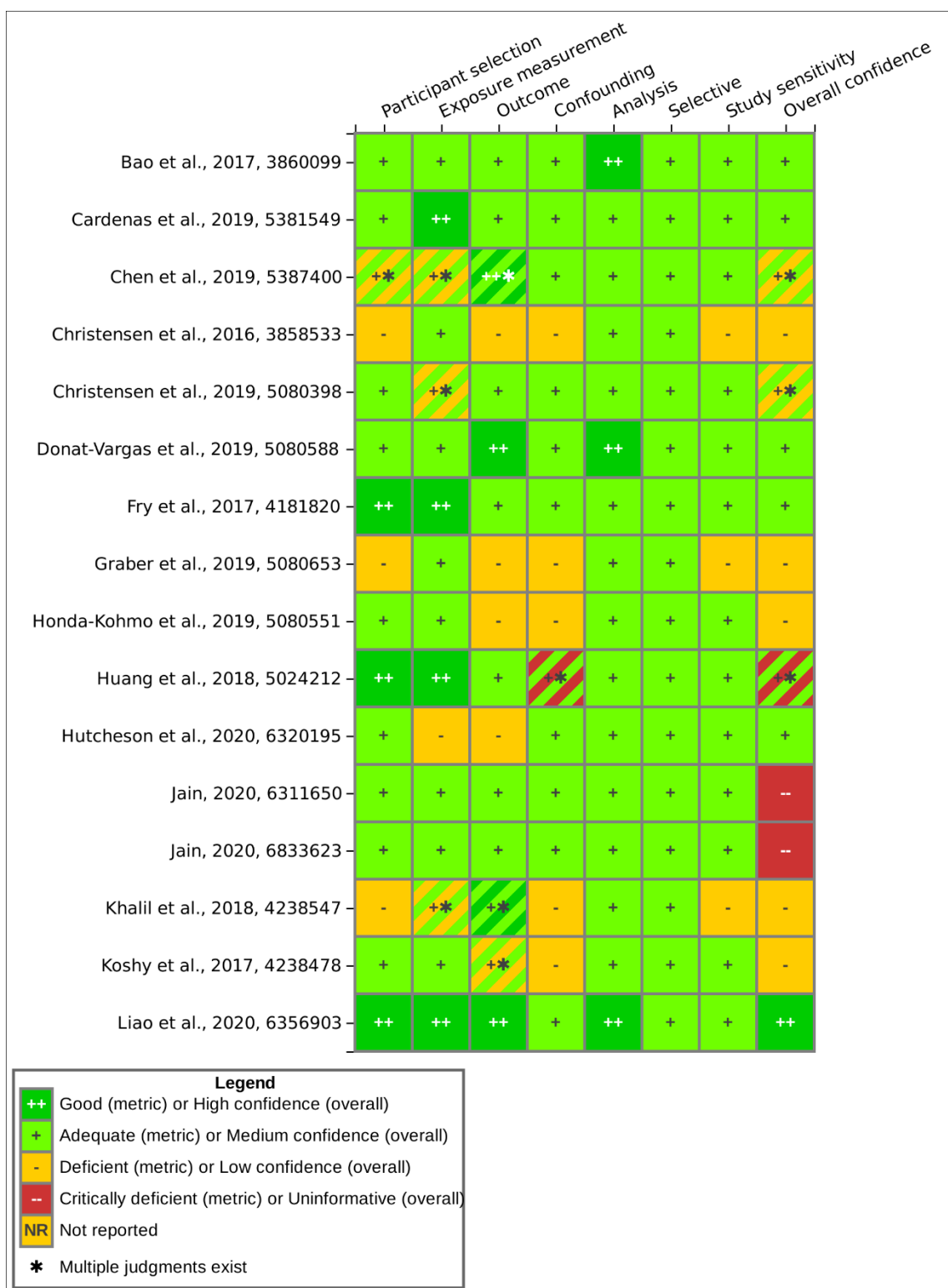


Figure 72. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on [HAWC](#).

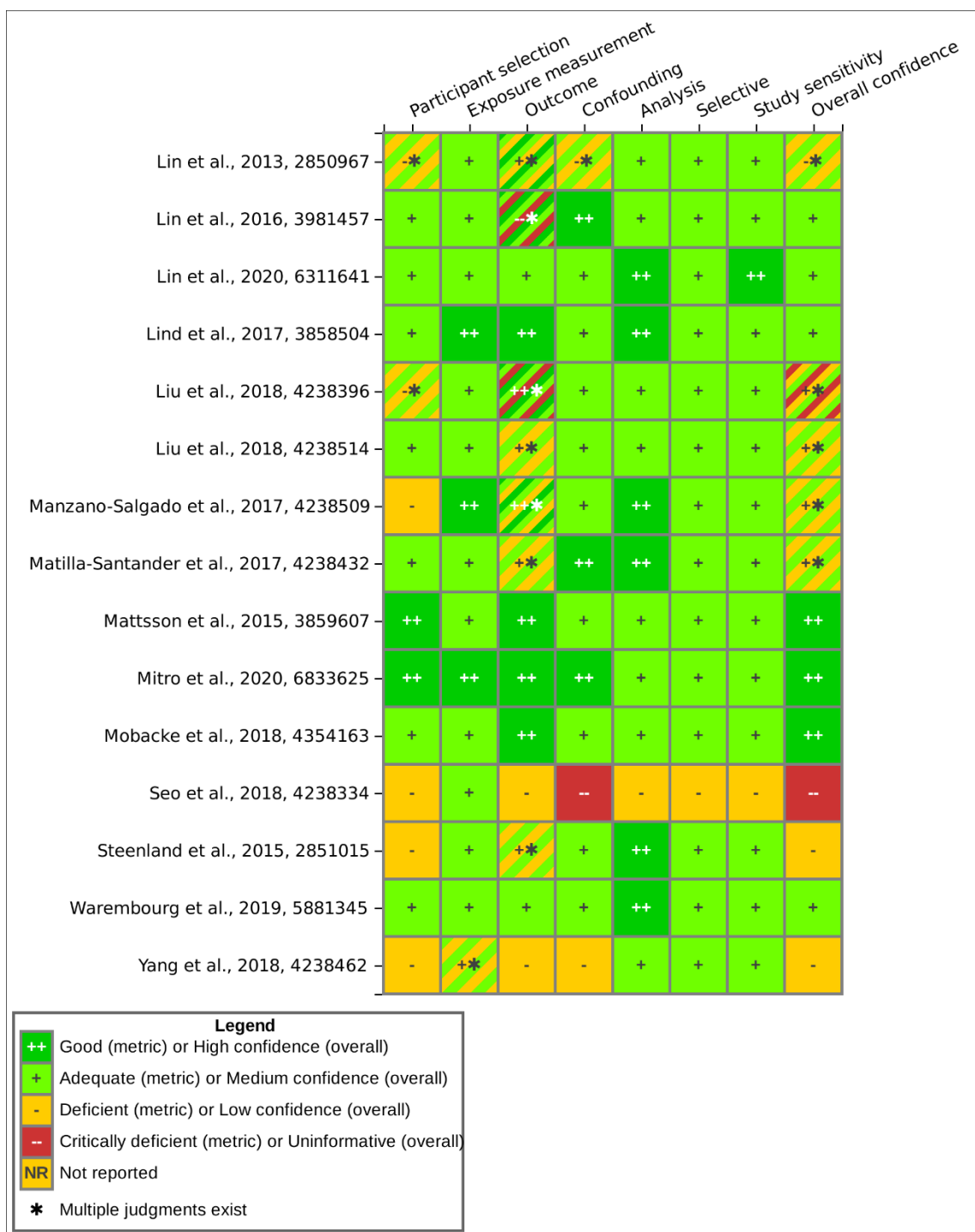


Figure 73. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on [HAWC](#).

3.3.5.1.1.3 Findings from Children

Of the three *medium* confidence studies that examined blood pressure in children and adolescents, one reported positive association with DBP only {Ma, 2019, 5413104; Manzano-Salgado, 2017, 4238509; Warembourg, 2019, 5881345} (Table C-13). Among 2,251 NHANES (2003–2012) adolescents (mean age 15.5 years) Ma (2019, 5413104) observed a positive association with DBP, but significant only in boys (0.025; 95% CI: 0.001, 0.049). The study also reported that male adolescents with PFOS levels in the highest quintile (>18 ng/mL) had mean DBP values that were 2.70% greater (95% CI: 0.32%, 5.02%) than the lowest quartile (<6.2 ng/mL). No association was observed for DBP among female adolescents, or for SBP among all adolescents. Manzano-Salgado (2017, 4238509) reported that maternal PFOS was not associated with blood pressure in combined or in gender-stratified analyses at age 4 and 7 years. In a cohort of 1,277 children (age 6–11 years), Warembourg et al. (2019, 5881345) observed that PFOS measured in maternal blood during the pre-natal period, and in plasma during the post-natal period were not associated with blood pressure in single-pollutant models.

Two *low* confidence studies did not observe associations between serum PFOS and blood pressure {Khalil, 2018, 4238547; Lin, 2013, 2850967}.

Other cardiovascular conditions reported in the recent literature include carotid artery intima-media thickness (CIMT) and brachial artery distensibility. Two *medium* confidence studies examined CIMT among 664 {Lin, 2013, 2850967} and 848 {Lin, 2016, 3981457} adolescents and young adults from the Young Taiwanese Cohort Study. Both studies observed a statistically significant increase in the mean CIMT with higher serum PFOS levels ($p < 0.001$ in test for trend). A *low* confidence study of children and adolescents from the World Trade Center Health Registry (WTCHR) reported that the association between PFOS and brachial artery distensibility was borderline significant ($p = 0.06$), with no association reported for pulse wave velocity {Koshy, 2017, 4238478}. However, concerns for residual confounding by age and SES contributed to the *low* confidence.

Overall, the limited evidence available among children and adolescents indicates PFOS is not associated with blood pressure. The evidence for an association between PFOS and other CVD-related endpoints assessed in this study population was limited and inconsistent.

3.3.5.1.1.4 Findings from the General Adult Population

Most of the studies identified since the last assessment were conducted among general population adults (Table C-13). A total of 12 studies examined PFOS in association with SBP, DBP, hypertension, and elevated blood pressure {Bao, 2017, 3860099; Chen, 2019, 5387400; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Donat-Vargas, 2019, 5080588; Mitro, 2020, 6833625; Liao, 2020, 6356903; Lin, 2020, 6311641; Liu, 2018, 4238514; Liu, 2018, 4238396; Mi, 2020, 6833736; Yang, 2018, 4238462}.

Of the eight studies that examined blood pressure as a continuous measure, five observed statistically significant positive associations {Liao, 2020, 6356903; Mitro, 2020, 6833625; Bao, 2017, 3860099; Mi, 2020, 6833736; Liu, 2018, 4238396}. However, the results were not always consistent between SBP and DBP. A *high* confidence study in 6,967 NHANES (2003–2012) participants 20 years and older reported a statistically significant positive association with SBP (per 10-fold change in PFOS: 1.35; 95% CI: 0.18, 2.53) {Liao, 2020, 6356903}. Using a

generalized additive model and restricted cubic splines, a non-linear (J-shaped) relationship between PFOS and DBP was observed, with the inflection point of PFOS at 8.20 ng/mL. Each 10-fold increase in PFOS was inversely associated with DBP (OR: -2.62; 95% CI: -4.73, -0.51) on the left side of the inflection point and positively associated on the right side of the inflection point (OR: 1.23; 95% CI: -0.42, 2.88). A *high* confidence study {Mitro, 2020, 6833625} conducted in 761 women that examined associations between PFOS concentrations measured during pregnancy and blood pressure assessed at 3 years post-partum reported significantly higher SBP levels among all women (beta per doubling of PFOS: 1.2; 95% CI: 0.3, 2.2) and among women 35 years or older (percent difference per doubling of PFOS: 2.3; 95% CI: 0.9, 3.6). No association was observed with DBP.

Two *medium* confidence cross-sectional studies with overlapping data from the “Isomers of C8 Health Project”, a high-exposed population of Shenyang, China {Mi, 2020, 6833736; Bao, 2017, 3860099} also reported positive associations for blood pressure. In adults with very high PFOS levels (median 24.22 ng/mL), Bao et al. (2017, 3860099) observed statistically significant increases in DBP (2.70; 95% CI: 1.98, 3.42) and SBP (4.84; 95% CI: 3.55, 6.12). A positive trend for the association between PFOS, linear (n-PFOS), and branched isomers, and blood pressure was highly significant ($p < 0.001$). In adults with high PFOS levels (median 10.33 ng/mL) Mi et al. (2020, 6833736) reported statistically significant increases in SBP (2.23; 95% CI: 0.58, 3.89). After stratification by sex, significant positive associations were observed in women only for SBP, the estimate was 3.08 (95% CI: 1.53, 4.62; p -value for interaction by sex = 0.03). For DBP, the associations were positive but non-significant overall or among women. Lin, 2020, 6311641 using data from the Diabetes Prevention Program, a randomized controlled health intervention trial, reported that higher baseline PFOS concentrations were significantly associated with a decrease in SBP over time (year 2: -2.13 mmHg/yea; 95% CI: -3.54, -0.71) among participants assigned to the lifestyle intervention arm, but no association was observed in participants in the placebo-medication arm. However, the study authors attribute the negative findings for BP trajectories (decreases over time) in the lifestyle group to regression towards the mean, a statistical phenomenon where a more extreme value from the population mean can experience a greater change toward the mean; however, it is unclear why this phenomenon would only apply to the lifestyle arm.

In a weight loss-controlled trial population (POUNDS Lost study) Liu et al. (2018, 4238396) observed that baseline that PFOS was positively correlated with DBP ($p < 0.001$) but at 6- and 24-month follow-up assessments no associations were observed for SBP or DBP.

No association was observed for blood pressure in two *low* confidence studies {Chen, 2019, 5387400; Yang, 2018, 4238462}.

Of the eight studies that examined risk of elevated blood pressure (hypertension), two reported statistically significant associations {Bao, 2017, 3860099; Mi, 2020, 6833736}. Hypertension was defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed anti-hypertensive medication. Mi, 2020, 6833736 and Bao, 2017, 3860099 with overlapping data on high exposed Isomers of C8 Health Project participants reported significant associations. Bao et al. (2017, 3860099) reported significantly higher odds of hypertension (OR: 1.24; 95% CI: 1.08, 1.44) for PFOS, and for several PFOS isomers. The associations remained significant in women for PFOS (OR: 1.63; 95% CI: 1.24, 2.13; p -value for interaction by sex = 0.016), and some isomers. These results suggest branched PFOS isomers have a stronger

association with increased risk of hypertension compared to linear isomers (n-PFOS). Mi et al. (2020, 6833736) reported a significant positive association for hypertension (OR: 2.52; 95% CI: 1.91, 3.33) overall, and in women (OR 2.32; 95% CI: 1.38, 3.91; p-value for interaction by sex < 0.01).

The *high* confidence study {Liao, 2020, 6356903} reported in a fully adjusted analysis that the OR among adults exposed to PFOS levels in the highest tertile compared to the lowest tertile and the test of trend, respectively, were not significant. Additionally, a significant interaction was observed between gender and hypertension ($p = 0.016$), although the association between PFOS and hypertension was non-significant among males and females in stratified analysis. No association was observed for elevated blood pressure in two *medium* studies {Christensen, 2019, 5080398; Liu, 2018, 4238514} and for hypertension in one *medium* (Lin, 2020, 6311641) and one *low* confidence study {Christensen, 2016, 3858533}. One *medium* confidence study {Donat-Vargas, 2019, 5080588} reported a significant protective effect for hypertension (OR: 0.71; 95% CI: 0.56, 0.89).

Nine studies examined other CVD-related outcomes in adults, including CHD, stroke, carotid artery atherosclerosis, angina pectoris, C-reactive protein, CHF, microvascular disease, and mortality.

Graber, 2019, 5080653 reported a positive, borderline significant association with self-reported cardiovascular conditions (i.e., high blood pressure, CAD, stroke) (1.08; 95% CI: 0.98, 1.21). However, potential selection bias is a major concern for this study owing to the recruitment of volunteers who already knew their PFAS exposure levels and were motivated to participate in a lawsuit.

Among the four studies that examined CHD, the findings were mixed, with three studies reporting positive non-significant associations, and one study reporting negative associations. A *high* confidence study {Mattson, 2015, 3859607}, a *medium* confidence NHANES study {Huang, 2018, 5024212}, and a *low* confidence study {Christensen, 2016, 3858533} reported positive non-significant associations with CHD. A *low* confidence study from the C8 Health Project {Honda-Kohmo, 2019, 5080551} reported a significant inverse association between PFOS and CHD among adults with and without diabetes. However, study limitations that may have influenced these findings include the reliance on self-reporting of a clinician-based diagnosis for CHD outcome classification and residual confounding by SES.

A *medium* confidence study of 10,850 NHANES participants (1999–2014) {Huang, 2018, 5024212} reported significantly higher odds of heart attack for the third quartile (OR: 1.56; 95% CI: 1.01, 2.43) compared to the first quartile, and a very similar but not significant effect in the fourth quartile. No associations were observed with stroke, CHF, and angina pectoris. A *medium* confidence study {Hutcheson, 2020, 6320195} of 3,921 adults with and 44,285 without diabetes participating in the C8 Health Project found a significant inverse association with history of stroke (OR: 0.90; 95% CI: 0.82–0.98; $p = 0.02$). A significant inverse association with history of stroke (OR: 0.81; 0.70–0.90) was observed among people with diabetes. No association with stroke was observed among those without diabetes.

Cardenas, 2019, 5381549 reported significant increases in risk of any microvascular disease, that were significant (OR: 1.37; 95% CI: 1.04, 1.84) only in the lifestyle arm of a health

interventions-controlled trial (OR: 1.37; 95% CI: 1.04, 1.84). No associations were observed for nephropathy, retinopathy, or neuropathy.

Two studies assessed potential PFOS-associated changes in heart structure {Mobacke, 2018, 4354163} and carotid atherosclerosis {Lind, 2017, 3858504} in participants 70 years and older, with mixed results. Mobacke, 2018, 4354163 evaluated alterations of left ventricular geometry, a risk factor for CVD and reported that serum PFOS (linear isomer) was significantly associated with higher left ventricular end-diastolic diameter (0.47; 95% CI: 0.08, 0.87; $p = 0.02$) and lower relative wall thickness (-0.01 ; 95% CI: -0.01 , -0.001 ; $p = 0.03$). PFOS was not significantly associated with left ventricular mass. Lind, 2017 (3858504) reported that plasma PFOS was not associated with markers of carotid artery atherosclerosis, including atherosclerotic plaque, the intima-media complex, and the CIMT, a measure used to diagnose the extent of carotid atherosclerotic vascular disease.

No association between PFOS and C-reactive protein levels, a risk factor for CVD, were observed in two studies, one in women from Project VIVA {Mitro, 2020, 6833625} and in pregnant women from the INMA study {Matilla-Santander, 2017, 4238432}.

Mortality due to heart/cerebrovascular diseases was examined in one *medium* confidence study {Fry, 2017, 4181820}. Among a cohort of 1,043 NHANES participants 60 years and older PFOS was not associated with mortality due to heart/cerebrovascular diseases.

Overall, the findings from a single *high* confidence study and several *medium* confidence studies conducted among the general population provided consistent evidence for an association between PFOS and blood pressure. The directionality of this association was mostly positive, although a single medium confidence study {Lin, 2020, 6311641} reported an inverse association. The limited evidence for an association between PFOS and increased risk of hypertension was inconsistent. There was evidence suggesting an increased risk of hypertension among women {Liao, 2020, 6356903; Bao, 2017, 3860099} in the general adult population, but additional studies are needed to confirm this finding. Evidence for other CVD-related endpoints was also limited and inconsistent. No occupational studies examining PFOS exposure and CVD were identified.

3.3.5.1.2 Serum Lipids

3.3.5.1.2.1 Introduction

Serum cholesterol and triglycerides are well-established risk factors for CVDs. Major cholesterol species in serum include low-density-lipoprotein cholesterol (LDL) and high-density-lipoprotein cholesterol (HDL). Elevated levels of total cholesterol (TC), LDL, and triglycerides are associated with increased cardiovascular risks, while higher levels of HDL are associated with reduced risks.

In the 2016 Health Assessment for PFOS, the epidemiologic evidence overall supported an association between PFOS and increased TC. An association between PFOS and small increases in TC in the general population was observed. A positive association between PFOS and hypercholesterolemia also was observed in two cohorts. Cross-sectional occupational studies demonstrated an association between PFOS and TC. Evidence for associations between other

serum lipids and PFOS was mixed including HDL, LDL, very-low-density lipoprotein cholesterol (VLDL), non-HDL cholesterol, and triglycerides.

For this updated review, 42 new epidemiologic studies (41 publications)⁸ were identified. These studies examined the associations between PFOS and serum lipids in children (n = 13), in pregnant women (n = 4), in the general adult population (n = 22), and workers (n = 3). Except for six studies {Olsen, 2012, 2919185; Domazet, 2016, 3981435; Lin, 2019, 5187597; Liu, 2020, 6318644; Donat-Vargas, 2019, 5080588; Liu, 2018, 4238396}, all studies were cross-sectional. Most studies assessed exposure to PFOS using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but some studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on the clinical cut-points, medication use, or doctor's diagnosis.

3.3.5.1.2.2 Study Quality

All studies were evaluated for risk of bias, selective reporting, and sensitivity following the EPA IRIS protocol. Three considerations were specific to evaluating the quality of studies on serum lipids. First, because lipid-lowering medications strongly affect serum lipid levels, unless the prevalence of medication use is expected to be low in the study population (e.g., children), studies that did not account for the use of lipid-lowering medications by restriction, stratification, or adjustment were rated as *deficient* in the *participant selection* domain. Second, because triglycerides levels are sensitive to recent food intake {Mora, 2016, 9564968}, outcome measurement error is likely substantial when TG is measured without fasting. Thus, studies that did not measure triglycerides in fasting blood samples were rated *deficient* in the *outcome measures* domain for triglycerides. The *outcome measures* domain for LDL was also rated *deficient* if LDL was calculated based on triglycerides. Fasting status did not affect the *outcome measures* rating for TC, directly measured LDL, and HDL because the serum levels of these lipids change minimally after a meal {Mora, 2016, 9564968}. Third, 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. Furthermore, although reverse causation due to hypothyroidism {Dzierlenga, 2020, 6833691} or enterohepatic cycling of bile acids {Fragki, 2021, 8442211} has been suggested, there is yet clear evidence to support these reverse causal pathways.

Based on these considerations, 16 studies were rated *medium* confidence for all lipid outcomes, 4 studies were rated *medium* confidence for TC or HDL, but *low* confidence for triglycerides or LDL, 17 studies were rated *low* confidence for all lipid outcomes, and four studies were rated *uninformative* for all lipid outcomes {Seo, 2018, 4238334; Abraham, 2020, 6506041; Predieri, 2015, 3889874; Huang, 2018, 5024212}. The domain-specific and overall ratings for each study are shown in Figure 74 and Figure 75. Notably, Zeng et al. 2015, 2851005, Manzano-Salgado et al. 2017 4238509, Canova et al., 2020, 7021512 and Matilla-Santander et al. 2017 4238432 were rated *low* confidence specifically for triglycerides and/or LDL because these studies measured triglycerides in non-fasting blood samples. The *low* confidence studies had *deficiencies* in participant selection {Wang, 2012, 2919184; Khalil, 2018, 4238547; Lin, 2013, 2850967; Lin,

⁸ Dong 2019, 5080195 counted as two studies, one in adolescents and one in adults.

2020, 6315756; van den Dungen, 2017, 5080340; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Rotander, 2015, 3859842; Liu, 2018, 4238396}, outcome measures {Koshy, 2017, 4238478; Yang, 2018, 4238462; Christensen, 2016, 3858533; Kishi, 2015, 2850268; Graber, 2019, 5080653; Rotander, 2015, 3859842}, confounding {Wang, 2012, 2919184; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Olsen, 2012, 2919185; Lin, 2013, 2850967; Lin, 2020, 6315756; van den Dungen, 2017, 5080340; Li, 2020, 6315681; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653}, analysis {He, 2018, 4238388; Sun, 2018, 4241053; Liu, 2018, 4238396}, sensitivity {Wang, 2012, 2919184; Khalil, 2018, 4238547; Olsen, 2012, 2919185; Christensen, 2016, 3858533; Graber, 2019, 5080653; Rotander, 2015, 3859842; van den Dungen, 2017, 5080340}, or selective reporting {Dong, 2019, 5080195} (adolescent portion). The most common reason for a *low* confidence rating was concerns for participant selection. These concerns include a lack of exclusion based on use of lipid-lowering medications {Wang, 2012, 2919184; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Sun, 2018, 4241053; van den Dungen, 2017, 5080340; Liu, 2018, 4238396}, potential for self-selection {Li, 2020, 6315681; Christensen, 2016, 3858533; Graber, 2019, 5080653; Rotander, 2015, 3859842; van den Dungen, 2017, 5080340}, highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOS and lipids {Lin, 2013, 2850967}, and missing key information on the recruitment process {Khalil, 2018, 4238547; Yang, 2018, 4238462}. Another common reason for *low* confidence was a serious risk for residual confounding by SES {Wang, 2012, 2919184; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Olsen, 2012, 2919185; Lin, 2013, 2850967; Lin, 2020, 6315756; van den Dungen, 2017, 5080340; Li, 2020, 6315681; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653}. Frequently, deficiencies in multiple domains contributed to an overall *low* confidence rating. The *uninformative* studies had *critical deficiencies* in at least one domain. These *critical deficiencies* include a lack of control for confounding {Seo, 2018, 4238334; Huang, 2018, 5024212; Abraham, 2020, 6506041} and treating PFOS as an outcome of all lipids instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination {Predieri 2015, 3889874}. In the evidence synthesis below, *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

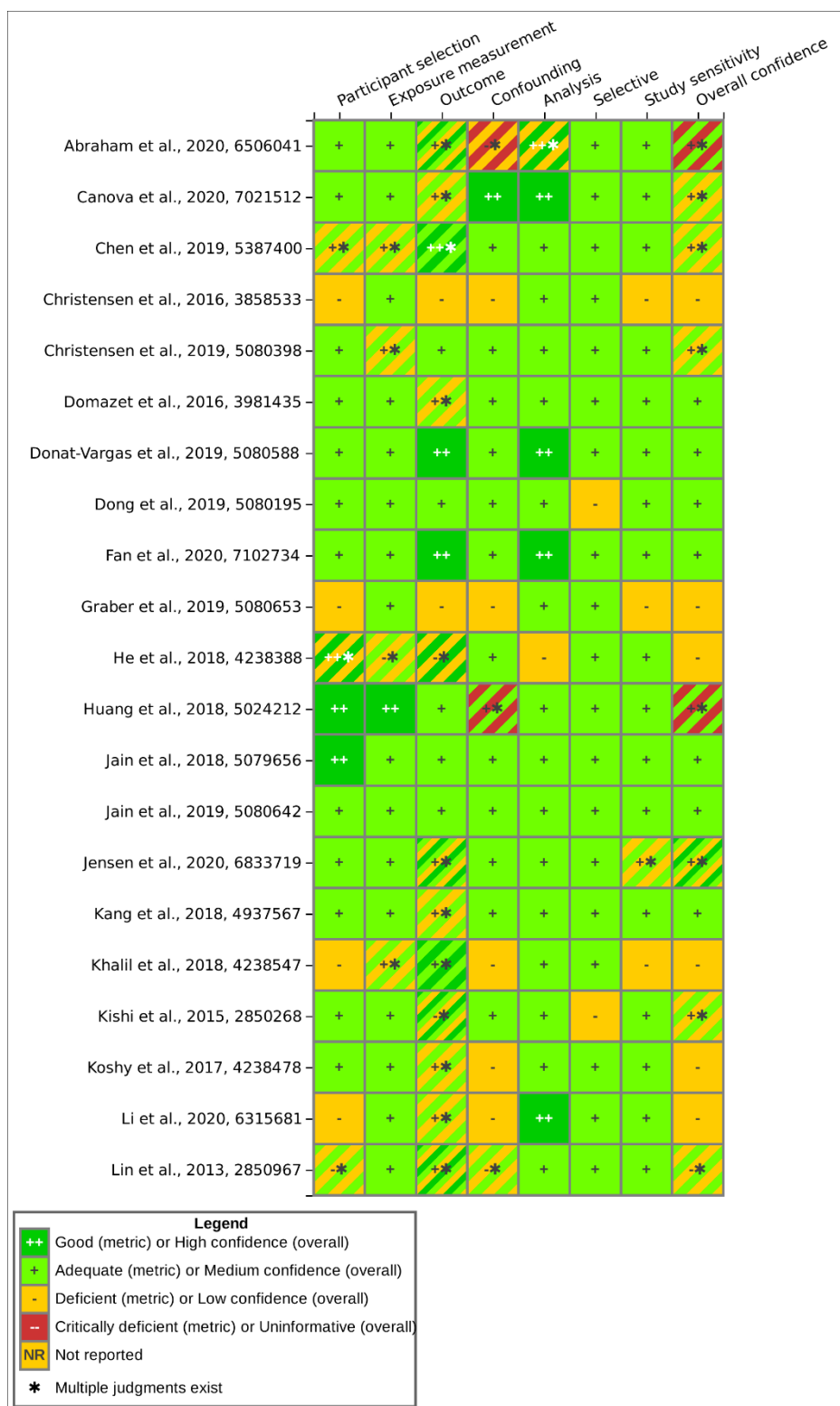


Figure 74. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids

Interactive figure and additional study details available on [HAWC](#).

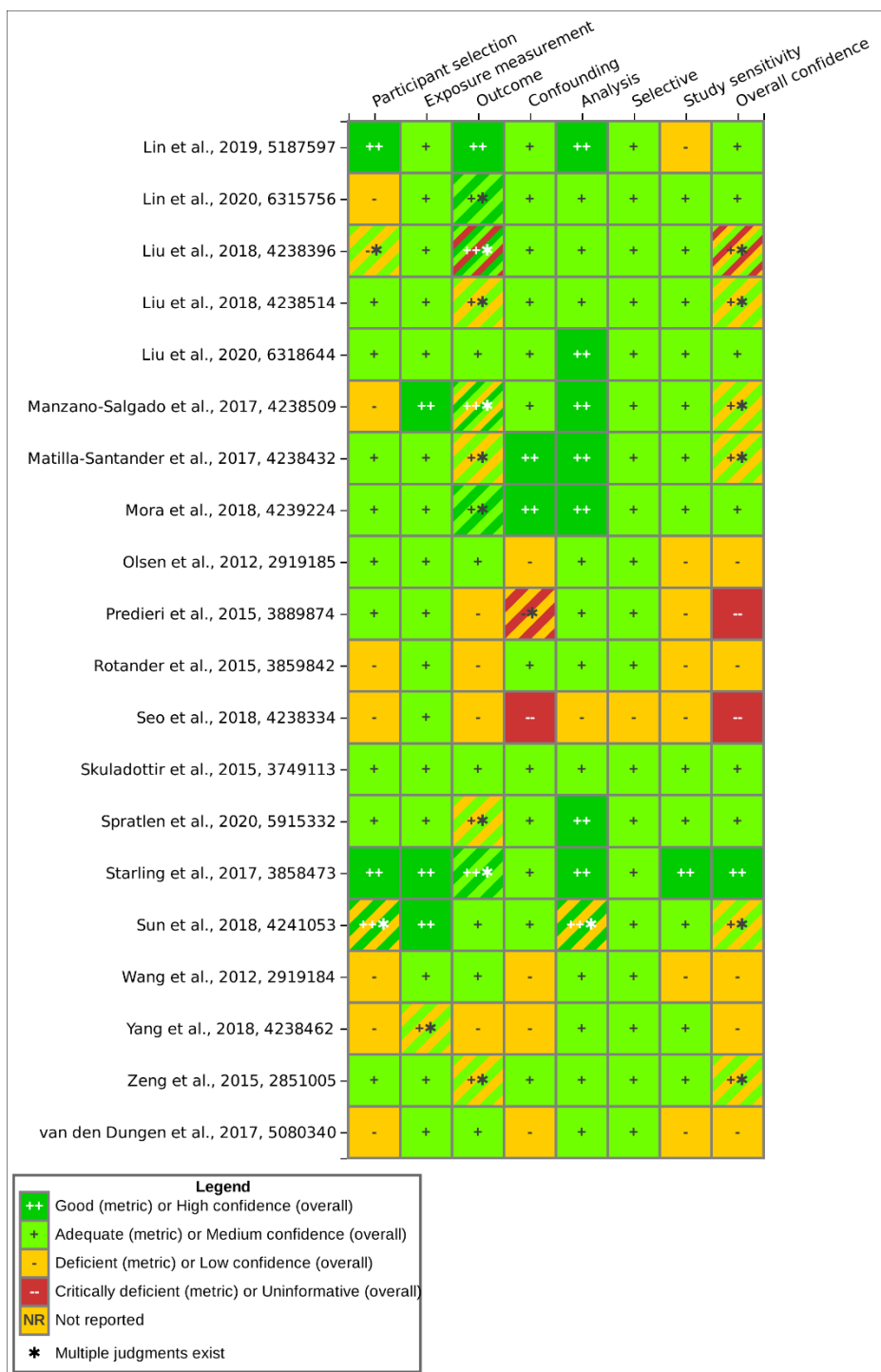


Figure 75. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on [HAWC](#).

3.3.5.1.2.3 Findings from Children

Results for the studies that examined TC in children are presented in Table C-14. Seven *medium* confidence and three *low* confidence studies examined the association between PFOS and TC in children. Of these, four studies examined the association between prenatal PFOS exposure and TC in childhood {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224} and seven examined the association between childhood PFOS exposure and concurrent TC {Mora, 2018, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Dong, 2019, 5080195} (adolescent portion). Higher PFOS was significantly associated with higher TC in all children in two *medium* confidence studies {Jain, 2018, 5079656; Zeng, 2015, 2851005}. Positive associations were also found in four other *medium* confidence studies {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224}, but the associations were small and statistically not significant except for girls in mid-childhood {Mora, 2018, 4239224}. In two out of three *low* confidence studies, positive associations were reported, including a statistically significant finding in Koshy 2017, 4238478 {Khalil, 2018, 4238547; Koshy, 2017, 4238478}. However, residual confounding by SES may have positively biased the results of both studies. Taken together, these studies support a positive association between PFOS and TC in children, particularly for childhood exposure.

Three *medium* confidence and five *low* confidence studies examined the association between PFOS and LDL in children. Of these, three examined prenatal exposure {Jensen, 2020, 6833719; Manzano-Salgado 2017, 4238509; Mora, 2018, 4239224} and six examined childhood exposure {Mora, 2018, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Dong, 2019, 5080195} (adolescent portion). The medium studies generally found small, positive associations between PFOS and LDL, but none of the associations were statistically significant (Table C-14) {Jensen, 2020, 6833719; Mora, 2018, 4239224; Kang, 2018, 4937567}. Most *low* confidence studies found a positive association between PFOS and LDL {Khalil 2018, 4238547; Koshy, 2017, 4238478; Manzano-Salgado, 2017, 4238509; Zeng, 2015, 2851005}, including statistically significant findings in Khalil 2018, 4238547; Koshy 2017, 4238478. However, residual confounding by SES {Khalil, 2018, 4238547; Koshy 2017, 4238478} and the use of non-fasting samples {Zeng, 2015, 2851005; Manzano-Salgado, 2017, 4238509} were concerns in these studies. Overall, increases in LDL with increasing PFOS were observed in children, but the magnitudes were small.

Five *medium* confidence and three *low* confidence studies examined the association between PFOS and HDL in children. Of these, three examined prenatal exposure {Jensen 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224} and six examined childhood exposure {Mora, 2018, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Dong, 2019, 5080195} (adolescent portion). Higher PFOS was significantly associated with higher HDL in children in mid-childhood in one *medium* confidence study {Mora, 2018, 4239224}. Other *medium* confidence studies found positive (Jain 2018, 5079656), inverse (HDL at 18 months in Jensen 2020, 6833719; Manzano-Salgado 2017, 4238509; Zeng, 2015, 2851005), or close to zero (HDL at 3 months in Jensen 2020, 6833719) associations; none of these associations were statistically significant. Two of the three *low* confidence studies found positive associations between PFOS and HDL {Khalil, 2018, 4238547; Koshy, 2017, 4238478}. In summary, mixed associations between PFOS and HDL were found in children.

Five *medium* confidence studies and four *low* confidence studies examined the association between PFOS and triglycerides in children. Of these, four examined prenatal exposure {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224} and six examined childhood exposure {Domazet, 2016, 3981435; Mora, 2018, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478}. Higher mid-childhood PFOS exposure was significantly associated with lower triglycerides in one *medium* confidence study {Mora, 2018, 4239224}. The other *medium* confidence studies reported positive {Spratlen, 2020, 5915332; Kang, 2018, 4937567}, inverse (triglycerides at 3 months in Jensen 2020, 6833719; PFOS exposure at age 9 years in Domazet 2016, 3981435), or close to zero associations (triglycerides at 18 months in Jensen 2020, 6833719; PFOS exposure at age 15 years in Domazet 2016, 3981435); none of these associations were statistically significant. Of note, in Jensen 2020, 6833719 and Domazet 2016, 3981435, the direction of association changed depending on the timing of outcome or exposure assessment. The *low* confidence studies all reported positive associations between PFOS and triglycerides, but all associations were small and not statistically significant {Manzano-Salgado, 2017, 4238509; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478}. The use of non-fasting samples and residual confounding by SES may have biased these results upwards. Overall, mixed associations were found between PFOS and triglycerides in children.

In summary, the available evidence supports positive associations between PFOS and TC and LDL in children. The associations with HDL and triglycerides were mixed.

3.3.5.1.2.4 Findings from Pregnant Women

Two *medium* confidence studies examined the association between PFOS and TC in pregnant women and reported positive associations between PFOS and TC (Table C-14) {Mattila-Santander, 2017, 4238432; Skuladottir, 2015, 3749113}. Skuladottir 2015, 3749113, reported a statistically significant linear trend of increasing TC with increasing PFOS. These findings suggest a consistently positive association between PFOS and TC in pregnant women.

No studies examined PFOS and LDL in pregnant women. One *medium* confidence study examined PFOS and HDL and reported a positive, though statistically non-significant, association between PFOS and HDL (Table C-14) {Starling, 2017, 3858473}.

One *medium* confidence and two *low* confidence studies examined the association between PFOS and triglycerides in pregnant women. The *medium* confidence study reported no association between PFOS and triglycerides (Table C-14) {Starling, 2017, 3858473}. Both *low* confidence studies reported statistically significant, inverse associations between PFOS and triglycerides {Mattila-Santander 2017, 4238432; Kishi, 2015, 2850268}. Both *low* confidence studies were limited by their use of non-fasting blood samples. Given that recent food intake is associated with increased triglycerides and may be a source of PFOS, using non-fasting blood samples is expected to positively bias the PFOS- triglycerides association. That inverse associations were still observed in the *low* confidence studies provides support for an inverse association between PFOS and triglycerides. This inverse association is inconsistent with the finding in the only *medium* confidence study. In sum, the available evidence suggests an inverse association between PFOS and triglycerides in pregnant women. However, high-quality evidence is lacking to confirm this association.

Kishi 2015, 2850268 additionally examined the association between PFOS and select fatty acids in serum. Except for stearic acid and EPA, PFOS was inversely associated with serum fatty acids; most of these associations were statistically significant {Kishi, 2015, 2850268}. This study suggests PFOS may disrupt fatty acid metabolism in pregnant women, but additional studies are needed to confirm this finding.

In summary, the available evidence supports a positive association between PFOS and TC in pregnancy. The available evidence does not support a consistent, positive association between PFOS and triglycerides. Finally, the available evidence is too limited or non-existent to determine the association between PFOS and HDL and LDL in pregnant women.

3.3.5.1.2.5 Findings from the General Adult Population

Eight *medium* confidence and eight *low* confidence studies examined PFOS and TC or hypercholesterolemia in adults. All studies examined the cross-sectional association {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Donat-Vargas, 2019, 5080588; Wang, 2012, 2919184; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Liu, 2018, 4238396; Canova, 2020, 7021512; Fan, 2020, 7102734}; two studies additionally examined the association between baseline PFOS and changes in TC or incident hypercholesterolemia {Liu, 2020, 6318644; Lin, 2019, 5187597}.

Of the eight *medium* confidence studies, seven reported positive associations. In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova 2020, 7021512 reported statistically positive associations with TC. Canova 2020, 7021512 also reported a concentration-response curve when PFOS was categorized in deciles, with a higher slope at lower PFOS concentrations in the lower two deciles.

Four *medium* studies using overlapping data from NHANES 2003–2014 reported positive associations between PFOS and TC in adults {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514; Fan, 2020, 7102734} (Table C-14). The association was statistically significant when data from all cycles were pooled in analyses {Dong, 2019, 5080195}. PFOS also was associated with slightly higher TC at baseline in the POUNDS-Lost cohort {Liu, 2020, 6318644} and the DPPOS {Lin, 2019, 5187597}, but neither association was statistically significant. The DPPOS also reported that PFOS was associated with a slightly higher prevalence of hypercholesterolemia at baseline (OR = 1.02, 95% confidence interval (CI): 0.85, 1.21)) and a slightly higher incidence of hypercholesterolemia prospectively (HR = 1.01, 95% CI: 0.91, 1.12). In contrast to these findings, Donat-Vargas 2019, 5080588 reported inverse associations between PFOS and concurrently measured TC. Further, it reported positive associations between PFOS averaged between baseline and follow-up and TC at follow-up {Donat-Vargas, 2019, 5080588}. All associations in Donat-Vargas 2019, 5080588 were small and few were statistically significant. It is noteworthy that all participants in Lin 2019, 5187597 were prediabetic, all participants in Liu 2020, 6318644 were obese and enrolled in a weight loss trial, and all participants in Donat-Vargas 2019, 5080588 were free of diabetes for at least 10 years of follow-up. It is unclear if differences in participants' health status explained the studies' conflicting findings. In *low* confidence studies, positive associations between PFOS and TC or hypercholesterolemia were reported in seven of eight studies {Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018,

4241053; Liu, 2018, 4238396}. However, oversampling of persons with potentially high PFOS exposure and health problems was a concern in three of these studies {Li, 2020, 6315681; Christensen, 2016, 3858533; Graber, 2019, 5080653}. Further, He 2018, 4238388 used similar data as the four *medium* NHANES studies and thus added little information. Considering *medium* and *low* confidence studies together, small increases in TC with increased PFOS were observed, though less consistently.

Five *medium* confidence studies examined PFOS and LDL in adults and all reported positive associations. The four studies using overlapping data from NHANES 2003-2014 reported positive associations between PFOS and LDL {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514}, but the association was statistically significant in obese women only {Jain, 2019, 5080642} (Table C-14). The association was inverse, but not statistically significant, in non-obese persons {Jain, 2019, 5080642}. Positive association between PFOS and LDL also was reported at baseline in the DPPOS, but this association was not statistically significant {Lin, 2019, 5187597}. This study additionally reported that PFOS was significantly associated with higher VLDL and non-HDL {Lin, 2019, 5187597}, which are cholesterol species related to LDL and known to increase cardiovascular risks. Liu 2020, 6318644 reported that PFOS was associated with slightly higher cholesterol in combined fractions of intermediate-density (IDL) and low-density lipoproteins (LDL) that contained apolipoprotein C-III (ApoC-III), but this association was not statistically significant. ApoC-III-containing IDL and LDL are strongly associated with increased cardiovascular risks. Thus, the positive associations with cholesterol in ApoC-III-containing fractions of IDL and LDL were coherent with the positive associations found for LDL in the other *medium* confidence studies. Consistent with these findings, six of the eight *low* confidence studies reported positive associations between PFOS and LDL {Lin, 2020, 6315756; Lin, 2013, 2850967; Li, 2020, 6315681; He, 2018, 4238388; Canova, 2020, 7021512; Liu, 2018, 4238396}. However, residual confounding by SES {Lin, 2020, 6315756; Lin, 2013, 2850967} and oversampling of persons with potentially high PFOS and health problems {Li, 2020, 6315681} were major concerns in these studies. In addition, He 2018, 4238388 provided little new information because it used similar data as the four *medium* confidence NHANES studies. Altogether, the available evidence supports a positive association between PFOS and LDL. Few available findings were statistically significant, however, suggesting that the association between PFOS and LDL may be relatively small.

Eight *medium* confidence and eight *low* confidence studies examined PFOS and HDL or clinically defined low HDL in adults. All studies examined the cross-sectional association {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019 5187597; Wang, 2012, 2919184; van den Dungen, 2017, 5080340; Lin, 2020 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Fan, 2020, ; 7102734; Canova 2020, 7021512; Liu 2018, 4238396}. Two studies additionally examined the association between baseline PFOS and changes in HDL (Liu 2020, 6318644; Liu 2018, 4238396). In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova 2020, 7021512 reported statistically positive associations with HDL. Canova 2020, 7021512 also reported a concentration-response curve when PFOS was categorized in deciles. PFOS was associated with lower HDL at baseline in the DPPOS, but this association was not statistically significant {Lin, 2019, 5187597} (Table C-14). The POUNDS-Lost study {Liu, 2020, 6318644} and most cycles of NHANES 2003–2014 {Dong, 2019, 5080195} reported no association

between PFOS and HDL. In *low* confidence studies, PFOS was positively associated with HDL in five of eight studies {Lin, 2020, 6315756; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Liu, 2018, 4238396} (association with concurrent HDL). Of note, in Lin 2020 6315756, the positive association was limited to linear PFOS only; the association between branched PFOS and HDL was inverse and statistically significant {Lin, 2020, 6315756}. The *low* confidence studies had limitations in participant selection, residual confounding by SES, and analysis. It is unclear to what extent these limitations explained the inconsistent findings between *medium* and *low* confidence studies. Overall, the available evidence does not support a consistently inverse association between PFOS and HDL in adults.

Seven *medium* confidence and ten *low* confidence studies examined the association between PFOS and TG or hypertriglyceridemia. All studies examined the cross-sectional association {Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Donat-Vargas, 2019, 5080588; Wang, 2012, 2919184; Lin, 2013, 2850967; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Sun, 2018, 4241053; Canova, 2020, 7021512; Fan, 2020, 7102734; Liu, 2018, 4238396}; three studies additionally examined the association between baseline PFOS and changes in TG or incident hypertriglyceridemia {Liu, 2020, 6318644; Lin, 2019, 5187597; Liu, 2018, 4238396}. Higher PFOS was significantly associated with higher levels of TG in the DPPOS {Lin, 2019, 5187597} (Table C-14). This study also reported that PFOS was associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively; the prospective association was particularly strong in participants enrolled in the placebo arm of the DPPOS {Lin, 2019, 5187597}. In contrast, PFOS was not associated with triglycerides or changes in triglycerides in the POUNDS-Lost study {Liu, 2020, 6318644}. Furthermore, PFOS was inversely associated with TG in the three studies using overlapping NHANES data {Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514} and in Donat-Vargas 2019, 5080588. In this latter study, there was a statistically significant, linear trend of lower TG with increasing PFOS, regardless of whether PFOS was measured concurrently with TG or averaged between baseline and follow-up {Donat-Vargas, 2019, 5080588}. In *low* confidence studies, five reported inverse associations {Lin, 2013, 2850967; Lin, 2020, 6315756; Li, 2020, 6315681; He, 2018, 4238388; Liu, 2018, 4238396}, three reported essentially null associations {Chen, 2019, 5387400; Sun, 2018, 4241053; Canova, 2020, 7021512}, one reported a positive association {Yang, 2018, 4238462}, and one simply stated the association was not statistically significant {Wang, 2012, 2919184}. Altogether, the association between PFOS and TG was inconsistent.

In summary, in the general adult population, the available evidence generally supports positive associations between PFOS and TC and LDL, although some inconsistency exists. The available evidence does not support a consistent association between PFOS and reduced HDL and elevated TG.

3.3.5.1.2.6 Findings from Occupational Studies

Workers are usually exposed to higher levels of PFOS, in a more regular manner, and potentially for a longer duration than adults in the general population. At the same time, workers tend to be healthier than non-workers, which may lead to reduced susceptibility to toxic agents {Shah 2009, 9570930}. Because of these potential differences in exposure characteristics and host

susceptibility, occupational studies are summarized separately from studies among adults in the general population.

Three *low* confidence studies examined the association between PFOS and TC in workers. Of these, two examined the cross-sectional association between PFOS and TC in fluorochemical plant workers or firefighters exposed to aqueous film forming foam (AFFF) {Wang, 2012, 2919184; Rotander, 2015, 3859842}; one investigated the association between baseline PFOS and changes in TC over the course of a fluorochemical plant demolition project {Olsen, 2012, 2919185}. PFOS was positively associated with TC in Rotander 2015, 3859842, but the association was not statistically significant. The other cross-sectional study simply reported no significant association {Wang, 2012, 2919184}. Olsen 2012, 2919185 reported an inverse or positive association between changes in PFOA and changes in TC, depending on whether the outcome was log-transformed {Olsen, 2012, 2919185}. This pattern is unusual and suggests different data subsets may have been used for analyses with and without log-transformed outcome. Taken together, the occupational studies are limited in both quantity and quality. Based on these studies, it is difficult to discern the pattern of association between PFOS and TC in workers.

Two studies examined PFOS and LDL in workers. One study examined PFOS and non-HDL, of which LDL is a major component. All studies were considered *low* confidence. PFOS was positively associated with LDL in Rotander 2015, 3859842, but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found {Wang, 2012, 2919184}. The study examining non-HDL found that changes in PFOS during the fluorochemical plant demolition project were inversely associated with changes in non-HDL, but the association was not statistically significant {Olsen, 2012, 2919185}. Overall, these studies suggest no consistent association between PFOS and elevated LDL in workers.

The studies that examined LDL or non-HDL also examined the association between PFOS and HDL {Wang, 2012, 2919184; Rotander, 2015, 3859842; Olsen, 2012, 2919185}. PFOS was positively associated with HDL in Rotander 2015, 3859842, but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found {Wang, 2012, 2919184}. In Olsen 2012, 2919185, changes in PFOS over the demolition project was positively associated with changes in HDL {Olsen, 2012, 2919185}. Together, the occupational studies suggest a positive association between PFOS and HDL in workers, although these findings were limited by potentially unmeasured confounding {Rotander, 2015, 3859842; Olsen, 2012, 2919185} and self-selection of subjects {Rotander, 2015, 3859842}.

Two *low* confidence cross-sectional studies examined PFOS and TG in workers and found that PFOS was inversely associated with TG in Rotander 2015, 3859842, but this association was not statistically significant. Wang 2012, 2919184 only reported that no significant association was found. Given these limited data, it is not possible to determine the pattern of association between PFOS and TG in workers.

In summary, among workers, a positive association between PFOS and HDL was observed in some studies. There was not a consistent positive association between PFOS and elevated LDL. The evidence is too limited to determine the association between PFOS and TC and TG.

3.3.5.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 3 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 7 studies are shown in Figure 76.

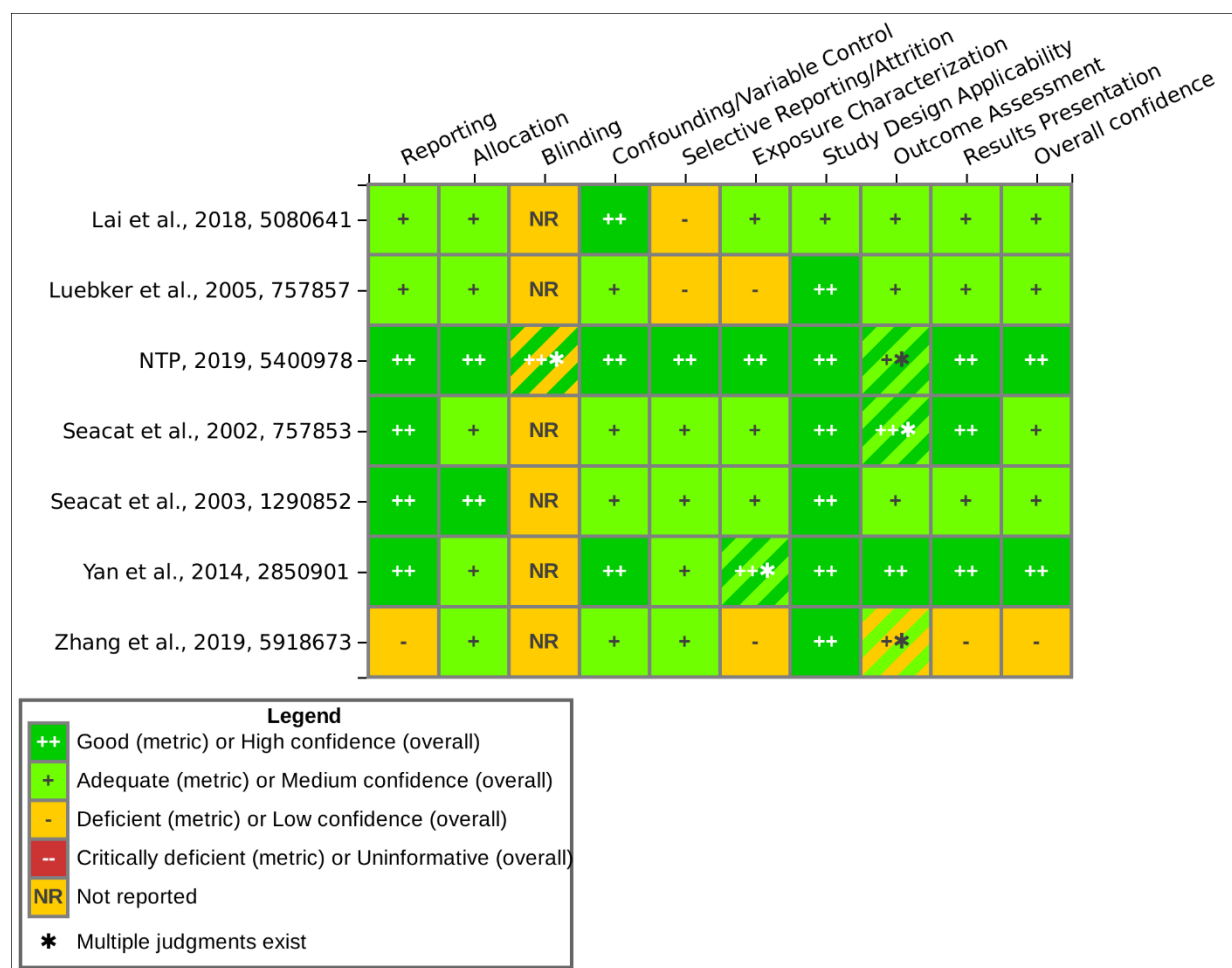


Figure 76. Summary of Study Evaluation for Toxicology Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on [HAWC](#).

Cardiovascular effects, including blood pressure, heart weight, heart histopathology, and serum lipid levels, following exposure to PFOS were minimal {Curran et al., 2008, 757871; NTP, 2019, 5400978; Rogers et al., 2014, 2149155}. In 10–11-week-old Sprague-Dawley rats exposed daily by gavage for 28 days, a decrease in absolute (14% relative to control animals) and relative (9% relative to control animals) heart weight were reported in females exposed to 5 mg/kg/day; no change was observed in male rats. The authors note that the biological significance of this is not clear. No alterations were observed in the heart following histopathological analysis in either sex {NTP, 2019, 5400978}. It should be noted that this study design (e.g., 28-day duration) is not sufficient to address whether PFOS exposure leads to injuries in the cardiovascular system like

plaque formation in atherosclerosis as this often requires 10–12 weeks for development to accurately be evaluated in a rodent model {Daugherty, 2017, 593243}.

Curran et al. (2008, 757871) measured blood pressure in 35–37-day old Sprague-Dawley rats exposed to PFOS in the diet (doses up to approximately 6.34 mg/kg/day for males and 7.58 mg/kg/day for females) for 28 days; no significant change in blood pressure measurements were observed across the groups. Adult Sprague-Dawley offspring of dams treated with PFOS (18.75 mg/kg/day) via oral gavage from GD2–GD6 had increased blood pressure measurements {Rogers, 2014, 2149155}. Male offspring exhibited an 18% and 12% increase in systolic blood pressure at 7 and 52 weeks of age, respectively. Female offspring exhibited a 24% and 19% increase in systolic blood pressure at 37 and 65 weeks of age, respectively; no change in blood pressure was noted at the 7-week timepoint. In male offspring, increased systolic blood pressure was associated with a significantly decreased number of nephrons in the kidney (measurements were taken at PND22; body weights and kidney weights were not significantly different compared to control animals). Rogers et al. (2014, 2149155) discussed that the association is a consequence of a higher load on the available nephrons. The higher load results in a cycle of sclerosis and pressure natriuresis that can increase blood pressure. However, the exact mechanisms have yet to be elucidated.

PFOS has been observed to cause perturbations in lipid homeostasis, which may have effects on the cardiovascular system. Alterations in serum lipid levels have been observed in non-human primates and rodent models in subchronic, chronic, and developmental studies of oral exposure to PFOS (Figure 77). Decreased serum TC, triglycerides, HDL, LDL, and/or VLDL levels occurred in rhesus monkeys {Goldenthal, 1979, 9573133}, cynomolgus monkeys (Seacat et al. 2002, 757853), rats (Seacat et al. 2003, 1290852; Thibodeaux et al. 2003, 5082311; Luebker et al., 2005, 757857; Curran, 2008, 757871; NTP, 2019, 5400978), and mice {Bijland, 2011, 1578502; Wan, 2012, 1332470; Wang, 2014, 2851252; Yan, 2014, 2850901; Lai, 2018, 5080641} following PFOS exposure. In Sprague-Dawley rats exposed daily by gavage for 28 days, significant decreases in serum TC (males) and triglyceride (females) levels were reported following PFOS exposure as low as 0.312 and 2.5 mg/kg/day, respectively {NTP, 2019, 5400978}. Serum triglyceride levels were significantly decreased in female CD-1 mice exposed daily by gavage to 3 mg/kg/day PFOS for 7 weeks {Lai, 2018, 5080641}. One study reported decreased serum HDL levels but an approximate 2-fold increase in serum LDL levels in male BALB/c mice following exposure to 5 mg/kg/day PFOS by gavage for 28 days {Yan, 2014, 2850901}. Conclusions from these studies are met with limitations as the difference in serum lipid composition between humans and commonly used rodent models may impact the relevance to human exposures (Getz et al., 2012, 1065480; Oppi et al., 2019, 5926372). Additionally, food consumption may confound these results, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection.

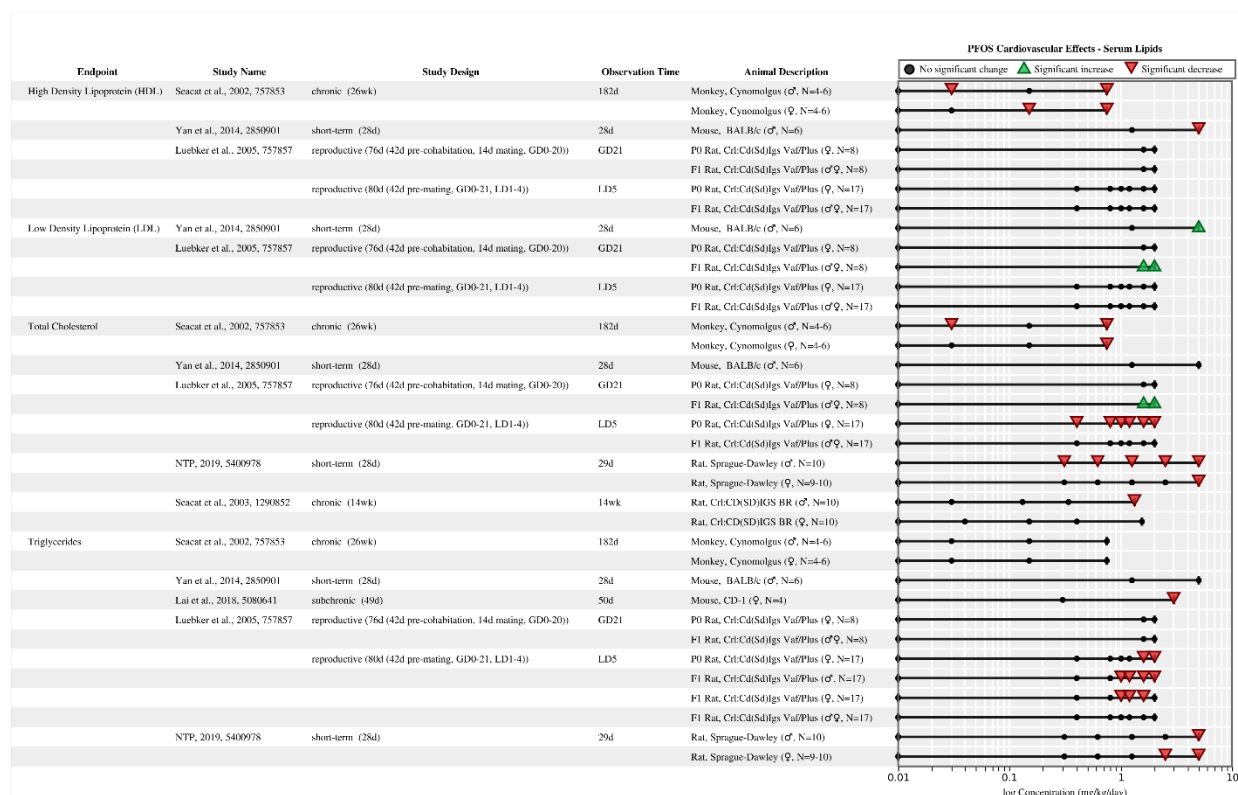


Figure 77. Serum Lipid Levels in Rodents Following Exposure to PFOS

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; P0 = parental generation; PND = postnatal day; PNW = postnatal week; F1 = first generation.

3.3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cardiovascular outcomes is discussed in Section 3.2.6 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 12 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to cardiovascular effects. A summary of these studies is shown in Figure 78.

Additional analysis on the mechanistic actions of PFOS on cardiovascular health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	0	1	0	1
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	0	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	6	7
Cell Signaling Or Signal Transduction	0	0	4	4
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Oxidative Stress	1	2	1	4
Other	1	0	0	1
Grand Total	3	2	7	12

Figure 78. Summary of Mechanistic Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.5.4 Evidence Integration

In summary, the human epidemiological studies identified since the 2016 assessments provided additional clarity on the association between PFOS and CVD. Most of the CVD evidence identified focused on blood pressure in general adult populations (12 studies). The findings from one *high* confidence study and five *medium* confidence studies provided evidence for a positive association between PFOS and blood pressure, although the results were not always consistent between SBP and DBP, and one study reported an inverse association. The limited evidence for an association between PFOS and increased risk of hypertension was inconsistent. There was evidence suggesting an increased risk of hypertension among women, but additional studies are needed to confirm this finding. One *high* confidence study in women with PFOS measured during pregnancy reported a positive association with blood pressure assessed at 3 years post-partum. Evidence in children and adolescents is also less consistent. The six studies available among children and adolescents suggest PFOS was not associated with elevated blood pressure. Evidence for other CVD-related outcomes across all study populations was more limited and inconsistent. The limited evidence for CVD outcomes discussed in the 2016 assessment also indicated association with blood pressure among children.

Based on this systematic review of 42 epidemiologic studies, the available evidence supports a positive association between PFOS and TC in the general population, including children and pregnant women. The available evidence also generally supports a positive association between PFOS and LDL in children and adults in the general population. Although PFOS appeared not associated with elevated TC and LDL in workers, this conclusion is uncertain as the occupational studies included in this review are limited in both quantity and quality. Finally, for all populations, the association between PFOS and HDL and TG were mixed, suggesting no consistent associations between PFOS and reduced HDL and elevated TG. Overall, these findings are largely consistent with the 2016 Health Assessment.

Results are most consistent for TC and LDL in adults, and multiple medium confidence studies reporting a positive association for this outcome are available. These include several large high-quality studies {Canova, 2020, 7021512; Fan, 2020, 7102734; Li, 2020, 6315681; Dong, 2019, 5080195}. From the original assessment, Steenland (2009) remains a strong source of evidence, especially since Canova 2020 is limited to ages 20–39 adults. These studies involved mostly general population-based samples, used serum levels to assess both PFOS and lipid levels, included adjustments for multiple relevant confounders, and involved excellent statistical power. Overall, these studies provide strong evidence that PFOS alters serum lipid levels. As a result of this strong evidence base, the TC endpoint from Dong et al. (2019, 5080195) was considered for the derivation of a POD.

In animal studies, no effects or minimal alterations were noted for blood pressure, heart weight, and histopathology in the heart. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism. Based on known differences between the serum lipid composition in human and animals and a lack of observed effects in other endpoints, no studies or endpoints from the available animal studies were considered for the derivation of PODs.

3.3.6 Endocrine

3.3.6.1 Human Evidence

3.3.6.1.1 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 PFASs 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 (34 publications) report on the association between PFOS exposure and endocrine effects. Six of the publications were studies in pregnant women {Aimuzi, 2020, 6512125; Berg, 2017, 3350759; 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}, six cohort and cross-sectional studies {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 Dugen, 2018, 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 seven studies measured PFOS in maternal blood or serum during pregnancy {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 {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).

3.3.6.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies. First, timing of exposure and hormone concentration measurements was important. Several studies on mother-child dyads examined relationships between maternal serum PFOS measurements and thyroid hormones in both mothers (i.e., a cross-sectional analyses) and in cord blood or children's serum (i.e., a longitudinal analyses). Longitudinal comparisons between maternal PFOS concentrations measured during pregnancy and thyroid hormone levels in cord blood or the child's blood attenuate any concerns for potential reverse causality. Measuring PFOS and thyroid hormone 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.

Of the 34 studies identified since the 2016 assessment, four studies were classified as *high* confidence, 16 as *medium* confidence, 10 as *low* confidence, and 3 studies {Abraham, 2020, 6506041; Predieri, 2015, 3889874} as *uninformative* (Figure 79, Figure 80).

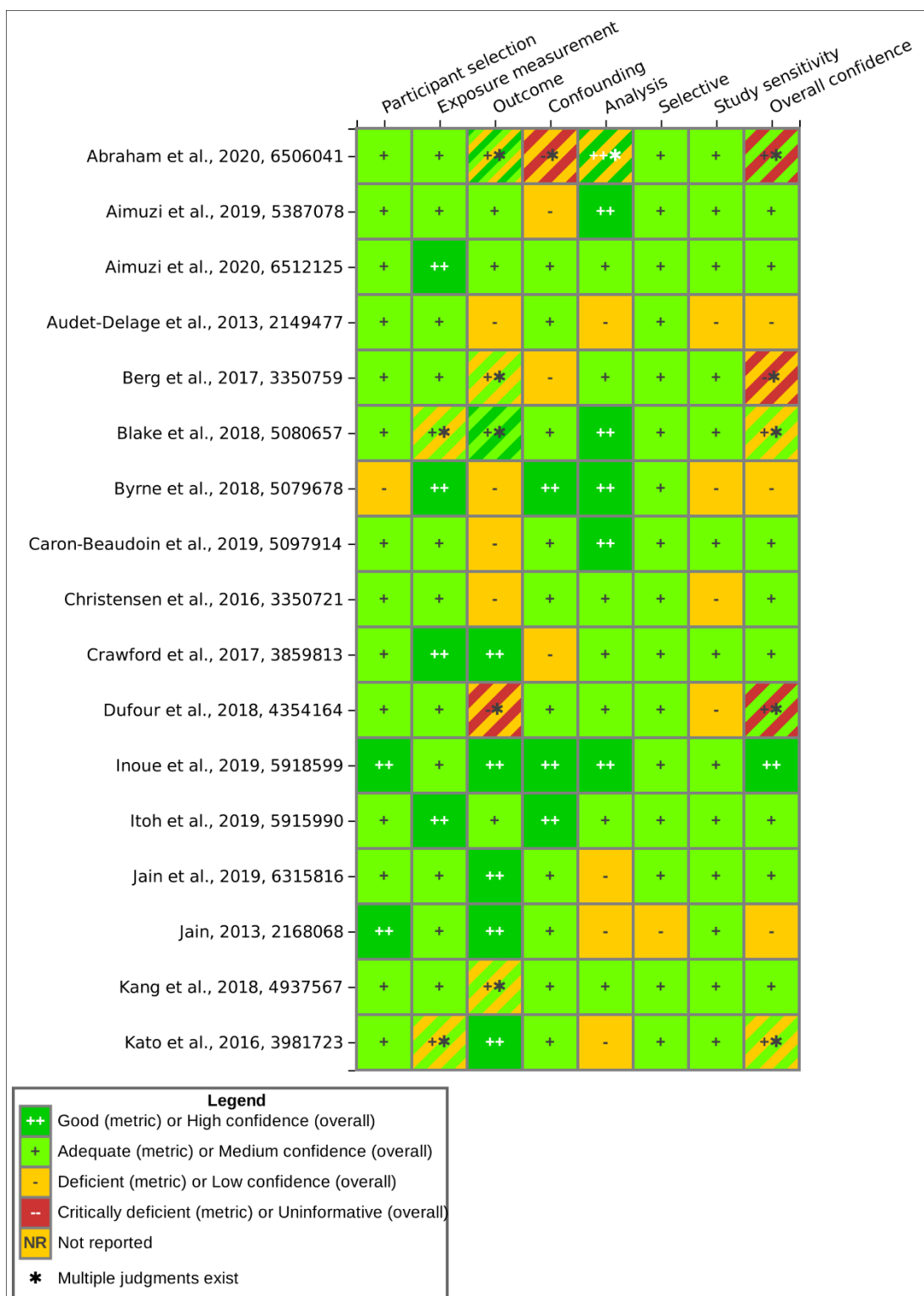


Figure 79. Summary of Study Evaluation for Epidemiology Studies of PFOS and Endocrine Effects

Interactive figure and additional study details available on [HAWC](#).

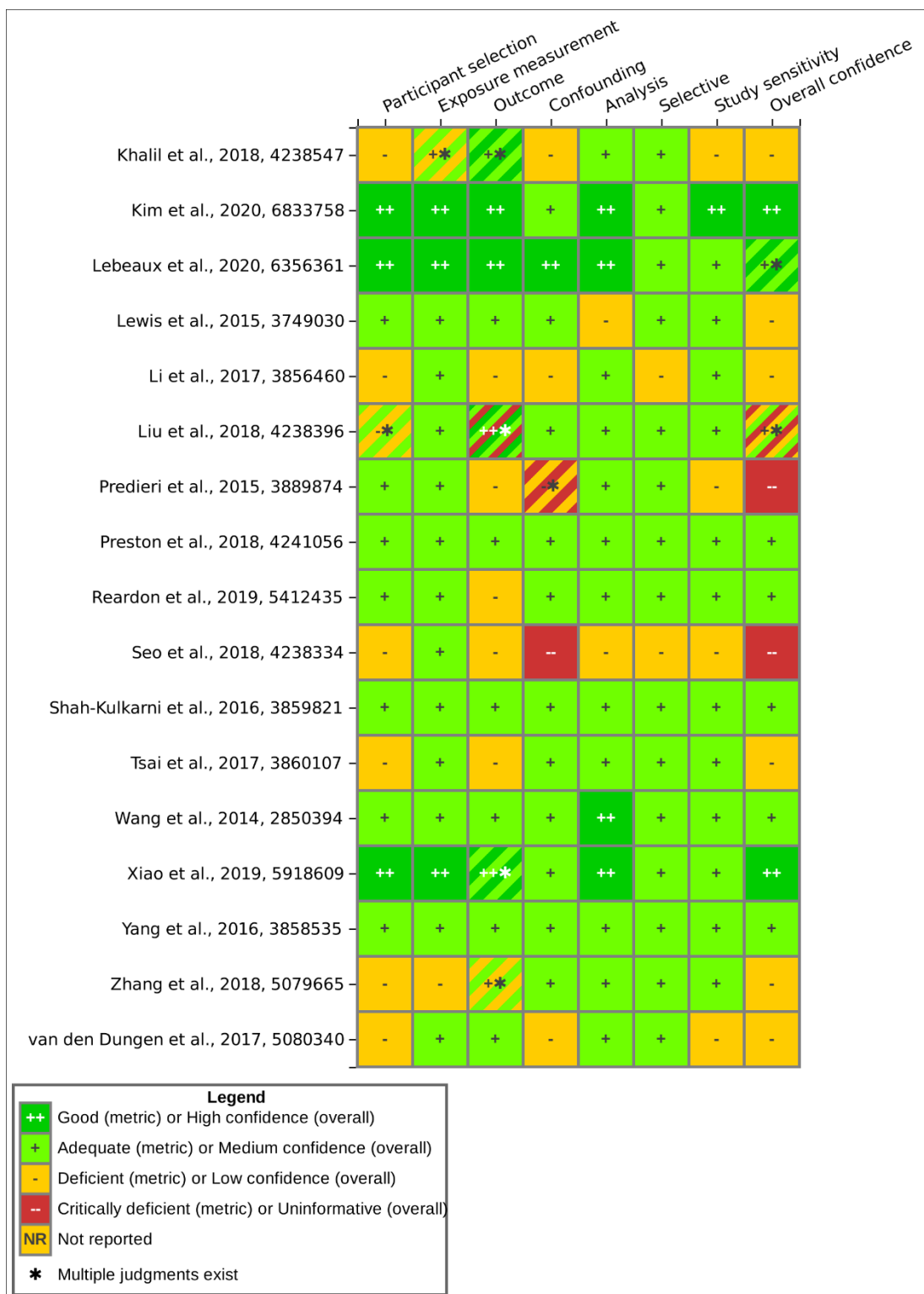


Figure 80. Summary of Study Evaluation for Epidemiology Studies of PFOS and Endocrine Effects (Continued)

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.

3.3.6.1.3 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, 2018, 3860107; Wang, 2014, Xiao, 2019, 5918609; Yang, 2016, 3858535} and five observed significant effects (Table C-15). 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 negative 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 negative 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 quartile to the reference in the overall population. A significant effect was also seen among boys in the second quartile (30–59th) 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.

3.3.6.1.4 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 (Table C-15). 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.

3.3.6.1.5 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; 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 (Table C-15). 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.

3.3.6.2 Animal Evidence

There are 5 studies from the most recent literature search conducted in 2020 and 3 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and endocrine effects. Study quality evaluations for these 8 studies are shown in Figure 81.

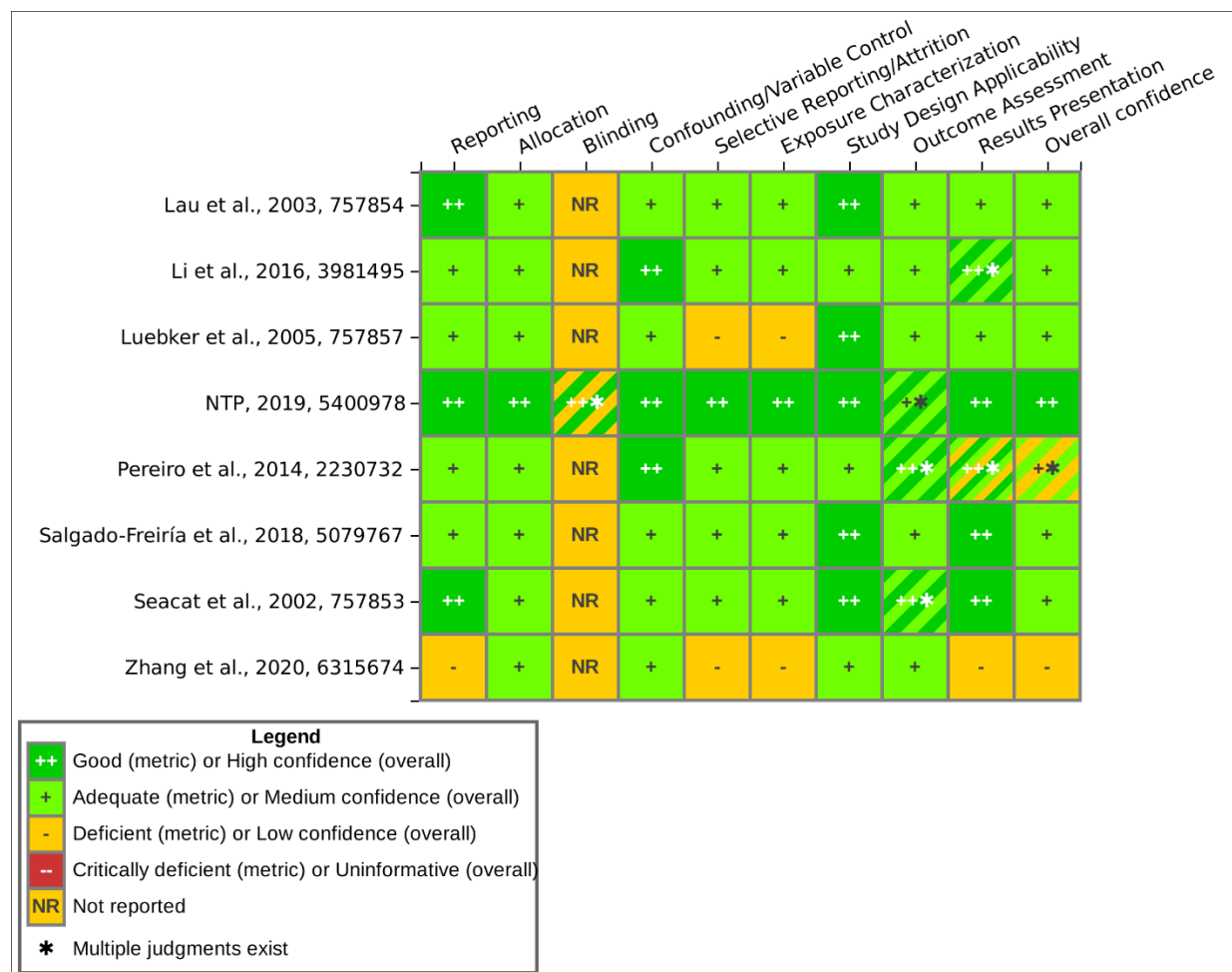


Figure 81. Summary of Study Evaluation for Toxicology Studies of PFOS and Endocrine Effects

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 are described in Section 3.3.17.2. 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 et al., 2005, 757857; NTP, 2019, 5400978; Lau et al., 2003, 757854), as well as reductions in adrenocorticotrophic 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}. Endocrine effects related to reproductive organs and hormone levels are discussed in Section 3.3.2.2.

3.3.6.2.1 Thyroid Hormones Levels

Several 28-day studies provide evidence that exposure to PFOS can result in adverse effects on rat thyroid hormone levels (Table 11). 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, 0.312, 0.625, 1.25, 2.5, 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, 1.7, 5.0, 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 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 F₁ rodents (Table 11). Lau et al. (2003, 757854) analyzed thyroid hormones in offspring of pregnant rats exposed by gavage to PFOS at 0, 1, 2, or 3 mg/kg/day from GD2–GD21. The authors reported statistically significant reductions in TT4 and FT4 on PND5 in rat pups treated with 2 and 3 mg/kg/day during gestation. Signs of recovery in TT4 were noted at weaning, while reduced FT4 persisted through PND35. 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 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 PND21 and PND35. 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 (GD20, PND4, or PND21) following oral gavage exposure of pregnant Sprague-Dawley rats to PFOS (0, 0.1, 0.3, or 1.0 mg/kg/day) from GD0–PND20. 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, 0.8, 1.0, 1.2, 1.6, or 2.0 mg/kg/day for 42 days prior to mating through LD4. 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 0.4-1.6 mg/kg/day (2.0 mg/kg/day group not assessed due to high pup mortality). The authors noted that the contributions of prenatal versus postnatal effects of PFOS on thyroid hormones were not clear {Luebker, 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}.

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, 1, 5, 10, 15, or 20 mg/kg/day PFOS from GD1–GD17 and evaluated TT4 in sera of pooled mouse pups of each sex at several timepoints across postnatal development. Due to mortality in the 15 and 20 mg/kg/day groups, TT4 was only assessed in the 1, 5, and 10 mg/kg/day groups. TT4 levels varied across the different time points with different trends based on treatment group. On PND7, 14, and 28 there was a general trend for decreased TT4 in the 5 and 10 mg/kg/day exposure groups when compared to control animals (Lau et al., 2003, 757854). However, this was not observed on PND3 or PND21. 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, 0.03, 0.15, or 0.75 mg/kg/day for 182 days {Seacat, 2002, 757853}. Recovery animals from the 0, 0.15, 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 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 11. Summary of Results for Thyroid 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 (26wk)	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
Lau et al., 2003, 757854 ^{c,d}	CD-1 Mice	Developmental (GD1-17)	F ₁ Pups (PND28)	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 (28d)	Adult	M	0	6.27 ± 0.92	NA
					2	5.19 ± 1.14	-17.3
					20	1.11 ± 0.32*	-82.3
					50	1.00 ± 0.21*	-84.1
					100	1.03 ± 0.20*	-83.6
				F	0	2.92 ± 1.19	NA
					2	2.51 ± 0.81	-14.1
					20	1.52 ± 0.19*	-48.0
					50	1.17 ± 0.15*	-60.1
					100	1.27 ± 0.36*	-56.5
NTP, 2019, 5400978 ^c	Sprague-Dawley Rats	Short-term (28d)	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

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change
Yu et al., 2009, 757872 ^c	Sprague-Dawley Rats	Subchronic (91d)	Adult	M	2.5	0.35 ± 0.09*	-84.2
					5	0.38 ± 0.05*	-82.8
					0	4.09 ± 0.18	NA
					0.0017	2.39 ± 0.13*	-41.6
					0.005	1.64 ± 0.54*	-59.9
Lau et al., 2003, 757854 ^{c,d}	Sprague-Dawley Rats	Developmental (GD2–21)	F ₁ Adult (PND35)	M/F	0.015	0.85 ± 0.16*	-79.2
					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	CrI:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80d (42d pre-mating, GD0–21, LD1–4))	P ₀ Adult (LD5)	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 (PND5) ^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
					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 (GD0–PND35)	F ₁ Pups (PND14)	M/F	0	6.78 ± 0.35	NA
					3.2 (Gestation Only)	6.36 ± 0.25	-6.2

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change					
				F ₁ Pups (PND21)	M/F	3.2 (Lactation Only)	5.97 ± 0.39	−11.9				
						3.2 (Gestation & Lactation)	4.29 ± 0.17*	−36.7				
						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 (PND35)	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 (28d)	Adult	M	0	0.00253 ± 0.00022
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										
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 (91d)	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					

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change
Lau et al., 2003, 757854 ^{c,d}	Sprague-Dawley Rats	Developmental (GD2–21)	F ₁ Adult (PND35)	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	CrI:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80d (42d pre-mating, GD0–21, LD1–4))	P ₀ Adult (LD5)	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 (PND5)	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	—	—
Free Triiodothyronine (FT3)							
Luebker et al., 2005, 757857 ^b	CrI:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80d (42d pre-mating, GD0–21, LD1–4))	F ₁ Pups (LD5)	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)		
Seacat et al., 2002, 757853 ^b	Cynomolgus Monkey	Chronic (26wk)	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	F	0	0.135 ± 0.031	NA
					0.03	0.12 ± 0.024	−11.1
					0.15	0.097 ± 0.008*	−28.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change
Curran et al., 2008, 757871 ^b	Sprague-Dawley Rats	Short-term (28d)	Adult	M	0.75	0.085 ± 0.012*	-37.0
					0	10.39 ± 2.14	NA
					2	11.75 ± 1.23	13.1
					20	8.83 ± 1.69	-15.0
					50	8.38 ± 8.38	-19.4
					100	7.86 ± 1.49*	-24.4
				F	0	11.88 ± 1.10	NA
					2	11.17 ± 0.91	-6.0
					20	11.36 ± 1.75	-4.4
					50	9.15 ± 1.43*	-23.0
					100	8.25 ± 1.30*	-30.6
NTP, 2019, 5400978 ^c	Sprague-Dawley Rats	Short-term (28d)	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 (91d)	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 (GD2–21)	F ₁ Adult (PND35)	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 (80d (42d pre-mating, GD0–21,	P ₀ Adult (LD5)	F	0.0	0.0760 ± 0.0185	NA
					0.4	0.0729 ± 0.0135	-4.1
					0.8	0.0638 ± 0.00668	-16.1

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change	
		LD1–4))			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	
				F ₁ Pups (PND5) ^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 (PND5) ^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 (GD0–PND35)	F ₁ Pups (PND14)	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 (PND21)	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 (PND35)	M/F	0	0.059 ± 0.003	NA	
					3.2 (Gestation Only)	0.052 ± 0.003	–11.9	

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change		
Yu et al., 2009, 757880 ^{c,h}	Wistar Rats	Reproductive (GD0–PND35)	F ₁ Pups (PND14)	M/F	3.2 (Lactation Only)	0.049 ± 0.004	−16.9		
					3.2 (Gestation & Lactation)	0.055 ± 0.002	−6.8		
			Reverse Triiodothyronine (rT3)						
			F ₁ Pups (PND21)	M/F	0	—	—		
					3.2 (Gestation Only)	—	—		
					3.2 (Lactation Only)	—	—		
					3.2 (Gestation & Lactation)	—	—		
					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 (PND35)	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 (26wk)	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		

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change
NTP, 2019, 5400978 ^c	Sprague-Dawley Rats	Short-term (28d)	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
					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 (91d)	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 (GD0–PND20)	P ₀ Adult (GD20)	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 (PND4)	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 (PND21)	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 (PND21)	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 (PND21)	F	0	0.880 ± 0.06	NA
					0.1	0.889 ± 0.074	1.0

Study Name	Species	Study Design	Life Stage	Sex	Dose (mg/kg/day)	Value (µg/dL) ^a	Percent Change
Lau et al., 2003, 757854 ^{c,d}	Sprague-Dawley Rats	Developmental (GD2–21)	F ₁ Pups (GD20)	M/F	0.3	0.865 ± 0.07	–1.7
					1	0.840 ± 0.065	–4.5
					0	1.212 ± 0.134	NA
					0.1	1.053 ± 0.08	–13.1
					0.3	0.934 ± 0.075	–22.9
					1	0.969 ± 0.075	–20.0
			F ₁ Pups (PND4)	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
					0	0.62 ± 0.08	NA
			F ₁ Adult (PND35)	M/F	1	0.73 ± 0.16	17.7
					2	0.65 ± 0.06	4.8
					3	0.29 ± 0.02	–53.2
					0.0	0.163 ± 0.096	NA
Luebker et al., 2005, 757857 ^b	CrI:CD®(SD)IGS VAF/Plus® Rats	Reproductive (80d (42d pre-mating, GD0–21, LD1–4))	P ₀ Adult (LD5)	F	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 (PND5)	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

M = male; F = female; NA = not applicable; GD = gestation day; PND = postnatal day; LD = lactation day; F1 = first generation; P0 = parental generation.

*Statistically significant at $p \leq 0.05$.

^a Values were converted to µg/dL for Seacat et al., 2002, 757853 (ng/dL TT3; 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).

^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 $\mu\text{U/mL}$.

3.3.6.2.2 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 82). 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, 0.5, 3.0, 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, 5, and 20 mg/kg/day from GD12–GD18 via gavage {Li, 2016, 3981495}. Fetal serum corticosterone levels were significantly increased in animals treated with 5 and 20 mg/kg/day.

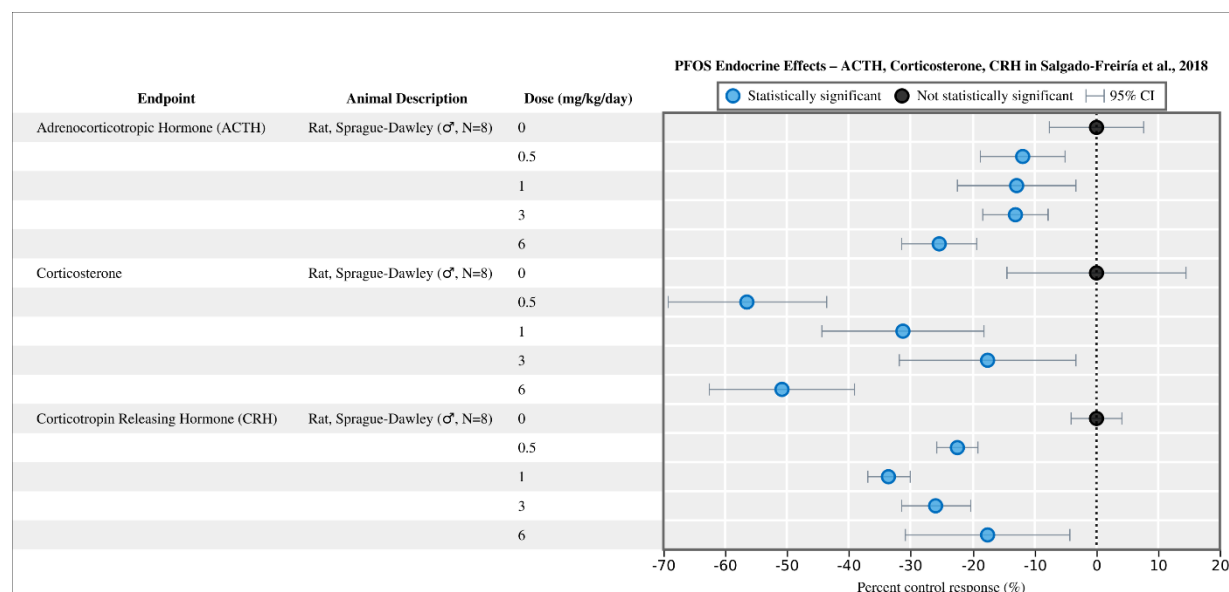


Figure 82. Percent Change in Adrenal Hormones Relative to Controls in Male Rats Following 28-Day Exposure to PFOS, as Reported by Salgado-Freiría et al. (2018, 5079767)^a

Interactive figure and additional study details available on [HAWC](#).

ACTH = adrenocorticotrophic hormone; CRH = corticotropin releasing hormone; CI = confidence interval.

^aAn additional publication (Pereiro et al., 2014, 2230732) reported on the same data as Salgado-Freiría et al., 2018, 5079767 and is not shown in the figure.

3.3.6.2.3 Organ Weights

No adverse effects on male and female thyroid weights (Table 12) 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, 1.7, 5.0, or 15 mg/L for 91 days {Yu, 2009, 757872}.

PFOS exposure was associated with changes in adrenal gland weights in rats and non-human primates (Table 12). 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 0–5 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}. Effects on the relative weight of the hypothalamus were observed by Salgado et al. (2015, 3981583) and are discussed in Section 3.3.8.2.

Seacat et al. (2002, 757853) measured absolute and relative adrenal and thyroid/parathyroid weights in male cynomolgus monkeys exposed to PFOS at doses of 0, 0.03, 0.15, 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.

Table 12. 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.
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.
	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.

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
Thyroid Weight, 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.
	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.

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

3.3.6.2.4 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 GD0–PND20 (Chang et al., 2009, 757876). On GD20, 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 GD20 or in neonates at PND4 or PND21 {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, 0.5, 1.0, 3.0 and 6.0 mg/kg/day for 28 days. Fasciculated 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, 0.03, 0.15, or 0.75 mg/kg/day for 182 days, no treatment related effect on cell proliferation of the pancreas was observed {Seacat, 2002, 757853}.

3.3.6.3 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 36 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to endocrine effects. A summary of these studies is shown in Figure 83. Additional analysis on the mechanistic actions of PFOS on endocrine health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	2	14	16
Cell Signaling Or Signal Transduction	2	12	13
Extracellular Matrix Or Molecules	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	4	5
Hormone Function	11	14	24
Inflammation And Immune Response	0	1	1
Oxidative Stress	3	0	3
Xenobiotic Metabolism	1	3	4
Other	0	1	1
Not Specified (Review Article)	1	0	1
Grand Total	13	25	36

Figure 83. Summary of Mechanistic Studies of PFOS and Endocrine Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.6.4 Evidence Integration

In summary, evidence from human epidemiological studies was inconsistent regarding associations between PFOS exposure and endocrine outcomes. The 2016 HESD found two studies supporting positive associations with thyroid disease in multiple cycles of NHANES participants. Since the 2016 Health Assessment, 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} found evidence of 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*. Interestingly, two general population studies identified seemingly sexually dimorphic effects for TSH {Blake, 2019, 5080657} and T3 {Byrne, 2019, 5079678}. Regarding pregnant women, the 2016 Health Assessment found three studies reporting positive associations between serum PFOS and TSH. In contrast, only one *medium* confidence study found a positive association while there was inconsistent evidence among *low* 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, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), PFHxS, and PFDoA) and found them to be

moderately correlated ($r = 0.37\text{--}82$). Results for PFOS were not significant, however, the direction and magnitude of effect were similar in single-pollutant and multi-pollutant models.

Findings on associations between PFOS and endocrine outcomes were inconsistent among *high* and *medium* confidence studies. No studies or endpoints from the available epidemiological literature were considered for the derivation of PODs.

Several studies in animal models provide further evidence suggesting that the endocrine system is a target of PFOS exposure. Decreases in free and TT4 and TT3 were observed in rats 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. EPA identified NTP, 2019, 5400978 and Seacat et al., 2002, 757853 as studies to be considered for the derivation of PODs due to dose-dependent decreases observed in T3 and T4 among each study. More specifically, the endpoints of TT4, FT4, and TT3 in male and female rats were selected from the NTP (2019, 5400978) study because these were the most sensitive (significant effects at the lowest dose tested, 0.325 mg/kg/day) and displayed the greatest magnitude of change (reductions up to 87%). Dose-dependent reductions in TT3 were observed by Seacat et al. (2002, 757853) in male and female cynomolgus monkeys in the only available chronic study and was therefore considered for the derivation of PODs.

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. Studies or endpoints related to adrenocortical hormones from the available animal studies were not considered for the derivation of PODs.

3.3.7 Metabolic/Systemic

3.3.7.1 Human Evidence

3.3.7.1.1 Introduction

Diabetes is a category of diseases caused by either insulin resistance or beta-cell dysfunction, or both. Type 1 diabetes is characterized by insulin deficiency and beta-cell destruction, while type 2 diabetes is characterized by beta-cell dysfunction 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) $\geq 6.5\%$, 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 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 et al., 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, BMI or ponderal index, body weight, gestational weight gain, body fat, and anthropometric measurements. Details of each study can be found in Table C-16.

3.3.7.1.2 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, 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.

Based on these considerations, 10 studies were classified as *high* confidence, 36 as *medium* and 14 as *low* confidence for all metabolic outcomes. Eight studies have split ratings and were classified as *medium* confidence for one outcome and *low* confidence for other outcomes), and 4

were considered *uninformative*. One study {Liu, 2018, 4238396} was considered *uninformative* for insulin resistance and *medium* confidence for other metabolic outcomes (Figure 84, Figure 85, Figure 86).

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 et al., 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; Heffernan, 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.

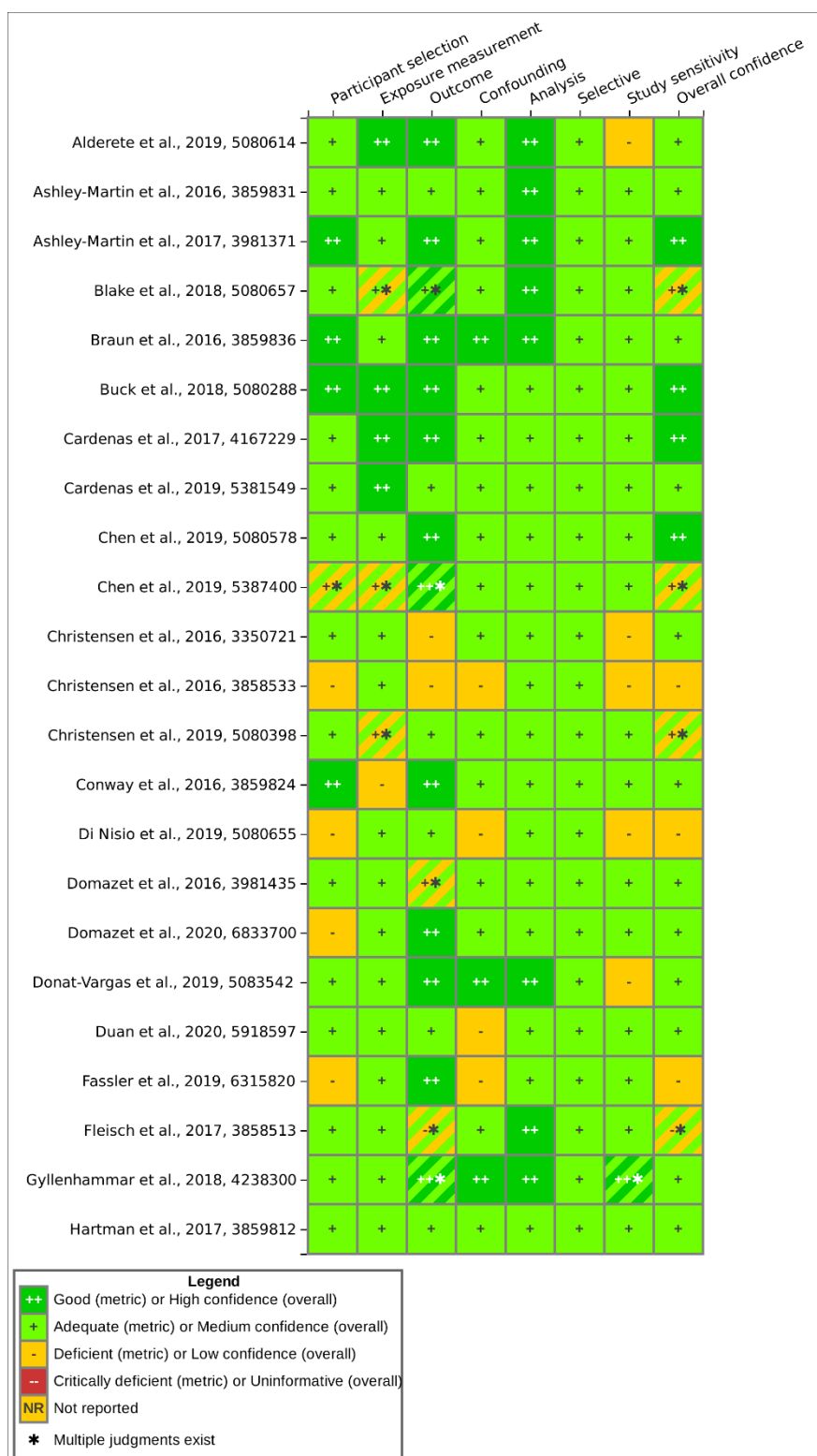


Figure 84. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic Effects

Interactive figure and additional study details available on [HAWC](#).



Figure 85. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic Effects (Continued)

Interactive figure and additional study details available on [HAWC](#).

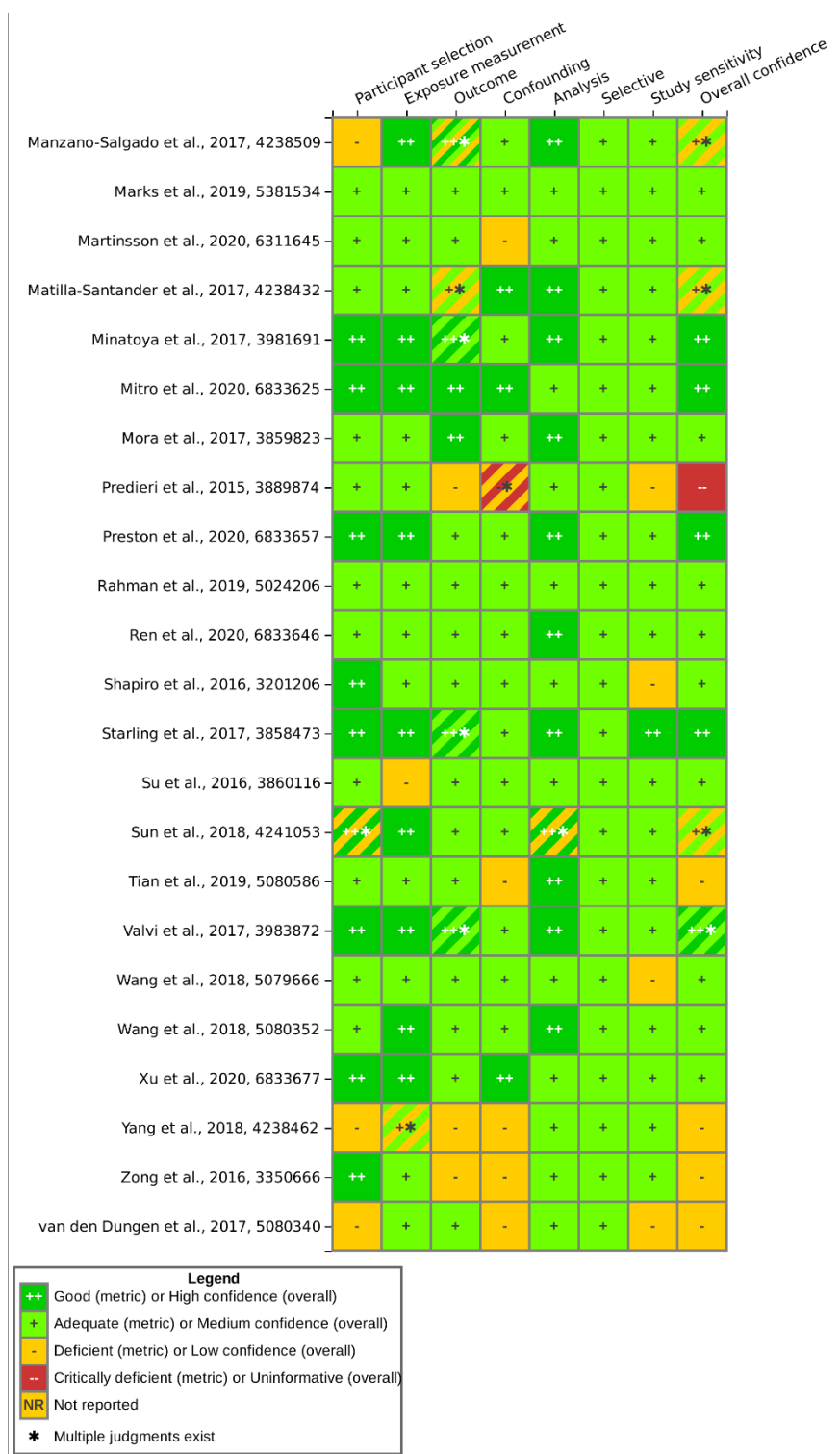


Figure 86. Summary of Study Evaluation for Epidemiology Studies of PFOS and Metabolic Effects (Continued)

Interactive figure and additional study details available on [HAWC](#).

3.3.7.1.3 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}. (Table C-16).

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 homeostasis model assessment of beta-cell function (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 et al., 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 et al., 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 Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study, Gyllenhammar et al. (2018, 4238300) observed a positive, significant association with BMI z-score among 4- and 5-years old children; 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 et al., 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 years 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}.

3.3.7.1.4 Findings from Pregnant Women

Ten studies examined diabetes or gestational diabetes and overall results were mixed, with no significant associations (Table C-16).

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* confidence study observed an increased, non-significant risk of gestational diabetes among women with a family history of type 2 diabetes and women who 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 negative, non-significant associations with gestational diabetes {Xu, 2020, 6833677; Wang, 2018, 5079666; Valvi, 2017, 3983872; Zong, 2016, 3350666; Shapiro, 2016, 3201206}. Shapiro et al. (2016, 3201206) observed non-significant, decreased 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 et al., 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}.

3.3.7.1.5 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 et al., 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} (Table C-16).

Three *low* confidence studies reported non-significant positive associations with diabetes {Lind, 2014, 2215376; Christensen, 2016, 3858533; He, 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 et al., 2018), while another reported lower non-significant odds of blood glucose ≥ 1.6 mmol/L for participants with serum n-PFOS > 3 ng/mL compared with those with serum n-PFOS ≤ 3 ng/mL {Yang, 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 50803542 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 et al., 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 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}.

3.3.7.1.6 Findings from Occupational Studies

No occupational studies examined metabolic outcomes and PFOS.

3.3.7.2 Animal Evidence

3.3.7.2.1 Metabolic Homeostasis

There are 2 studies from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and metabolic homeostasis. Study quality evaluations for these 4 studies are shown in Figure 87.

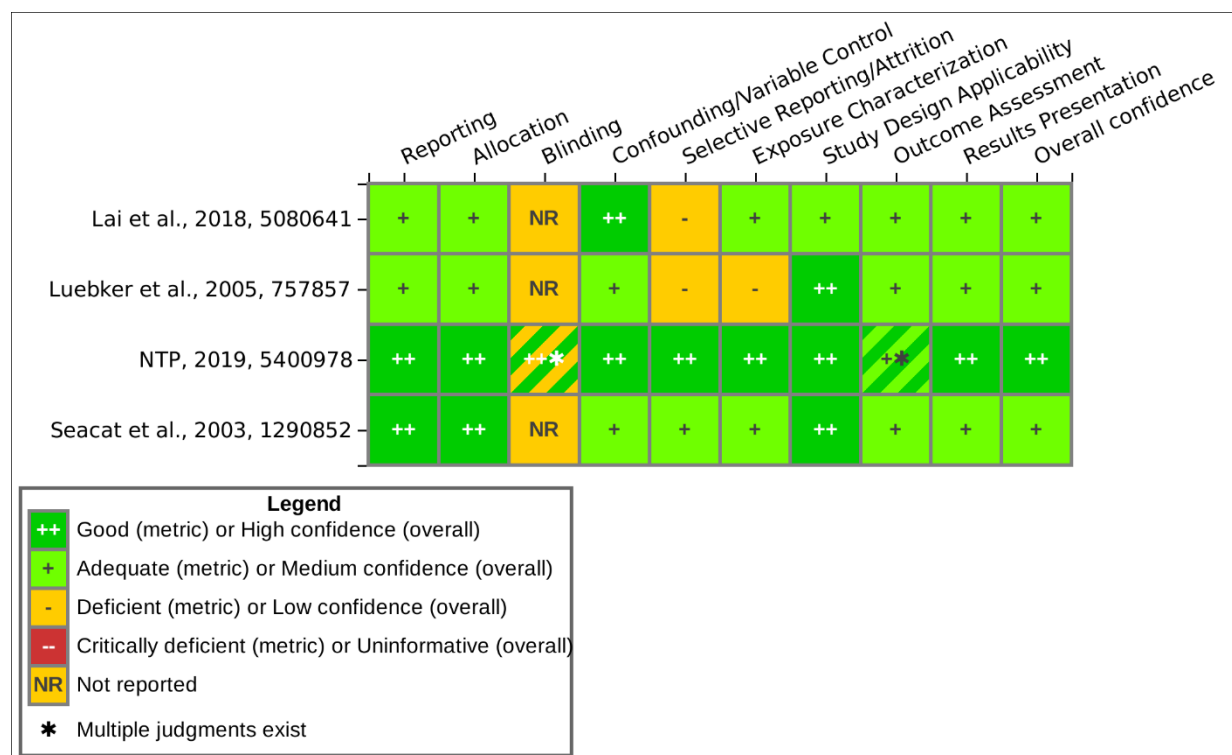


Figure 87. Summary of Study Evaluation for Toxicology Studies of PFOS and Metabolic Effects

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 of 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 GD21. Serum glucose levels were not significantly altered in fetuses at GD21 or in pups at LD5. 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 GD0–PND20 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, 0.3, or 3 mg/kg/day PFOS from GD3–PND21. Offspring were then fed either a standard or high-fat diet from PND21–PND63. At PND21, 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 PND21, though males from both the 0.3 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 PND21. In pups fed a standard diet, at PND63, 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 PND63 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 PNW6, PNW11, or PNW20 in wild-type or tumorigenic transgenic C57BL/6J-*Min*/+ mice offspring gestationally exposed to 0, 0.01, 0.1, or 3 mg/kg/day PFOS from GD1–GD18, 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 and 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 and 3 mg/kg/day dose groups which could indicate reduced gluconeogenesis.

3.3.7.2.2 Survival, Clinical Observations, Body Weight, and Food Consumption

There are 13 studies from the most recent literature search conducted in 2020 and 5 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association

between PFOS and systemic effects. Study quality evaluations for these 18 studies are shown in Figure 88.

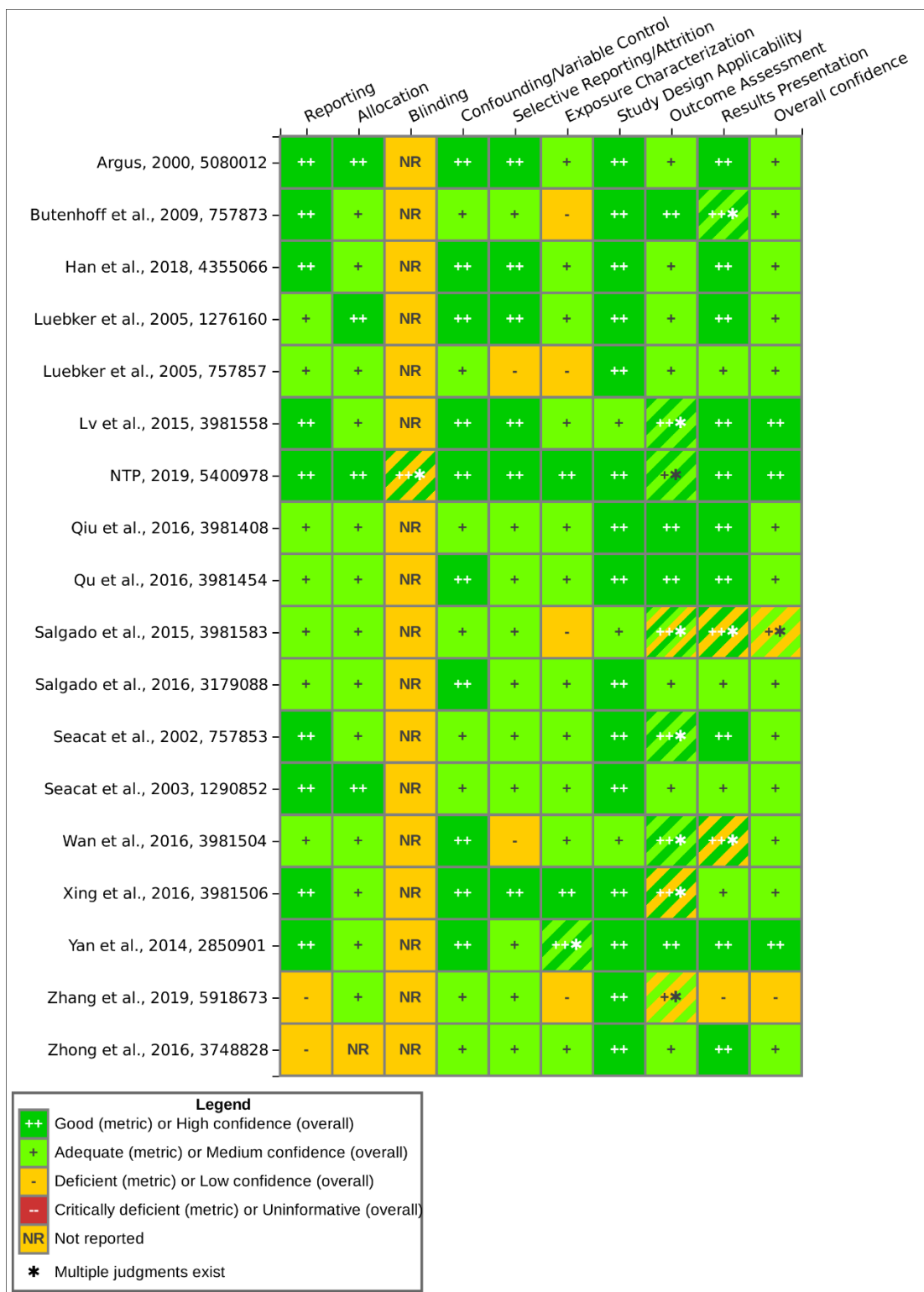


Figure 88. Summary of Study Evaluation for Toxicology Studies of PFOS and Systemic

Effects

Interactive figure and additional study details available on [HAWC](#).

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.

3.3.7.2.2.1 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 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 42 days. No mortality was observed in C57 male mice exposed to 0.5 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, Xing et al. (2016, 3981506) did not observe an effect on mortality in C57BL/6J male mice exposed to PFOS at 2.5, 5, 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 P₀ male Crl:CD(SD)lgS rats following 6 weeks of pre-mating exposure to 1.6, 2.0, or 3.2 mg/kg/day {Luebker, 2005, 1276160}. No mortality was observed in the P₀ 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}. Changes in the P₀ females during gestation and lactation and fetal mortality in offspring following perinatal exposure are discussed in Section 3.3.1.2.

3.3.7.2.2.2 Body Weight in Adults

Many studies with rodent models report reductions in body weight following short term to subchronic PFOS exposure (Figure 89). A dose-dependent reduction in body weight change was observed in C57BL/6J male mice exposed to PFOS at 2.5, 5, 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, 0.5, 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, 5, 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 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 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)Igs 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 2.0 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, 0.5, 2, 5, or 20 ppm (equivalent to 0, 0.05, 0.18, 0.37, or 1.51 mg/kg in males and 0, 0.05, 0.22, 0.47, 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, 0.03, 0.15, or 0.75 mg/kg/day PFOS (equivalent to cumulative doses of 0, 4.6, 22.9, 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, 0.5, 2, 5, and 20 ppm (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) 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}.

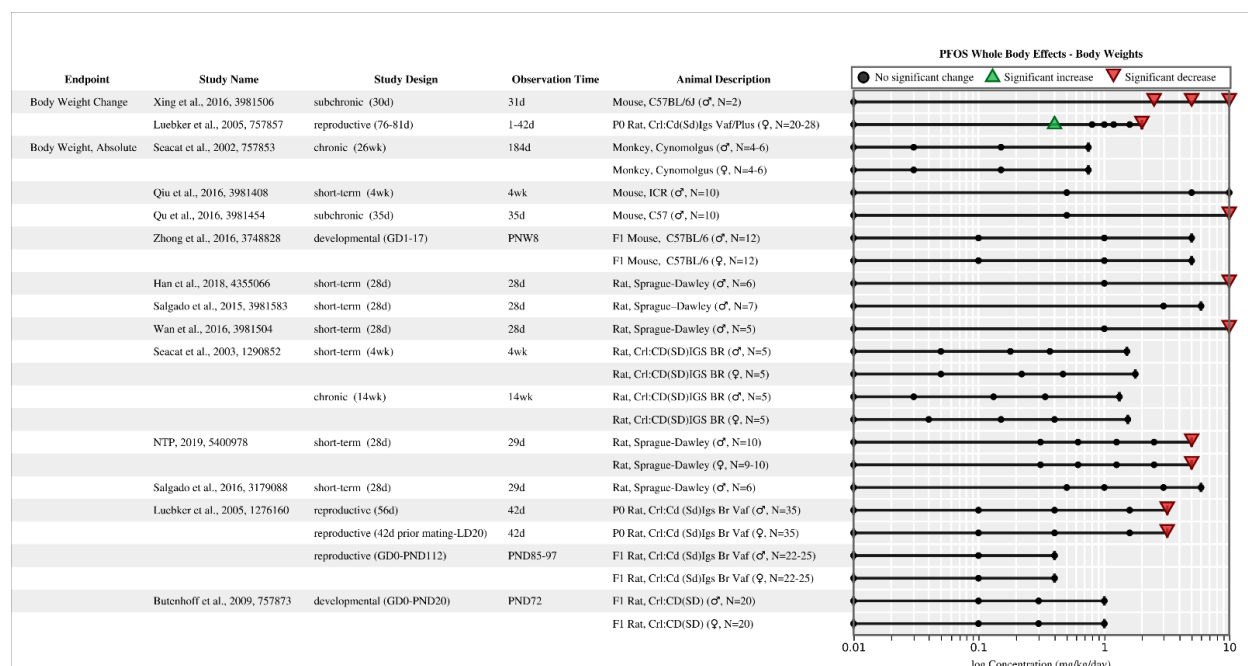


Figure 89. Effects on Body Weight in Rodents and Non-Human Primates Following Exposure to PFOS (logarithmic scale)

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.

3.3.7.2.2.3 Body Weight in Adults Following Developmental Exposure

Offspring body weights during developmental periods have been reported and are described in detail in Section 3.3.1.2. However, the effects on body weight may not persist into adulthood. No change was observed in adult body weight (PND85–PND97) compared to control in male and female Crl:CD(SD)Igs Br Vaf rats exposed perinatally through adulthood to 0.1 and 0.4 mg/kg/day PFOS {Luebker, 2005, 1276160}. Developmental (GD1–GD17) PFOS exposure in C57BL/6 mice at 0.1, 1, 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 PND70 were not statistically different from controls in the offspring of female Sprague-Dawley rats exposed to 0, 0.1, 0.3, or 1 mg/kg/day PFOA from GD0–PND20 {Butenhoff, 2009, 757873}.

3.3.7.2.2.4 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)Igs Br Vaf rats following a 6 week exposure via gavage to 1.6 or 3.2 mg/kg/day {Luebker, 2005, 1276160}, and in female Crl:CD(Sd)Igs Vaf/Plus rats following a 6 week exposure to 2.0 mg/kg/day {Luebker, 2005, 757857} (Section 3.3.1.2).

Food and water consumption was not observed to be affected in Sprague Dawley rats exposed to PFOS via gavage at doses of 1 or 10 mg/kg/day {Han, 2003, 4355066}, 3 or 6 mg/kg/day {Salgado, 2015, 3981583}, nor 0.5, 1, 3, or 6 mg/kg/day {Salgado, 2016, 3179088} for 28 days. Seacat et al. (2003, 1290852) fed CrI: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.

3.3.7.3 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 31 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to metabolic effects. A summary of these studies is shown in Figure 90. Additional analysis on the mechanistic actions of PFOS on metabolic health outcomes is pending and is expected to be completed after the EPA SAB review.

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	6	1	8	14
Hormone Function	1	4	4	9
Oxidative Stress	2	1	2	5
Xenobiotic Metabolism	0	0	2	2
Other	2	0	0	2
Not Specified (Review Article)	1	0	0	1
Grand Total	10	7	16	31

Figure 90. Summary of Mechanistic Studies of PFOS and Metabolic Effects

Interactive figure and additional study details available on [Tableau](#).

Mechanistic evidence linking PFOS exposure to adverse systemic outcomes is discussed in Section 3.3.4 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 31 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to systemic effects. A summary of these studies is shown in Figure 91.

Additional analysis on the mechanistic actions of PFOS on systemic health outcomes is pending and is expected to be completed after the EPA SAB review.

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	3	1	5	9
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	5	1	6	10
Hormone Function	1	0	0	1
Inflammation And Immune Response	0	0	2	2
Oxidative Stress	6	1	6	12
Xenobiotic Metabolism	4	1	1	5
Other	0	0	4	4
Not Specified (Review Article)	1	0	0	1
Grand Total	10	3	21	31

Figure 91. Summary of Mechanistic Studies of PFOS and Systemic Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.7.4 Evidence Integration

The 2016 EPA Health Advisory did not report associations between PFOS and metabolic health outcomes. In this review of 69 human epidemiological studies, evidence for any association with PFOS and metabolic outcomes was inconsistent, but suggestive evidence was observed for diabetes, gestational weight gain, HOMA-IR, HOMA-B, leptin, and adiponectin.

The 2016 EPA Health Assessment for PFOS concluded that there is no evidence of an association for diabetes in humans. More recent evidence suggests a possible association between PFOS and diabetes, including gestational diabetes. Since the 2016 Health Advisory, 21 studies were identified that examine diabetes in the general population, 10 of which examined gestational diabetes. No studies examining diabetes among children were identified. Of the 10 studies on gestational diabetes, non-significant positive associations were observed in 5 of them {Preston, 2020, 6833657; Rahman, 2019, 5024206; Wang, 2018, 5080352; Liu, 2019, 5881135; Matilla-Santander, 2017, 4238432}. Eleven studies evaluated diabetes in the general population, six of which reported a positive association {Sun, 2018, 4241053; Su, 2016, 3860116; Mancini, 2018, 5079710; Lind, 2014, 2215376; Christensen, 2016, 3858533; He, 4238388}. Sun et al. (2018, 4241053) reported significantly increased odds of type 2 diabetes for plasma PFOS levels

between 26.3–421 ng/mL among 1586 female nurses. Among Taiwanese adults aged 20–60, a significant positive association was observed between PFOS and type 2 diabetes {Su, 2016, 3860116}. Although the 2016 EPA Health Advisory for PFOS concluded that there is no evidence of an association for diabetes, new evidence suggests a possible association between PFOS and diabetes.

Three epidemiological studies observed positive associations with gestational weight gain among pregnant women, with one association being significantly significant. Two studies observed that the risk of increased gestational weight gain is greater among normal-weight women than overweight women. Jaacks et al. (2016, 3981711) reported a positive, non-significant association with gestational weight gain among mothers with a BMI < 25, but a negative association was reported among mothers with a BMI ≥ 25. Another study observed a significant positive association between PFOS and gestational weight gain among under- or normal weight mothers, and a non-significant positive association among mothers who are overweight or obese {Marks, 2019, 5381534}. While this evidence suggests a potential association with gestational weight gain, more evidence is needed.

Since 2016, 15 human epidemiological studies examining HOMA-IR were identified; six studies were on children, two were on pregnant women, and seven were on the general population. Seven studies reported non-significant positive associations with HOMA-IR in pregnant women {Jensen, 2018, 4354143; Wang, 2018, 5079666} and in general populations {Liu, 2018, 4238514; Donat-Vargas, 2019, 5083542; Lind, 2014, 2215376; Chen, 2019, 5387400; Lin, 2013, 2850967}. Additionally, Cardenas et al. (2017, 4167229) reported a significant positive association with HOMA-IR among 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 {Domazet, 2016, 3981435}. Overall, there is evidence of an association between PFOS and HOMA-IR among adults, but not among children.

Six human epidemiological studies examining HOMA-B were identified since the 2016 Health Advisory, four of which reported positive associations with HOMA-B. Among children, one study reported a negative, non-significant association with HOMA-B {Domazet, 2016, 3981435}, while the single study in pregnant women reported a non-significant positive association {Jensen, 2018, 4354143}. Of the four studies on HOMA-B in the general population, three observed positive associations {Cardenas, 2017, 4167229; Liu, 2018, 4238514; Chen, 2019, 5387400}. Cardenas et al. (2017, 4167229) reported a significant positive association with beta-cell function among 956 adults at high risk for type 2 diabetes. These studies suggest a possible association between PFOS and HOMA-B, but more evidence is needed.

Evidence from the human epidemiological studies identified since the 2016 Health Advisory suggest a potential association between PFOS and adiponectin among children. Four studies reported positive associations with adiponectin in children {Minatoya, 2017, 3981691; Buck et al., 2018, 5080288; Domazet, 2020, 6833700; Fleisch, 2017, 3858513}. Minatoya et al. (2017, 3981691) reported a significant positive association between maternal PFOS serum levels and total adiponectin in cord blood {Minatoya, 2017, 3981691}. Results among adults were mixed; two non-significant positive associations {Ashley-Martin, 2017, 3981371; Buck, 2018, 5080288} and three non-significant negative associations {Mitro, 2020, 6833625; Cardenas, 2017, 4167229; Sun, 2018, 4241053} with adiponectin were observed in pregnant women {Mitro, 2020, 6833625; Ashley-Martin, 2017, 3981371} and the general population {Buck,

2018, 5080288; Ashley-Martin, 2017, 3981371; Cardenas, 2017, 4167229; Sun, 2018, 4241053}. Overall, this evidence suggests a potential association between PFOS and adiponectin in children, but not adults.

No association with metabolic syndrome in humans was reported in the 2016 Health Advisory. Findings were mixed among the four general population epidemiological studies identified since 2016: two reported negative associations with metabolic syndrome, and two reported positive associations. Based on these studies, there is no evidence to suggest an association between PFOS and metabolic syndrome.

Findings on associations between PFOS and metabolic outcomes were inconsistent among *high* and *medium* confidence studies. No studies or endpoints from the available epidemiological literature were considered for the derivation of PODs.

Similarly, though some alterations related to glucose homeostasis were reported in the available animal toxicity literature, the results are inconclusive as there are too few studies to assess possible difference across life stages, sexes, and species. 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) 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 PNW15 in this study. In addition, Wan et al. (2014, 2850405) did not report significantly altered results of the glucose tolerance test at PND63 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. No studies or endpoints related to metabolic effects from the available animal studies were considered for the derivation of PODs.

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. Relative to other endpoints, body weight may not be a sensitive indicator of PFOS toxicity. No studies or endpoints related to systemic effects from the available animal studies were considered for the derivation of PODs.

3.3.8 Nervous

3.3.8.1 Human Evidence

3.3.8.1.1 Introduction

The 2016 Health Assessment (U.S. EPA, 2016, 3603279) 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 and Olsen, 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; and Shin, 2020, 6507470), 2 nested case-control (Liew, 2015, 2851010; Lyall, 2018, 4239287), 26 cohort, and 5 cross-sectional studies (Table C-17). 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 (Table C-17). 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.

3.3.8.1.2 Study Quality

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, twenty-eight studies were classified as *medium* confidence, and five were *low* confidence (Figure 92, Figure 93). 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, 2016 (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, 2014 (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.

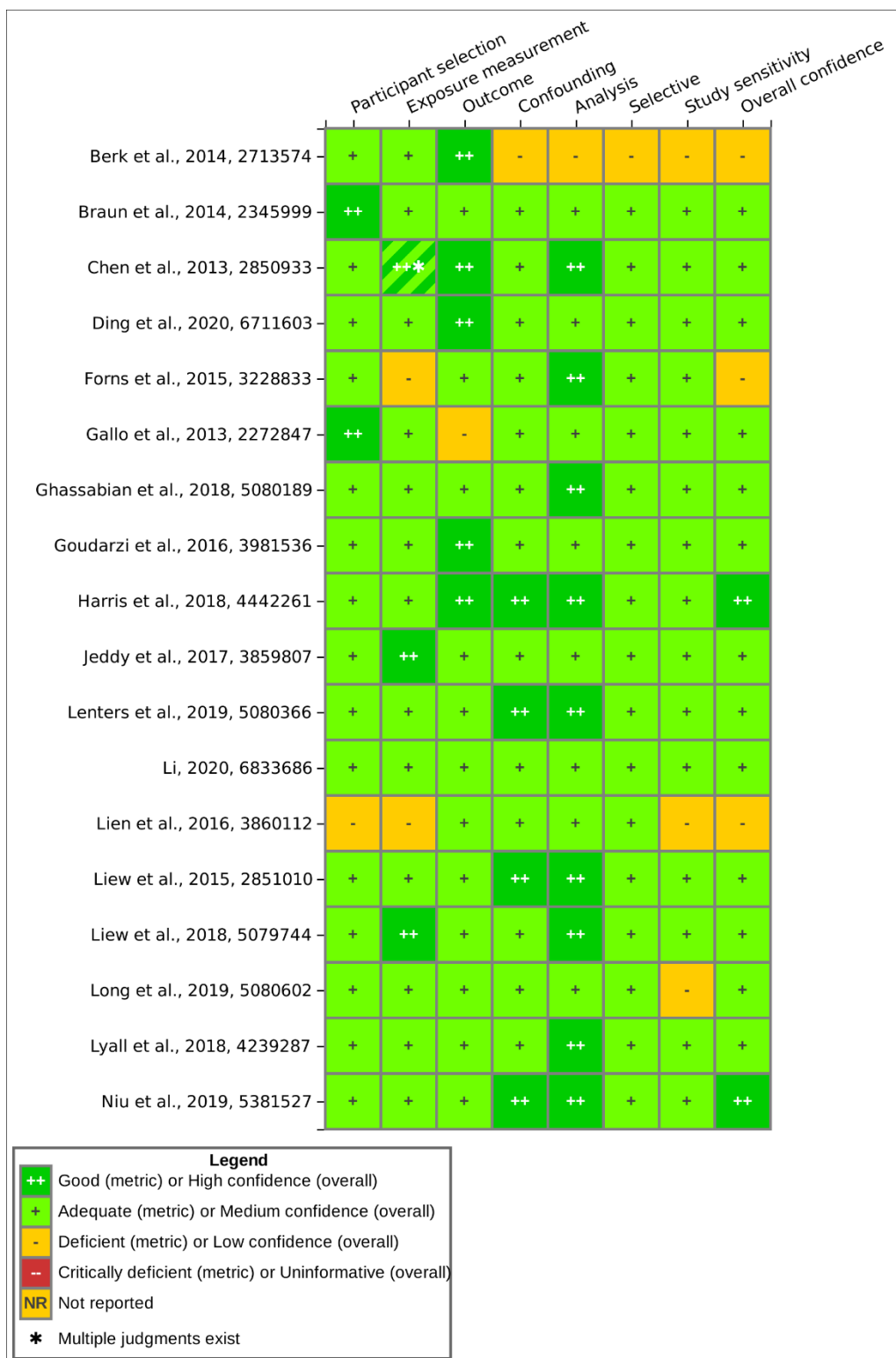


Figure 92. Summary of Study Evaluation for Epidemiology Studies of PFOS and Neurological Effects

Interactive figure and additional study details available on [HAWC](#).

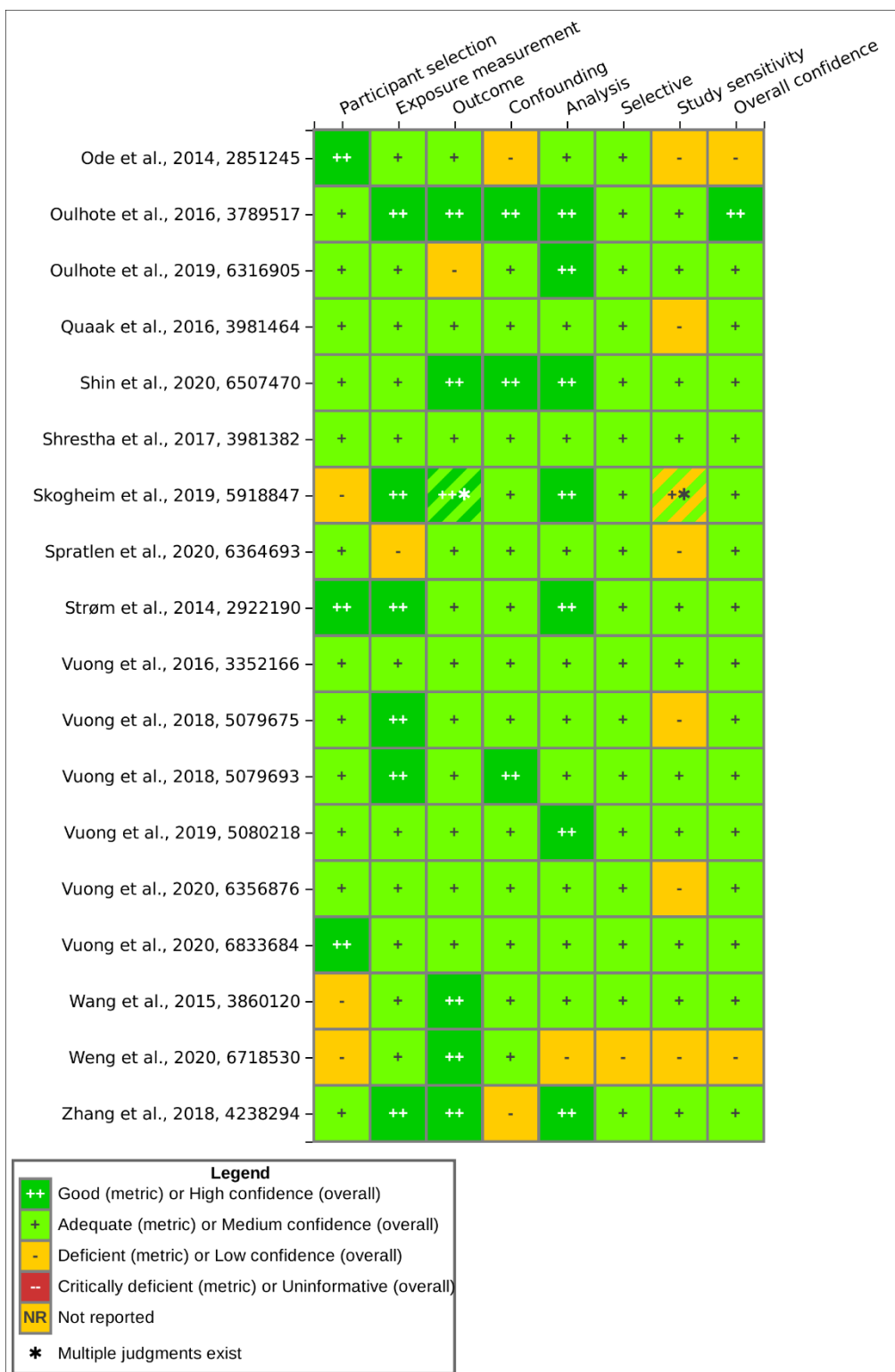


Figure 93. Summary of Study Evaluation for Epidemiology Studies of PFOS and Neurological Effects (Continued)

Interactive figure and additional study details available on [HAWC](#).

3.3.8.1.3 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; 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, 3781464; 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).

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, 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, 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, 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 and Lien, 2016, 3860112) examined PFOS exposures in relation to ADHD. Ode, 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.

Six *medium* confidence studies since the 2016 assessment evaluated PFOS exposures in relation to autism, autistic behaviors, and ID (Braun, 2014, 2345999; Liew, 2015, 2851010; Oulhote,

2016, 3789517; 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).

3.3.8.1.4 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).

3.3.8.1.5 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, 2020, 6711603 observed no significant associations.

3.3.8.2 *Animal Evidence*

There are 8 studies from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and nervous effects. Study quality evaluations for these 10 studies are shown in Figure 94.

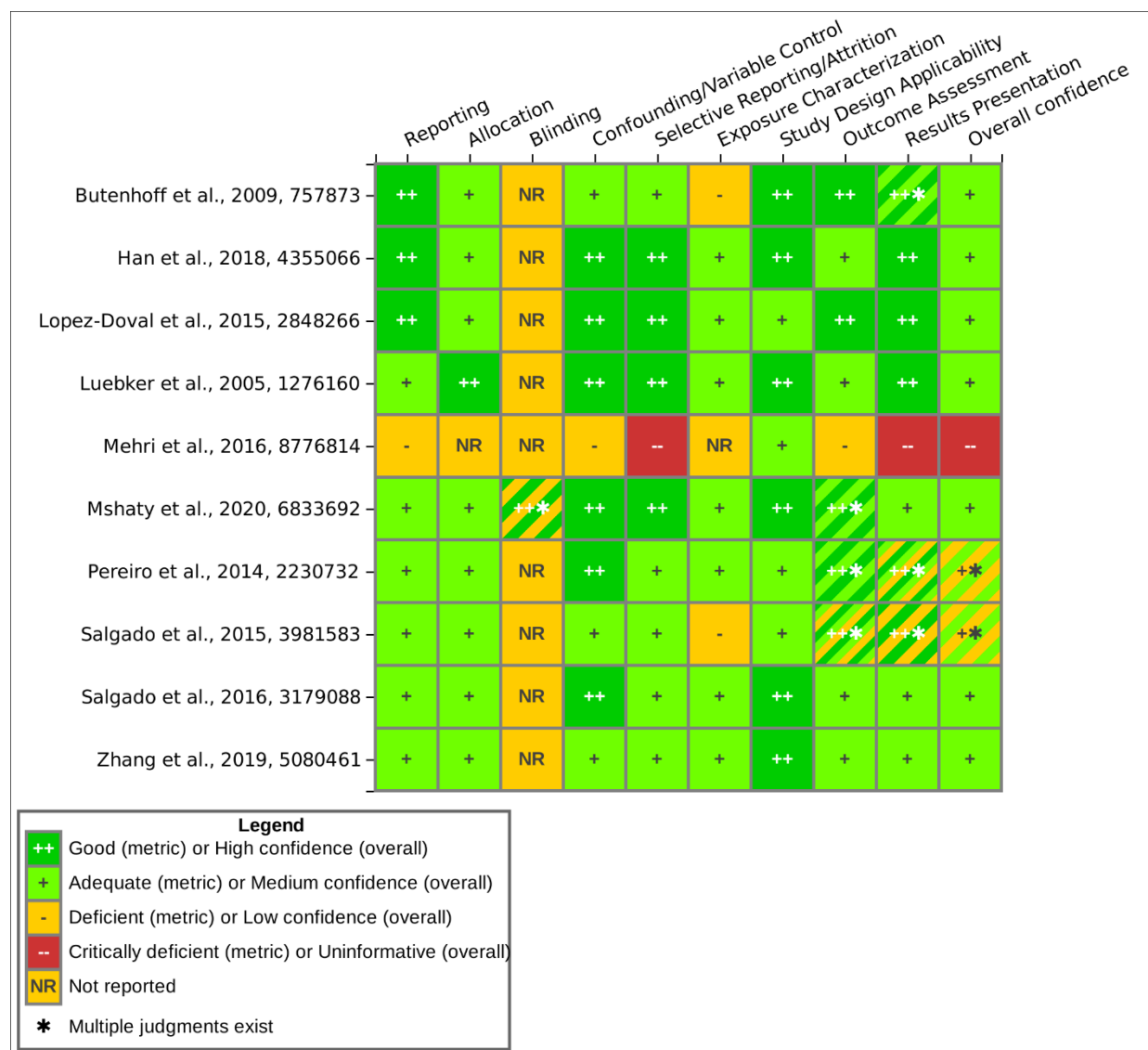


Figure 94. Summary of Study Evaluation for Toxicology Studies of PFOS and Nervous Effects

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 13). 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 one short-term study in rats. Absolute and relative brain weights were unchanged in the offspring of rats dosed with 0.1–1 mg/kg/day PFOS during gestation and lactation {Butenhoff, 2009, 757873}. The relative weights

of the amygdala, hippocampus, and prefrontal cortex were also unchanged in male rats dosed with 0.5–6 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 0.1–5 mg/kg/day PFOS {Butenhoff, 2009, 757873; NTP, 2019, 5400978}.

One developmental {Mshaty, 2020, 6833692} and one subchronic study {Long, 2013, 2850984} in mice and several reproductive {Luebker, 2005, 1276160} and developmental studies {Butenhoff 2009 et al., 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 mice exposed to 0.1–1 mg/kg/day PFOS from PND1–PND4 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 mice dosed with 2.15 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}. Similar effects on spatial learning and memory were seen in the offspring of rat dams exposed to 15 mg/mL but not 5 mg/mL PFOS in drinking water throughout gestation and lactation (drinking water consumption not reported) {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 0.1–3.2 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 PND17 in males exposed to 1 mg/kg/day throughout development {Butenhoff, 2009, 757873}. In male mice given a single dose of 11.3 mg/kg at PND10, 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 PND10. In this study, locomotion, rearing, and total activity was significantly decreased in both the 0.75 and 11.3 mg/kg dose groups at 2 months of age. Another development study exposed mice to 6 mg/kg/day PFOS from GD12–GD18 and assessed neuromotor maturation with surface righting reflex, open-field test, and rotarod test {Fuentes, 2007, 757863}. Surface righting reflex was delayed at PND4 and PND8. Significant effects were also observed during the climb test, with PFOS exposure resulting in diminished resistance to backwards pull and reduced climb ability at PND10 and PND11 but not PND12. Climbing ability and forelimb grip strength was reduced with PFOS exposure at PND11 but not PND10 or PND12. 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.

Table 13. Associations Between PFOS Exposure and Neurobehavioral Effects in Rodents

Reference	Study Design	Learning and Memory	Acoustic Startle	Motor Activity/Coordination	Neuromaturation
Mice					
Fuentes et al., 2007 ^a	Developmental exposure (GD12–18) to 0 or 6 mg/kg/day	NT	NT	Open field: No effect Rotarod: No effect	Surface righting reflex: ↓ at 6 mg/kg/day Grip strength: ↓ at 6 mg/kg/day
Mshaty et al., 2020 ^b	Developmental exposure (PND1–14) to 0, 0.1, 0.25, or 1 mg/kg/day	Object location and recognition test, and pairwise visual discrimination task: ↓ at 1 mg/kg/day	NT	NT	NT
Johansson et al., 2008 ^b	Single dose (PND10) to 0, 0.75, or 11.3 mg/kg	Habituation: ↓ at 11.3 mg/kg	NT	Motor activity: ↓ at ≥0.75 mg/kg	NT
Long et al., 2013 ^c	Subchronic exposure (3 months) to 0, 0.43, 2.15, or 10.75 mg/kg/day	Morris water maze: ↓ at ≥2.15 mg/kg/day	NT	NT	NT
Rats					
Wang et al., 2015 ^d	Developmental exposure (gestational and lactational) to 0, 5, 15 mg/L	Morris water maze: ↓ at 15 mg/mL	NT	Morris water maze, swimming speed: No effect	NT
Butenhoff et al., 2009 ^a	Developmental exposure (GD0–PND20) 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	Males, motor activity: ↑ at 1 mg/kg/day Females: No effect	NT
Luebker et al., 2005 ^a	Reproductive exposure (GD0–PND112) to 0.0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day	Morris water maze: No effect Passive avoidance: No effect	NT	NT	NT

GD = gestation day; PND = postnatal day; NT = not tested.

^a Males and females analyzed separately.

^b Study conducted in males.

^c Sexes combined.

^d Sex was not specified.

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 14). Glutamine, glycine, and serotonin were each examined in only one study. Neither glutamine nor glycine were altered in the dorsal hippocampus of male mice exposed to 0.1–1 mg/kg/day PFOS from PND1–14 {Mshaty, 2020, 6833692}. Serotonin was increased in the anterior hypothalamus, mediobasal hypothalamus, and the median eminence of male rats dosed with 0.5–6 mg/kg/day for 28 days {Lopez-Doval, 2015, 2848266}. Serum cholinesterase was also examined in one study and was found to be significantly increased in male rats exposed to 10 mg/kg/day, but not 1 mg/kg/day PFOS for 28 days {Han, 2018, 4355066}. 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 mice dosed with 0.43–10.75 mg/kg/day PFOS {Long, 2013, 2850984}. However, GABA was increased in the dorsal hippocampus of male mice exposed to 1 mg/kg/day, but not 0.1 or 0.25 mg/kg/day, PFOS from PND1–14 {Mshaty, 2020, 6833692}. In adult male 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 rats dosed with 0.5–6 mg/kg/day PFOS for 28 days, dopamine was increased in the hippocampus in the 0.5, 1, 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 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 mice dosed with 0.43–10.75 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 mice exposed to 1 mg/kg/day PFOS from PND1–PND14 {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 14. Associations Between PFOS Exposure and Neurotransmitters in Rodents

Reference	Study Design	Glutamine/ Glutamate	Glycine	Serotonin	GABA	Dopamine
Mice						
Mshaty et al., 2020 ^a	Developmental exposure (PND1–14) to 0, 0.1, 0.25, or 1 mg/kg/day	Dorsal hippocampus, glutamate: ↑ at 1 mg/kg/day Dorsal hippocampus, glutamine: No effect	Dorsal hippocampus: No effect	NT	Dorsal hippocampus: ↑ at 1 mg/kg/day	NT
Long et al., 2013 ^b	Subchronic exposure (3 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

Reference	Study Design	Glutamine/ Glutamate	Glycine	Serotonin	GABA	Dopamine
Rats						
Salgado et al., 2015 ^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 ^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
Lopez-Doval et al., 2015 ^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

NT = not tested.

^a Study conducted in males.

^b Sexes combined.

Synaptic transmission and plasticity were assessed in one electrophysiology study in rats exposed to 0.35–2.17 mg/kg/day PFOS throughout development until PND90 {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. Induction of long-term potentiation was also decreased in a dose-dependent manner in the 0.72 and 2.17 mg/kg/day dose groups.

3.3.8.3 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 53 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to nervous effects. A summary of these

studies is shown in Figure 95. Additional analysis on the mechanistic actions of PFOS on nervous health outcomes is pending and is expected to be completed after the EPA SAB review.

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	6	0	5	10
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 Specified (Review Article)	3	0	0	3
Grand Total	25	1	32	53

Figure 95. Summary of Mechanistic Studies of PFOS and Nervous Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.8.4 Evidence Integration

In summary, the human epidemiological studies reviewed since the 2016 assessments provide mostly mixed results on the associations between PFOS and neurological outcomes. 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 somewhat mixed, and establishment of a relationship with PFOS warrants further study.

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. No studies or endpoints from the available epidemiological literature were considered for the derivation of PODs.

Of the studies available in animal models, no effects were noted for brain weight or 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. Therefore, the endpoint of object location recognition from Mshaty et al. (2020, 6833692) was considered for the derivation of a POD. Overall, the results from these studies in animals, as well as mixed results from epidemiological evidence highlights the need for further analysis of alterations in neurodevelopmental and neuropsychological outcomes with PFOS exposure in animals and humans.

3.3.9 Renal

3.3.9.1 Human Evidence

3.3.9.1.1 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 m²) 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} (Table C-18). 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.

3.3.9.1.2 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}.

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} (Figure 96). 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.

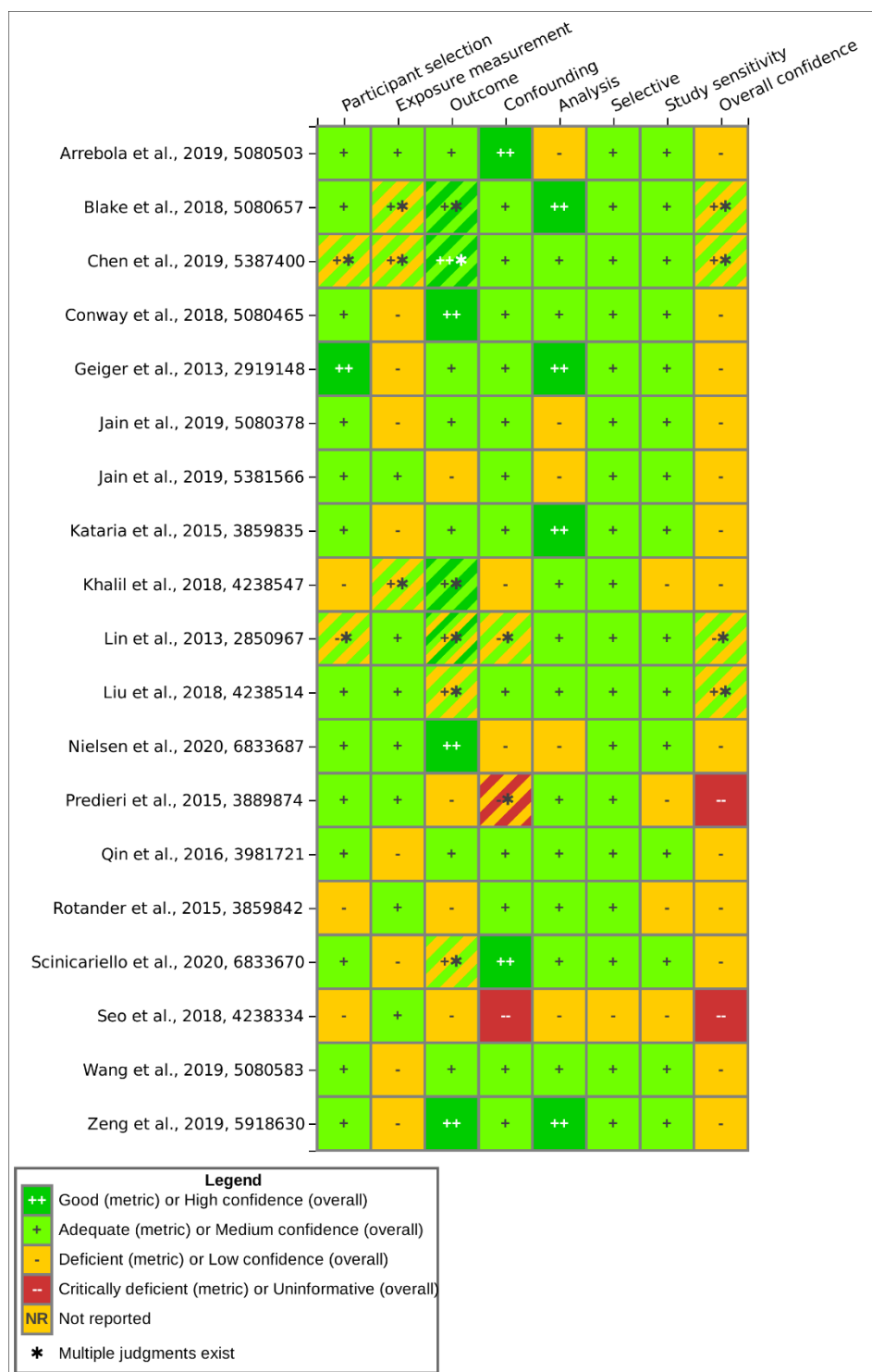


Figure 96. Summary of Study Evaluation for Epidemiology Studies of PFOS and Renal Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.9.1.3 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 (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, 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.

3.3.9.1.4 Findings from the General Adult Population

Two *low* confidence studies examined CKD in the general population {Conway, 2019, 5080465; Wang, 2019, 5080583} and both observed positive associations. CKD was defined as an eGFR of < 60 mL/min/1.73 m². 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, 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-significant increase in serum uric acid with increasing PFOS.

One *low* confidence occupational study examined serum uric acid levels among firefighters with past exposure to 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, 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.

3.3.9.2 Animal Evidence

There are 3 studies from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and renal effects. Study quality evaluations for these 5 studies are shown in Figure 97.

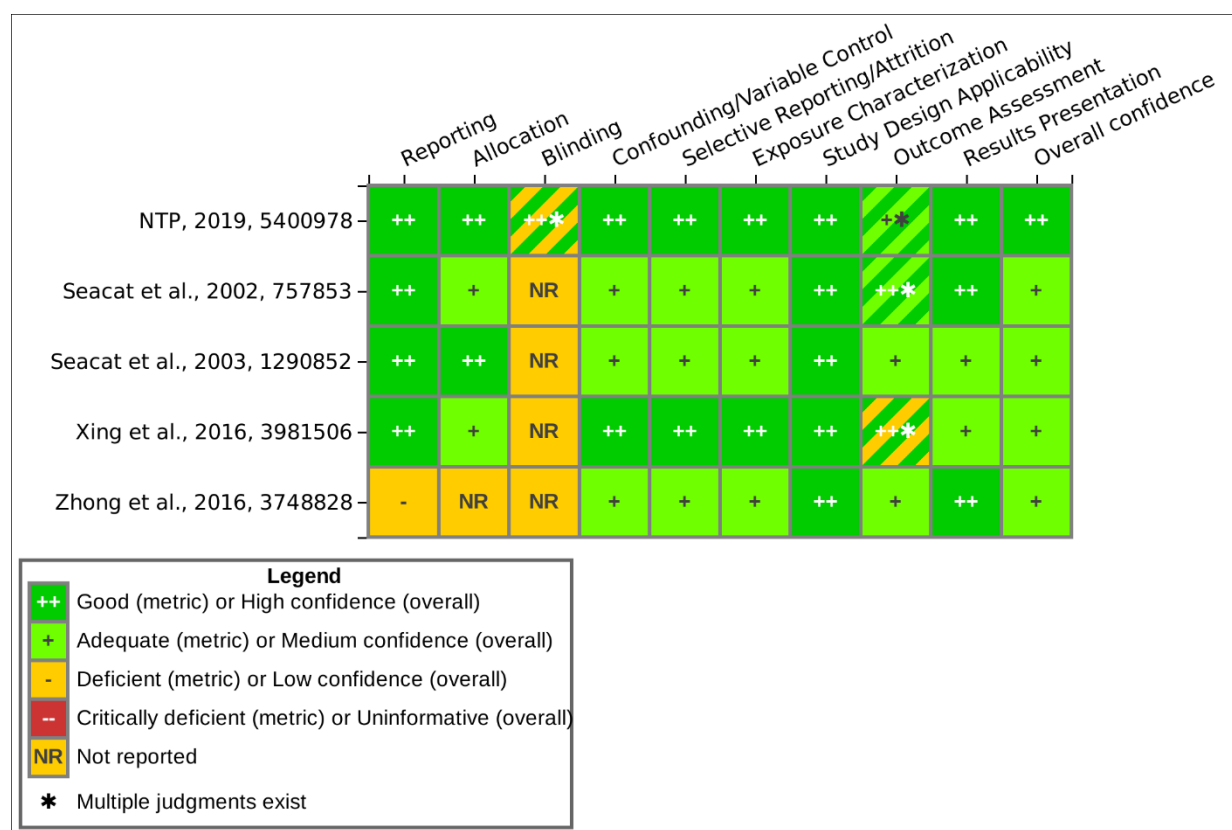


Figure 97. Summary of Study Evaluation for Toxicology Studies of PFOS and Renal Effects

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; Peden-Adams, 2008, 1424797; Yahia, 2008, 2919381; Zhong, 2016, 3748828; NTP, 2019, 5400978}. 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 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. In contrast to the mouse studies, two short-term/subchronic studies in male rats reported significant increases in relative kidney weight at doses as low as 5 mg/kg/day {Cui, 2009, 757868} and 6 mg/kg/day {Goldenthal, 1978, 1291068}. 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 both Goldenthal et al. (1978, 1291068) and Cui et al. (2009, 757868). Cui et al. (2009, 757868) also observed altered kidney histopathology 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). In addition, a chronic study in female rats reported significant increases in kidney weight relative to body weight with the highest dose tested (1.6 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}. Besides Cui et al. (2009, 757868), all other studies reported no treatment-related changes in histopathology {Seacat, 2003, 1290852; Yahia, 2008, 2919381; Butenhoff, 2012, 1276144; Xing, 2016, 3981506; NTP, 2019, 5400978}.

Several studies also analyzed clinical chemistry endpoints relevant to renal toxicity. At the highest dose tested in each study (1.3–5 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. 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 1.3 and 1.6 mg/kg/day, respectively) at 27 weeks and significantly increased BUN in doses ≥ 0.13 mg/kg/day in males and ≥ 0.4 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, 2002, 757853; Seacat, 2003, 1290852; 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 mice treated with up to 10 mg/kg/day PFOS for 30 days {Xing, 2016, 3981506} or in 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; NTP, 2019, 5400978}, urinary N-acetyl-b-glucosaminidase (NAG) {Xing, 2016, 3981506}, and urinalysis parameters including urine pH {Seacat, 2002, 757853; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144} were not widely assessed across multiple studies or did not show consistent responses between studies.

3.3.9.3 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 4 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to renal effects. A summary of these studies is shown in Figure 98. Additional analysis on the

mechanistic actions of PFOS on renal health outcomes is pending and is expected to be completed after the EPA Scientific Advisory Board review.

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	1	1
Extracellular Matrix Or Molecules	1	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	1	1
Inflammation And Immune Response	1	0	1
Renal Dysfunction	3	1	3
Xenobiotic Metabolism	0	1	1
Grand Total	4	2	4

Figure 98. Summary of Mechanistic Studies of PFOS and Renal Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.9.4 Evidence Integration

In summary, the present review of human epidemiological studies found limited evidence of an association between PFOS and decreased renal function. In contrast to the 2016 Health Assessment, the available epidemiological evidence in this review does not support an association with CKD; 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. Positive association between serum PFOS concentrations and CKD were only reported in *low* confidence studies {Conway, 2018, 5080465; Wang, 2019, 5080583}. 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.

Evidence from animal models similarly indicates that 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. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.10 Hematological

3.3.10.1 Human Evidence

3.3.10.1.1 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 outcome categories.

For this updated review, eight studies examined the association between PFOS hematological health outcomes (Figure 99). 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 used 2003–2010 NHANES data for adolescents and adults 12 years and older {Etzel, 2019, 5043582}, and Jain (2020, 6333438) and Jain, 2020, 6833623, used 2003–2016 NHANES data for adults 20 years and older {Jain, 2020, 6333438; Jain, 2020, 6833623}. Also in the United States, Khalil et al., 2018 {Khalil, 2018, 4238547} included 48 obese children 8–12 years old from a hospital lipid clinic in Dayton, Ohio. Abraham et al., 2020 {Abraham, 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 {Jiang, 2014, 2850910} recruited 141 pregnant women in Tianjin, China. Chen et al., 2019 {Chen, 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 {van den Dungen, 2017, 5080340} included 80 men aged 40–70 years in the Netherlands who regularly consumed eel.

3.3.10.1.2 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 versus plasma or serum) could also affect the interpretation of results. Measuring PFOS and serum lipids concurrently was considered adequate in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.5 years) {Li, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures.

Based on these considerations, three studies were classified as *medium* confidence, two as *low* confidence and three as *uninformative* (Figure 99). Two *low* confidence studies had deficiencies in participant selection, confounding, or sample size. Khalil et al., 2018 {Khalil, 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 {van den Dungen, 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, 2020b {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 {Abraham, 2020, 6506041} and Jiang et al., 2014 {Jiang, 2014, 2850910} only performed unadjusted correlation analyses and therefore did not consider the influence of potential confounding factors.

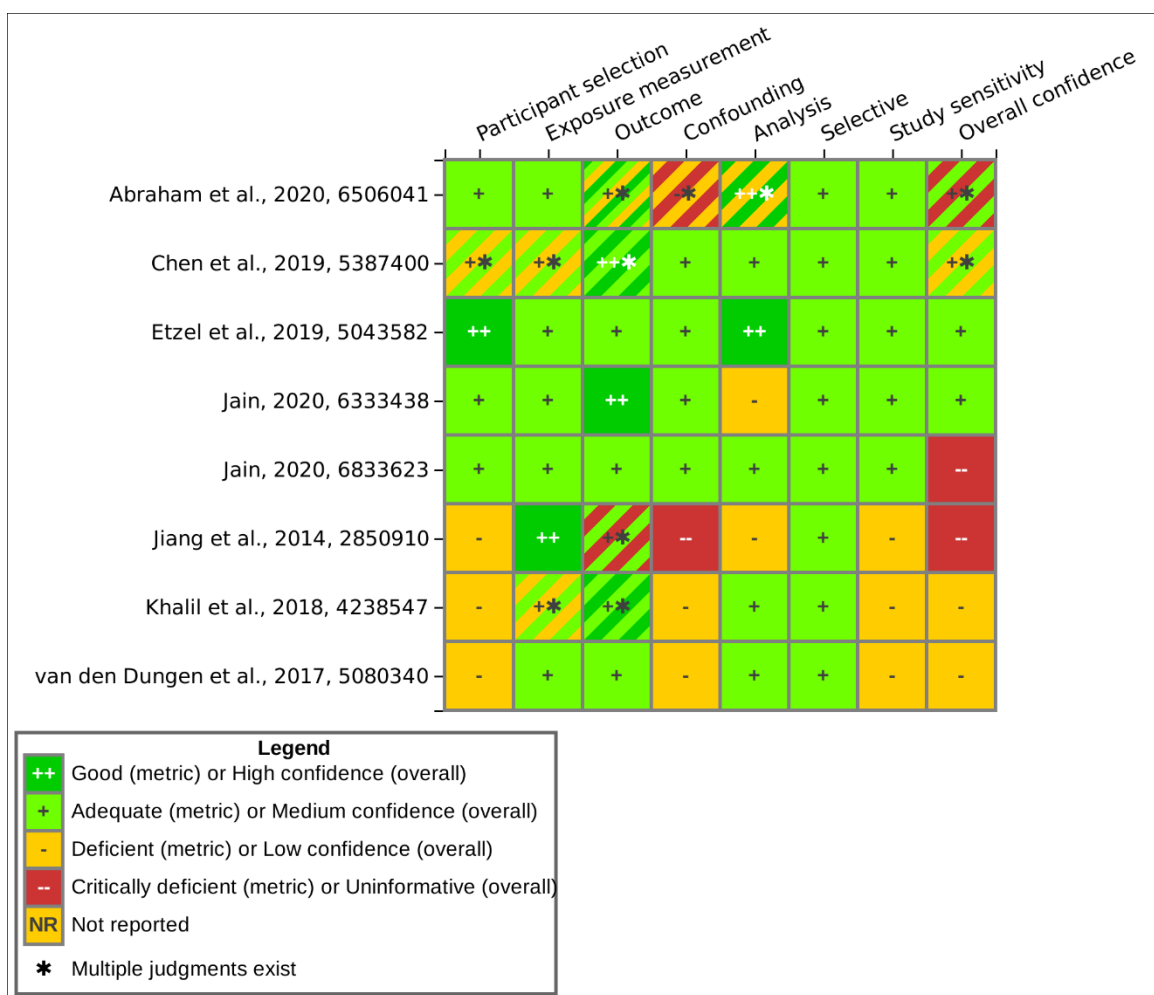


Figure 99. Summary of Study Evaluation for Epidemiology Studies of PFOS and Hematological Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.10.1.3 Findings

Two studies examined levels of 25-hydroxy vitamin D and vitamin D deficiency and observed no associations. In adolescents and adults from NHANES (2003–2010), Etzel et al., 2019 {Etzel, 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 (25.9–435.0 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 (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 (Table C-19). 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 \geq 60 mL/min/1.73 m²). 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 {Chen, 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.

3.3.10.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and hematological effects. Study quality evaluations for these 3 studies are shown in Figure 100.

	Reporting	Allocation	Blinding	Confounding/Variable Control	Selective Reporting/Attrition	Exposure Characterization	Study Design	Outcome Applicability	Results Presentation	Overall confidence
NTP, 2019, 5400978	++	++	+++*	++	++	++	++	++	++	++
Seacat et al., 2002, 757853	++	+	NR	+	+	+	++	+++*	++	+
Seacat et al., 2003, 1290852	++	++	NR	+	+	+	++	+	+	+

Legend

- ++ Good (metric) or High confidence (overall)
- + Adequate (metric) or Medium confidence (overall)
- Deficient (metric) or Low confidence (overall)
- Critically deficient (metric) or Uninformative (overall)
- NR Not reported
- * Multiple judgments exist

Figure 100. 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; discussions on histopathological impacts of PFOS exposure on the spleen and bone marrow can be found in Section 3.3.4.2. Four oral studies in rodents or monkeys with short-term to chronic exposure durations evaluated the effects of PFOS on the hematological system.

Significantly decreased reticulocyte counts were observed in male and female Sprague Dawley rats following 28-day oral gavage exposure to 2.5 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 (Section 3.3.4.2). 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 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 PFOA in the diet) and in female rats at 3.73 mg/kg/day (50 mg/kg of PFOA in the diet). In both males and females exposed to 100 mg/kg PFOS in the diet, equivalent to 6.34 and 7.34 mg/kg/day, respectively, the red blood cell deformability index was significantly reduced over a range of shear stress levels {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 0.03–1.33 and 0.04–1.56 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.

3.3.10.3 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 3 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to hematological effects. A summary of these studies is shown in Figure 101. Additional analysis of mechanistic actions of PFOS on hematological health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	Grand Total
Atherogenesis And Clot Formation	1	1	2
Other	1	0	1
Grand Total	2	1	3

Figure 101. Summary of Mechanistic Studies of PFOS and Hematological Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.10.4 Evidence Integration

Similar to the 2016 Health Effects Support Document for PFOS {U.S. EPA, 2016, 3603279}, there is no evidence of an association between PFOS exposure and general hematology parameters in human populations. Evidence from recent epidemiological studies does not support a relationship between PFOA exposure and hematological alterations. Many of the relevant outcomes were not studied in more than one instance. 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 and three studies examined hemoglobin and also reported mixed effects. Overall, the number of epidemiological studies and study quality were limited, suggesting further research is needed to draw conclusions about the hematological effects of PFOS in humans.

Similarly, alterations of hematological effects in animal models were also limited. Though 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 (Section 3.3.4). 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. Overall, there were minimal changes observed and effects were sometimes transient or only apparent at relatively higher doses compared to other health outcomes.

Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.11 Respiratory

3.3.11.1 Human Evidence

3.3.11.1.1 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 (FEV₁), forced vital capacity (FVC), FEV₁/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 (Table C-20).

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.

3.3.11.1.2 Study Quality

The five general population studies identified since the last assessment were all classified *medium* confidence (Figure 102). 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}.

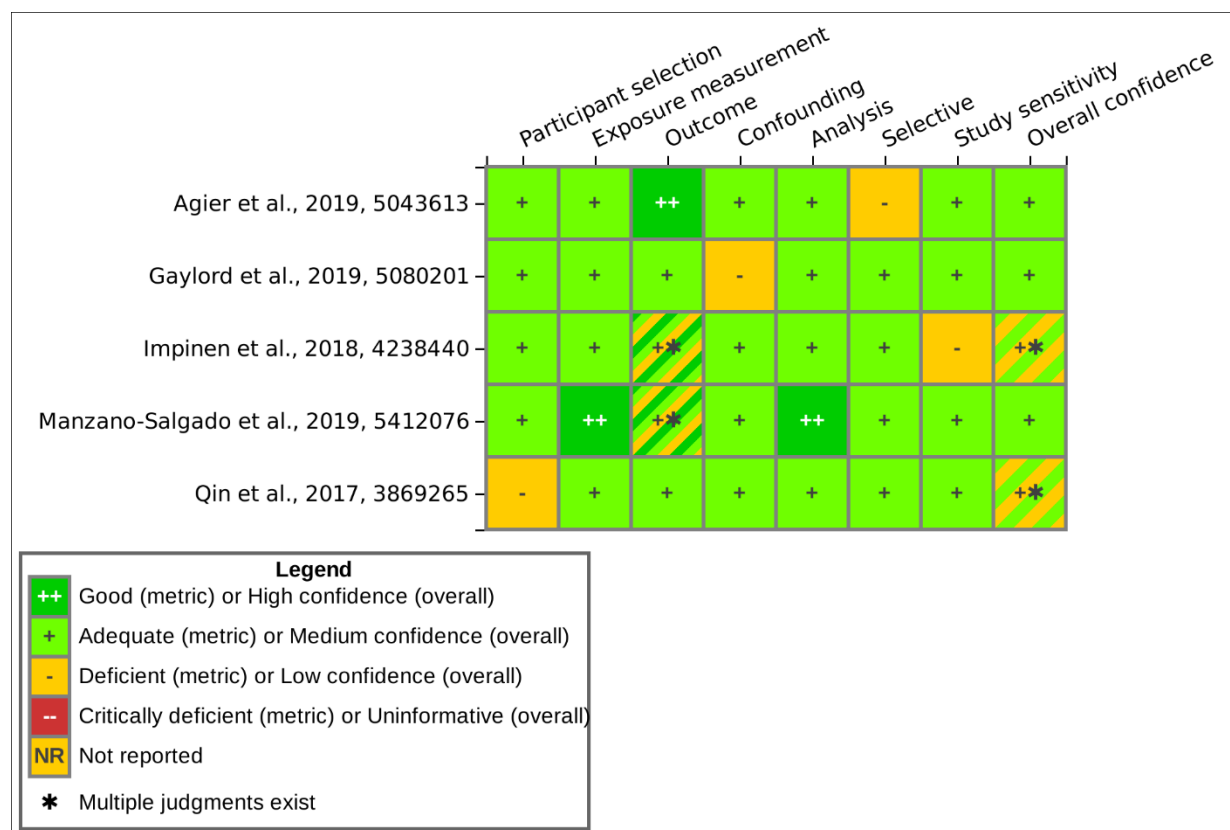


Figure 102. Summary of Study Evaluation for Epidemiology Studies of PFOS and Respiratory Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.11.1.3 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} (Table C-20).

Of the four studies examining FEV1, three reported negative associations (i.e., decrease in FEV1 with higher PFOA levels), while one reported a positive association. Qin, 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, 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, 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 log₂ 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/FEV₁, lung resistance, total lung capacity, functional residual capacity, and residual volume) in the remaining studies {Gaylord, 2019, 5080201; Manzano-Salgado, 2019, 5412076}.

3.3.11.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 that investigated the association between PFOS and respiratory effects. The study quality evaluation for this study is shown in Figure 103.

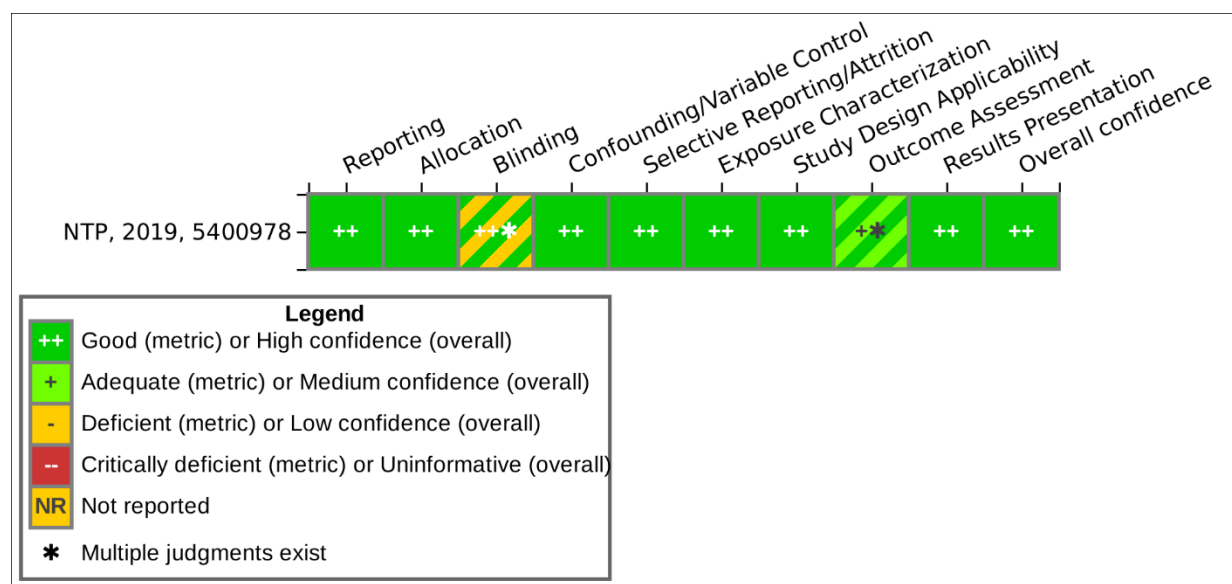


Figure 103. Summary of Study Evaluation for Toxicology Studies of PFOS and Respiratory Effects

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, 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}.

Yahia et al. (2008, 2919381) exposed mouse dams to 0, 1, 10, or 20 mg/kg/day PFOS from GD0–17 and assessed neonatal and maternal lung histopathology. Initially, a single surviving pup from each dam (n=5/treatment group) was analyzed at PND0; 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 GD0–GD17 or 10 mg/kg/day PFOS from GD0–GD18 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 GD18. The authors suggested an increase in the incidence of moderate to severe intracranial blood vessel dilation in fetuses at GD18 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 PND0 and PND21 after gestational exposure to 0, 0.1, or 2 mg/kg/day PFOS from GD1–GD21. With PFOS exposure of 2 mg/kg/day, pups showed marked alveolar hemorrhaging, thickened interalveolar septum, and focal lung consolidation at PND0 (incidence data not provided). These effects lasted through PND21, 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 PND0 and PND21 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.

In an attempt to identify the prenatal window of susceptibility to PFOS in neonatal rats, Grasty et al. (2003, 1332670) dosed dams with 0, 25, or 50 mg/kg/day PFOS during several 4-day gestational timepoints, including GD17–GD20, 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, 25, or 50 mg/kg/day PFOS from GD19–GD21 and sacrificed fetuses at GD21 or pups at PND0 to examine lung histology {Grasty, 2003, 1332670}. No histological differences between lung samples of control and treated fetuses sacrificed at GD21 were observed, though it appeared that PFOS reduced lung expansion and slowed or compromised lung maturation of pups by PND0; epithelial thickness of lungs of PFOS-treated pups at PND0 was similar to that of lungs from fetal control animals at GD21 (incidence data not provided). Grasty et al. (2005, 2951495) conducted a follow-up study with the same GD19–GD21 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 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 PND0

resembled the lungs of control fetuses at GD21 (incidence of 17% and 50% of pups from the 25 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 GD12–GD18 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 GD7–GD20 (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, 2.5, 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 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, 2.5, 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. There were no other reports of altered lung weight in the available literature.

3.3.11.3 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 the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to respiratory effects. A summary of these studies is shown in Figure 104. Additional analysis on the mechanistic actions of PFOS on respiratory health outcomes is pending and is expected to be completed after the EPA SAB review.

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 104. Summary of Mechanistic Studies of PFOS and Respiratory Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.11.4 Evidence Integration

In summary, as in the 2016 Health Assessment, there is insufficient evidence from human epidemiological studies to support an association between PFOS and respiratory health effects in children, adolescents, and young adults. No studies were available that assessed respiratory health effects in older adults. Two studies observed some evidence of an association between exposure to PFOS and reduced lung function in children, particularly in children with asthma. However, there were mixed results for similar lung function outcomes in different studies.

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 (Section 3.3.1.2), indicating that respiratory toxicity is not likely a highly sensitive health outcome for PFOS exposure. Observed effects in adult animals similarly occur at relatively high doses compared to other health outcomes. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.12 Musculoskeletal

3.3.12.1 Human Evidence

3.3.12.1.1 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.

3.3.12.1.2 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.

Based on these considerations, six studies were classified as *medium* confidence and two as *low* confidence (Figure 105). 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.

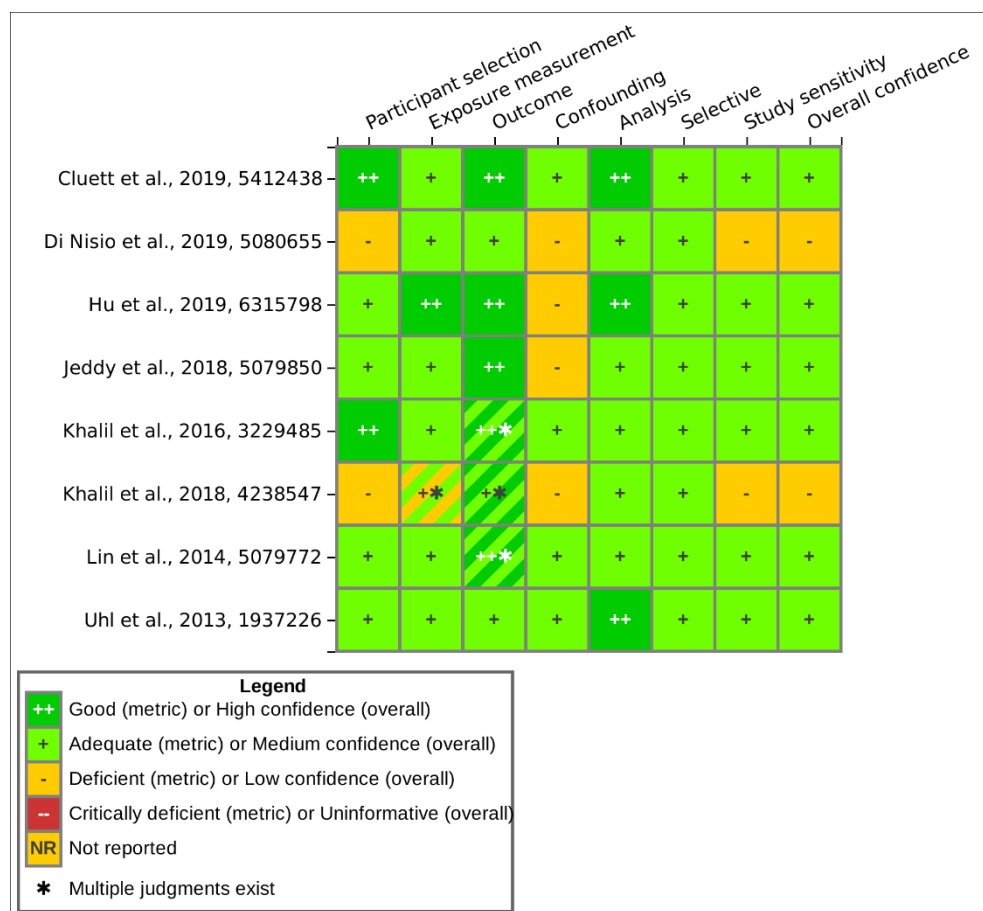


Figure 105. Summary of Study Evaluation for Epidemiology Studies of PFOS and Musculoskeletal Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.12.1.3 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 (Table C-21). Cluett et al., 2019 {Cluett, 2019, 5412438} observed a statistically significant negative 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. Negative non-significant associations were also observed with aBMD in boys and in girls with bone mineral content (BMC) z score. Jeddy et al., 2018 {Jeddy, 2018, 5079850} identified a statistically significant negative association between prenatal PFOS exposure and total lean body mass and height in 17-year-old girls. The same study initially showed negative 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.

3.3.12.1.4 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 (Table C-21).

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. {Uhl, 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 {Khalil, 2016, 3229485} observed a statistically significant negative 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 {Hu, 2019, 6315798} observed small but statistically significant negative 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.

3.3.12.2 Animal Evidence

Limited data are available on the effect of PFOS on the musculoskeletal system other than developmental skeletal defects resulting from gestational exposure that are discussed in Section 3.3.1.2. In a single-dose study, Onishchenko et al. (2011, 758427) exposed pregnant C57BL/6/Bkl mice to 0.3 mg/kg/day PFOS between GD1–PND0 and subsequently carried out a muscle strength test on pups. Gestational PFOS exposure resulted in a significantly shorter latency to fall period in male pups, potentially indicating lower muscle strength or endurance in these animals. The latency to fall period for PFOS-exposed female pups was not statistically different from female control pups. Since publication of the HESD for PFOS {U.S. EPA, 2016, 3603365}, no studies with a medium or high overall confidence rating during study quality evaluation report musculoskeletal effects outside of those associated with developmental toxicity.

3.3.12.3 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 5 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to musculoskeletal effects. A summary of these studies is shown in Figure 106. Additional analysis on the mechanistic actions of PFOS on musculoskeletal health outcomes is pending and is expected to be completed after the EPA Scientific Advisory Board review.

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	0	3	3
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	1	4	5

Figure 106. Summary of Mechanistic Studies of PFOS and Musculoskeletal Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.12.4 Evidence Integration

In summary, the present review of human epidemiological studies identified suggestive evidence of a harmful effect of elevated PFOS exposure on bone health, particularly measures of bone mineral density (four studies), with more statistically significant effects among females. 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 (Section 3.3.1.2). 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 moderately reduced confidence in most studies; common issues included cross-sectional design or a lack of information on SES.

Overall, the available literature showing effects of PFOS on the musculoskeletal system is limited. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.13 Gastrointestinal

3.3.13.1 Human Evidence

3.3.13.1.1 Introduction

Gastrointestinal 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 gastrointestinal 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}. Gastrointestinal outcomes only assessed in the context of immune system health, including ulcerative colitis and Crohn's disease, are discussed in Section **Error! Reference source not found..** 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 gastrointestinal 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 gastrointestinal 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.

3.3.13.1.2 Study Quality

Several considerations were specific to evaluating the quality of the studies of gastrointestinal 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 gastrointestinal disorders has developed substantially over recent decades and diagnostic indicators continue to rapidly evolve. Causal factors in developing gastrointestinal 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 gastrointestinal conditions lead to increased intake of PFOS from food packaging or preparation methods, increased PFOS absorption through the gastrointestinal tract, or reduced fecal excretion {Xu, 2020, 6315709}. Measuring PFOS and gastrointestinal 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.

Based on these considerations, one study was considered *medium* confidence {Timmermann, 2020, 6833710} and three as *low* confidence {Dalsager, 2016, 3858505; Hammer, 2019, 8776815; Xu, 2020, 6315709} (Figure 107).

The *medium* confidence study {Timmermann, 2020, 6833710} relied on retrospective reporting of gastrointestinal 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.

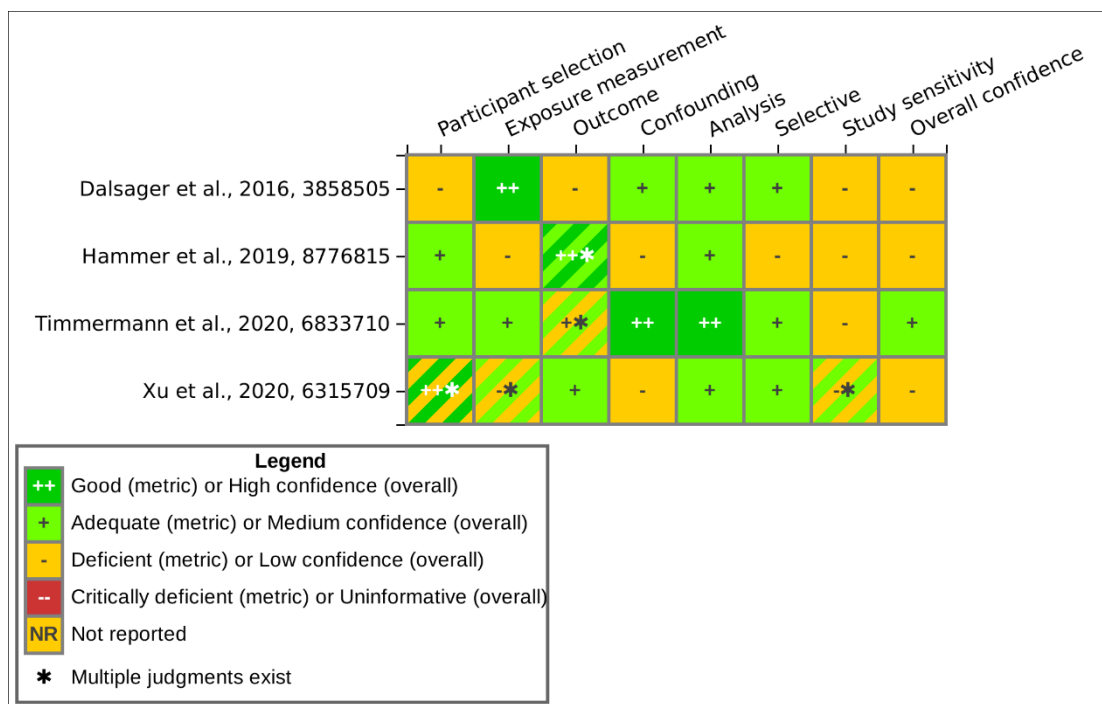


Figure 107. Summary of Study Evaluation for Epidemiology Studies of PFOS and Gastrointestinal Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.13.1.3 Findings

Results for the studies that examined gastrointestinal outcomes are presented in Table C-22. Both studies examining diarrhea observed non-significant increased association with PFOS.

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 increased odds and incidence of vomiting or diarrhea 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.

3.3.13.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 that investigated the association between PFOS and gastrointestinal effects. The study quality evaluation for this study is shown in Figure 108.

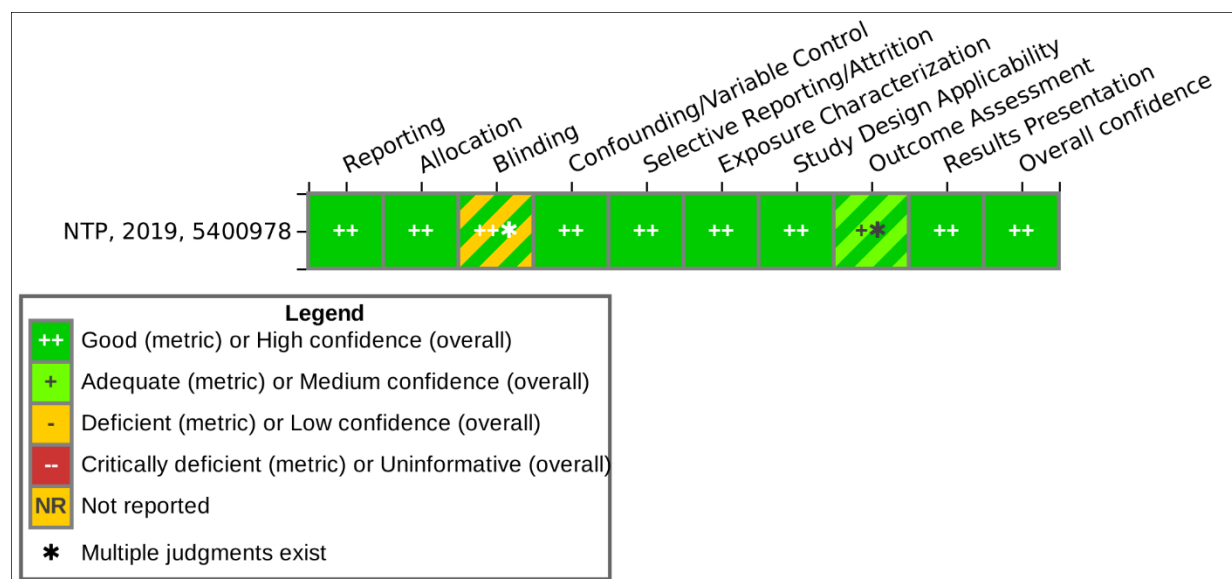


Figure 108. Summary of Study Evaluation for Toxicology Studies of PFOS and Gastrointestinal Effects

Interactive figure and additional study details available on [HAWC](#).

Studies on the gastrointestinal effects of PFOS exposure are limited. In a study conducted by NTP (2019, 5400978), male and female Sprague-Dawley rats were orally administered 0, 0.312, 0.625, 1.25, 2.5, 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, 100, 215, 464, 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 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 LD₅₀ 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, 0.5, 1.5, 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 gastrointestinal tract toxicity (anorexia, emesis, black stool) {Goldenthal, 1979, 9573133}.

3.3.13.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOS exposure to adverse gastrointestinal outcomes in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 10 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to gastrointestinal effects. A summary of these studies is shown in Figure 109. Additional

analysis on the mechanistic actions of PFOS on gastrointestinal health outcomes is pending and is expected to be completed after the EPA SAB review.

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 109. Summary of Mechanistic Studies of PFOS and Gastrointestinal Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.13.4 Evidence Integration

In the 2016 HESD for PFOS, gastrointestinal outcomes in humans were only assessed in the context of immune system health. Overall, the available evidence in this review of the epidemiological literature does not support an inverse association between PFOS exposure and gastrointestinal health effects. 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 general IBD, vomiting, or diarrhea. Similarly, the animal toxicity database for studies related to gastrointestinal effects is also limited. The few studies that demonstrated gastrointestinal effects in animal models appeared to only observe effects in moribund or deceased individuals.

Overall, the available literature showing effects of PFOS on the gastrointestinal system is limited. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.14 Dental

3.3.14.1 Human Evidence

3.3.14.1.1 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 {Puttige Ramesh, 2019, 5080517} assessed data from 2,869 12–19-year-old adolescents included in 1999–2012 NHANES and Wiener and Waters, 2019 (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.

3.3.14.1.2 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.

Based on these considerations, the two included studies were considered *medium* confidence (Figure 110), 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.

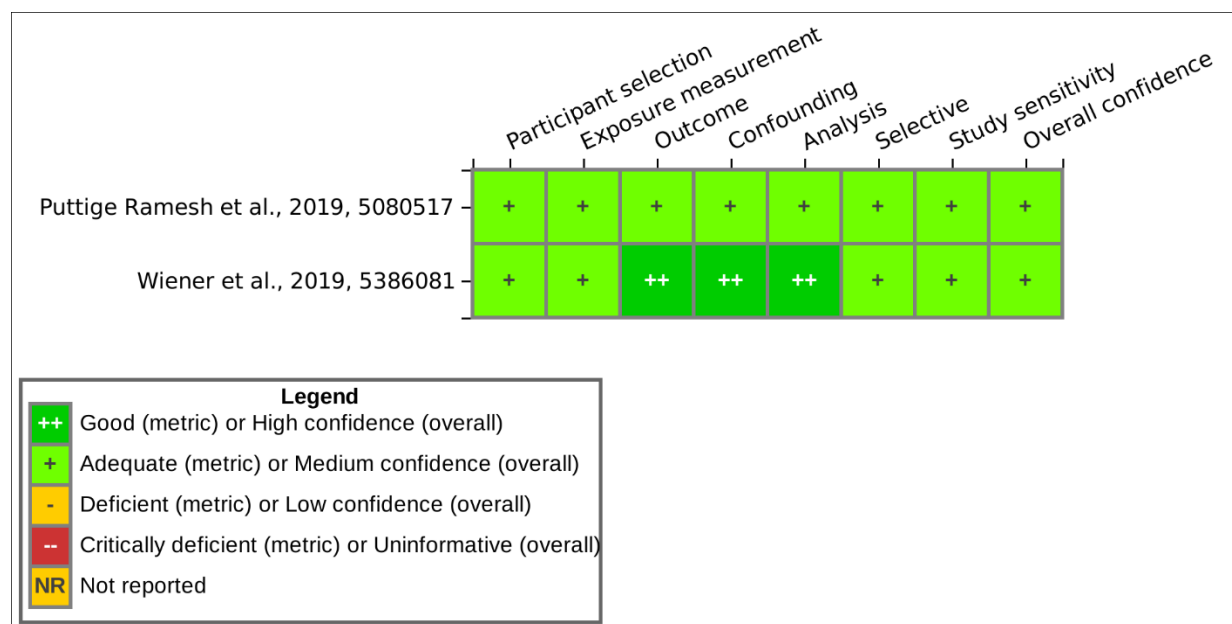


Figure 110. Summary of Study Evaluation for Epidemiology Studies of PFOS and Dental Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.14.1.3 Findings

Results for the studies that examined dental outcomes are presented in Table C-23. The two studies observed mixed effects {Puttige Ramesh, 2019, 5080517; Wiener, 2019, 5386081}. Wiener and Waters, 2019 (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 (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.

3.3.14.2 Animal Evidence

In the available literature, there is no reported biological consequence of PFOS exposure on dental tissue in animals. Therefore, the available data do not support dental tissue as a target of PFOS toxicity and no endpoints are recommended for dose-response modeling.

3.3.14.3 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 the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to dental effects.

3.3.14.4 Evidence Integration

Overall, the available epidemiological evidence in this review does not support an inverse association between PFOS and dental outcomes. Dental health outcomes were not previously reviewed in the 2016 HESD for PFOS. 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 mostly 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. There are no available studies in animal models examining the effects of PFOS exposure on dental outcomes.

Overall, the available literature showing effects of PFOS on dental health is limited. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.15 Ocular

3.3.15.1 Human Evidence

3.3.15.1.1 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.

3.3.15.1.2 Study Quality

Zeeshan et al. (2020, 6315698) was classified as *medium* confidence (Figure 111). 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.

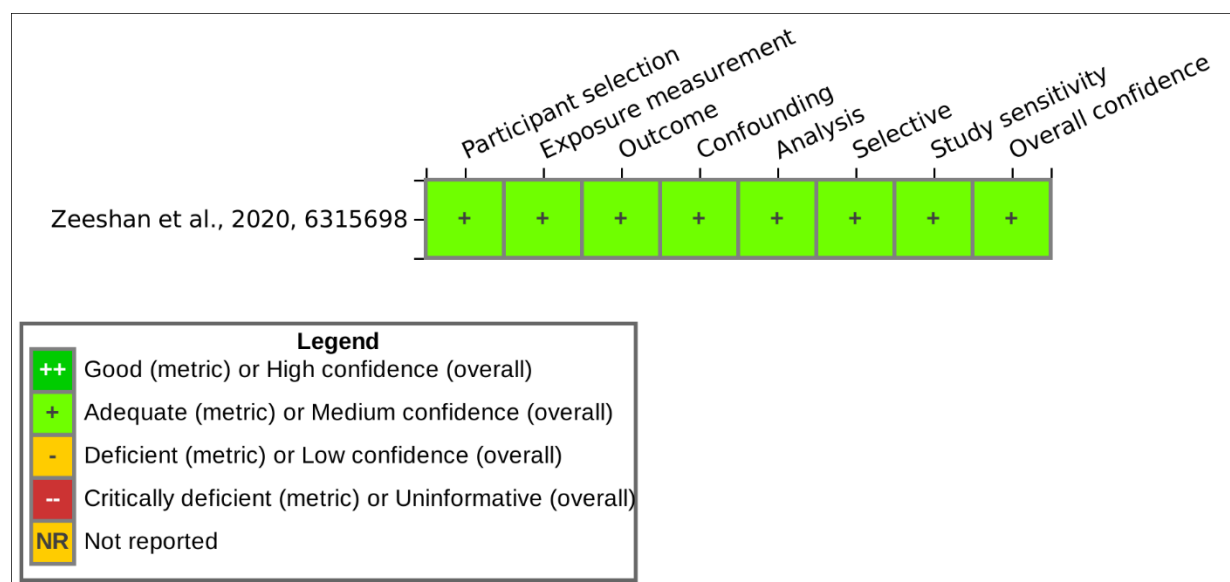


Figure 111. Summary of Study Evaluation for Epidemiology Studies of PFOS and Ocular Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.15.1.3 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 (Table C-24). Outcomes examined included ocular conditions, including 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.

3.3.15.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 that investigated the association between PFOS and ocular effects. The study quality evaluation for this study is shown in Figure 112.

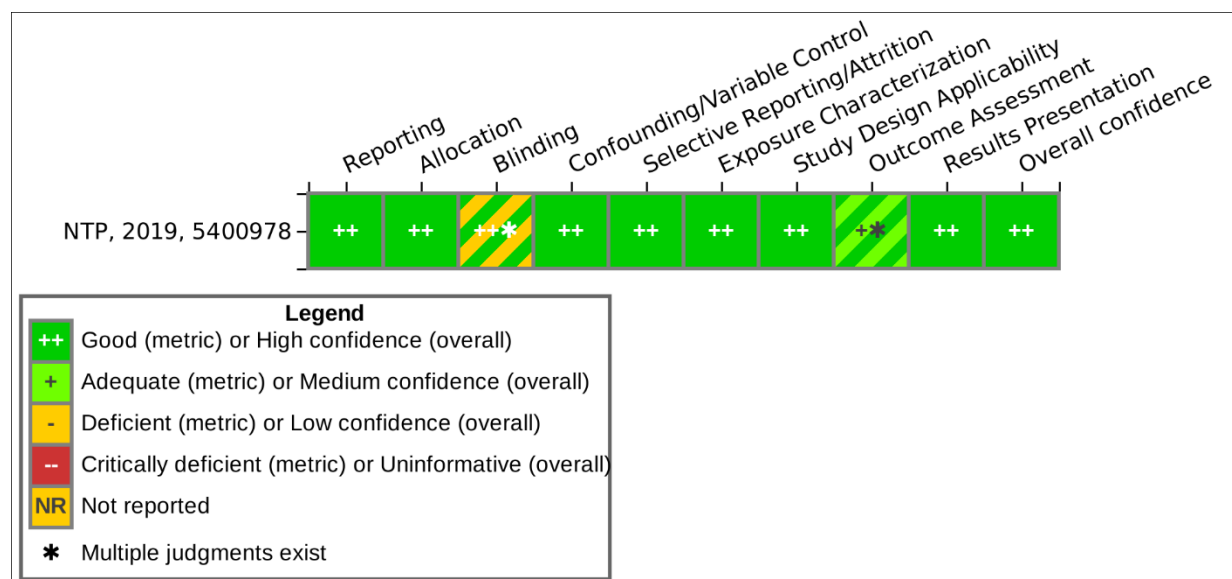


Figure 112. Summary of Study Evaluation for Toxicology Studies of PFOS and Ocular Effects

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}. Due to the limited evidence available, EPA is not considering ocular endpoints for dose-response modeling at this time.

3.3.15.3 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 are no studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to ocular effects.

3.3.15.4 Evidence Integration

In the 2016 Health Assessment for PFOS, no epidemiological evidence of an association between PFOS exposure and ocular health effects was examined. In this updated review, based on one epidemiological study, there is suggestive evidence of 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. Longitudinal studies are needed to ascertain causality between exposure to PFOS and ocular conditions.

Additionally, the one available study in an animal model did not report histopathological ocular abnormalities. Overall, the available literature showing effects of PFOS on the ocular system is

limited. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.16 Dermal

3.3.16.1 Human Evidence

3.3.16.1.1 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. {Ernst, 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.

3.3.16.1.2 Study Quality

Ernst et al. {Ernst, 2019, 5080529} was considered a *medium* confidence study (Figure 113), 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.

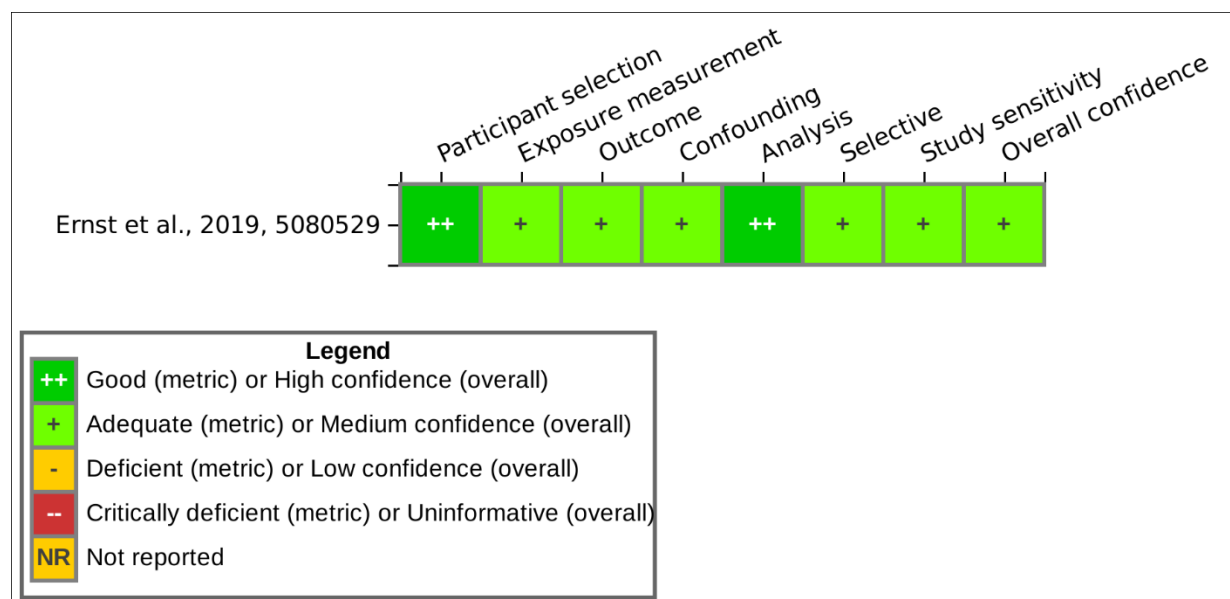


Figure 113. Summary of Study Evaluation for Epidemiology Studies of PFOS and Dermal Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.16.1.3 Findings

Results for the studies that examined dermal outcomes are presented in Table C-25. 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.

3.3.16.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 that investigated the association between PFOS and dermal effects. The study quality evaluation for this study is shown in Figure 114.

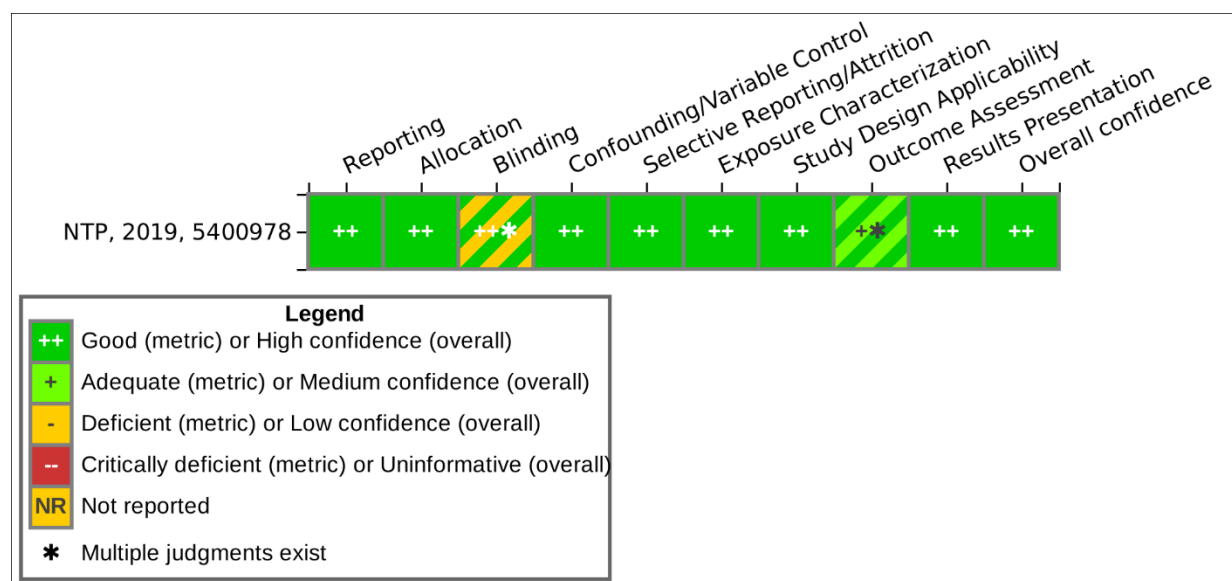


Figure 114. Summary of Study Evaluation for Toxicology Studies of PFOS and Dermal Effects

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}.

3.3.16.3 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 the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to dermal effects.

3.3.16.4 Evidence Integration

In the 2016 Health Effects Support Document, 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.

In the available literature from animal models, there is no reported biological consequence of oral PFOS exposure on dermal tissue. Therefore, these data do not support the skin as a target of PFOS toxicity. Further investigation is needed to fully characterize the relationship between PFOS and the range of dermal effects in humans and animals.

Overall, the available literature showing effects of PFOS on dermal health is limited. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

3.3.17 Cancer

3.3.17.1 Human Evidence

3.3.17.1.1 Introduction

The 2016 Health Effects Support Document for PFOS {U.S. EPA, 2016, 3603365} concluded that there was no evidence of carcinogenic effects for PFOS, but that the small number, breadth and scope of the studies were not adequate to make definitive conclusions. Although an elevated risk of bladder cancer mortality was observed in an occupational study {Alexander, 2003, 1291101}, a subsequent study to ascertain cancer incidence in the cohort observed elevated but non-significant incidence ratios that were 1.7- to 2-fold higher among exposed workers {Alexander, 2007, 4727072}. Mean PFOS serum levels were 94.1 ng/mL. No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment ranging 1–130.5 ng/mL {Eriksen, 2009, 2919344}. Elevated non-significant ORs for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007, 4727072) and the Danish population-based cohort examined by Eriksen et al. (2009, 2919344), and no association was reported by another case-control study in Denmark {Hardell, 2014, 2968084}. A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (1.5–172 ng/mL) reported an association of low magnitude that could not be separated from other perfluorosulfonated acids, and the association was not confirmed in a Danish population {Bonefeld-Jørgensen, 2011, 2150988; Bonefeld-Jørgensen, 2014, 2851186}. Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis and the impact of this potential exposure misclassification on the estimated risks is unknown {Bonefeld-Jørgensen, 2011, 2150988; Hardell, 2014, 2968084}. No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

For this updated review, there are 11 studies (11 publications) that investigated the association between PFOA and cancer that have been identified since the 2016 document (Table C-26). All

studies were conducted on the general population with one in a high-exposure community (i.e., C8 population). Different study designs were also used including 1 cohort study {Fry, 2017, 4181820}, 3 case-control studies {Wielsoe, 2017, 3858479; Tsai, 2020, 6833693; Lin, 2020, 6835434}, 5 nested case-control studies {Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Cohn, 2020, 5412451; Mancini, 2019, 5381529; Shearer, 2021, 7161466}, and 2 cross-sectional studies {Christensen, 2016, 3858533; Ducatman, 2015, 3859843}. The studies were conducted in different study populations including populations from China {Lin, 2020, 6835434}, Denmark {Ghisari, 2017, 3860243}, France {Mancini, 2019, 5381529}, Greenland {Wielsoe, 2017, 3858479}, Taiwan {Tsai, 2020, 6833693}, and the United States {Fry, 2017, 4181820; Christensen, 2016, 3858533; Ducatman, 2015, 3859843; Shearer, 2021, 7161466; Hurley, 2018, 5080646; Cohn, 2020, 5412451}. All the studies measured PFOA in study subject's blood components (i.e., serum or plasma) with one study measuring the levels in the maternal serum {Cohn, 2020, 5412451}. Cancers evaluated included breast {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Mancini, 2019, 5381529; Tsai, 2020, 6833693; Wielsoe, 2017, 3858479}, germ cell tumors {Lin, 2020, 6835434}, prostate {Ducatman, 2015, 3859843}, kidney {Shearer, 2021, 7161466}, and any {Christensen, 2016, 3858533; Fry, 2017, 4181820}.

3.3.17.1.2 Study Quality

Of the 11 studies identified since the 2016 assessment (Figure 115), eight were considered *medium* confidence and three were *low* confidence {{Christensen, 2016, 3858533; Lin, 2020, 6835434; Tsai, 2020, 6833693}}. The main concerns with the *low* confidence studies were the possibility of outcome misclassification, confounding or potential selection bias. Residual confounding was possible mainly due to lack of appropriately addressing SES (SES), which can be associated with both exposure and cancer outcome. Although PFOS has a long half-life in the blood, concurrent measurements may not be appropriate for cancers with long latencies. Temporality of exposure measure in terms of breast cancer development were noted to be an issue in Tsai, 2020, 6833693. Many of the *low* confidence studies also had sensitivity issues due to limited sample size.

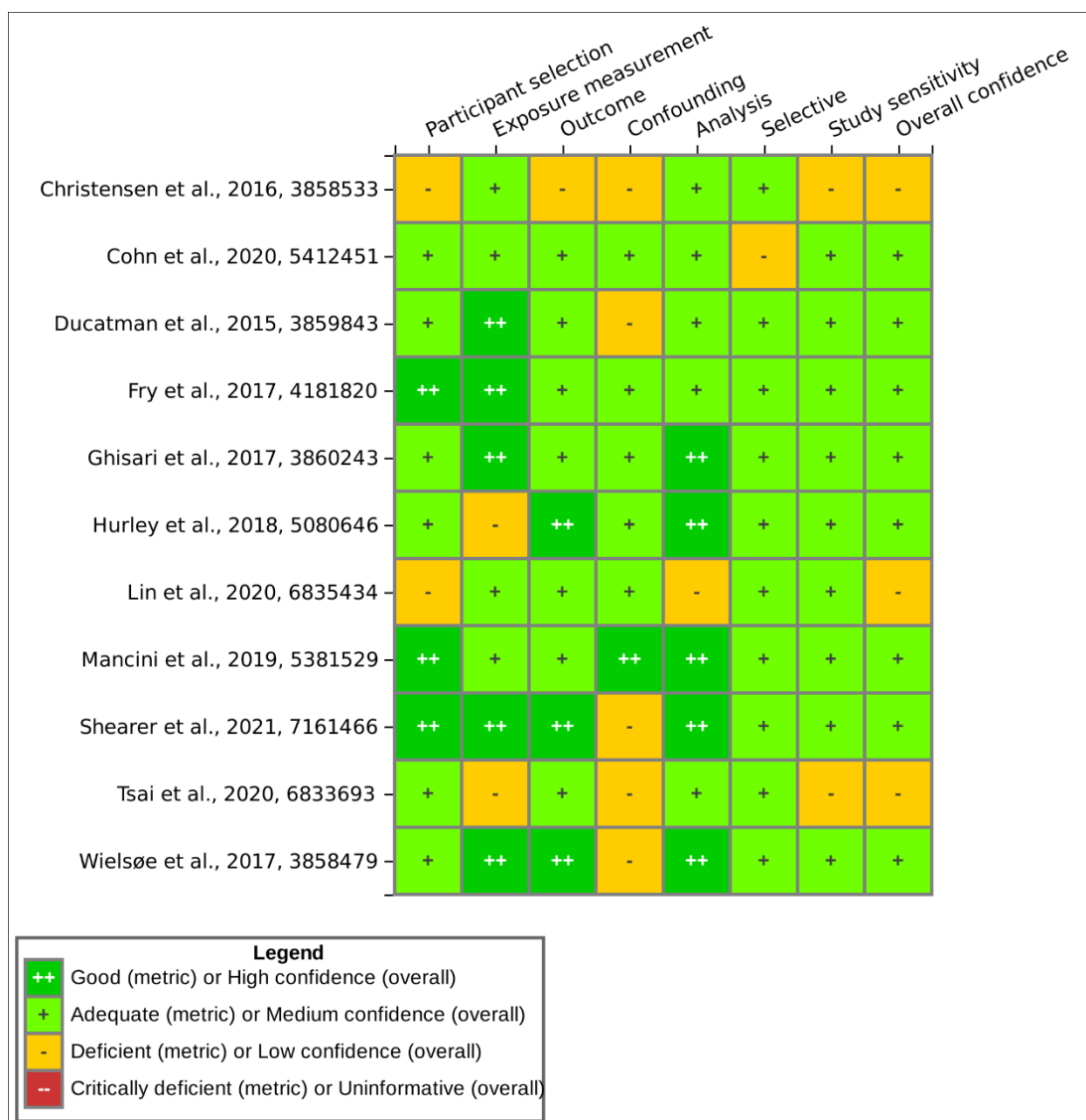


Figure 115. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on [HAWC](#).

3.3.17.1.3 Findings from Children

One *low* confidence study examined cancers in children {Lin, 2020, 6835434} and reported a statistically significant higher median PFOS concentration in 42 pediatric germ cell tumor cases compared to 42 controls in blood samples collected from the children one week after diagnosis. However, the study did not observe an increased risk of germ cell tumors when evaluated on a per ng/mL increase in blood PFOS.

3.3.17.1.4 Findings from the General Adult Population

PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a *medium* confidence study {Shearer, 2021, 7161466}. The study reported a statistically

significant increase in risk in the highest exposure quartile and per doubling of PFOS concentration. After adjusting for other PFAS the association remained elevated in the highest quartile (i.e., adjusted OR=1.14), but it was no longer statistically significant and was lower than the second quartile; additionally, there was no association when evaluated on a per doubling of PFOS.

Six of the general population studies since the 2016 assessment, evaluated PFOS and risk for breast cancer {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Mancini, 2019, 5381529; Tsai, 2020, 6833693; Wielsoe, 2017, 3858479} with mixed results. All studies were case-control studies (with some nested case-controls). One study was considered *low* confidence {Tsai, 2020, 6833693} because of concerns about temporality of exposure measurements and breast cancer development, the control status was not confirmed via examination or medical records, and not addressing SES. The remaining studies were all *medium* confidence. A nested case-control study did not find any association between breast cancer identified through California cancer registry and PFOS concentrations in serum after case diagnosis (Hurley, 2018, 5080646; max concentration of 99.8 ng/mL). Wielsoe, 2017, 3858479 observed a statistically significant increase in risk of breast cancer (most were histologically confirmed) with increasing PFOS (either per ng/mL increase or in the two highest exposure tertile) in a case-control study conducted in Greenland. Two nested case-control studies and one *low* confidence case-control study found associations between PFOS and breast cancer, but only in specific groups of participants {Ghisari, 2017, 3860243; Mancini, 2019, 5381529; Tsai, 2020, 6833693}. Ghisari, 2017, 3860243 reported an increased risk for breast cancer identified from the cancer registry with increasing PFOS concentrations only in participant with a CC genotype (n=36 cases and 47 controls) in the CYP19 gene (cytochrome P450 aromatase). Mancini, 2019, 5381529 reported that the risk for breast cancer (93% verified pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increasing trend in estrogen receptor positive (ER+) and progesterone receptor positive (PR+) breast cancers. The study also observed a significant increase in estrogen receptor- (ER-) and progesterone receptor- (PR-) breast cancers in the second quartile with elevated risks also observed in the other quartiles, but with no trend. The sample size was small with 26 participants having ER- breast cancers and 57 having PR- breast cancers and the number per quartile not provided. Tsai, 2020, 6833693 observed a statistically significant increase in risk for breast cancer with increasing log-transformed PFOS, but only in participants aged 50 years or younger and in ER+ breast cancer in participants aged 50 years or younger. A nested case-control study suggested maternal PFOS was associated with a decreased daughters' breast cancers risk in the first or fourth quartile of TC {Cohn, 2020, 5412451}, but the study did not examine breast cancer subtypes or genetic variants.

One *medium* confidence study based on the C8 Health Study {Ducatman, 2015, 3859843} examined prostate-specific antigen (PSA) as a biomarker for prostate cancer in adult males over age 20 years who lived, worked, or went to school in one of the six water districts contaminated by the DuPont Washington Works facility. No association was observed between PSA levels in either younger (i.e., aged 20–49 years) or older (i.e., aged 50–69 years) men and concurrent mean serum PFOS concentration up to 25 ng/mL. There were no other general population studies evaluating prostate cancer identified since the 2016 assessment.

Two studies examined all cancers together, but collected different information on cancer (i.e., incidence verses mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was not associated with PFOS exposure in a *medium* confidence study of participants over 60 years of age from NHANES (Fry, 2017, 4181820; median PFOA concentration 4.3 ng/g lipid). PFOS was also not associated with self-reported cancer incidence in a *low* confidence study on male anglers over 50 years (Christensen, 2016, 3858533; median concentration 19 ug/L). Christensen, 2016, 3858533 was considered *low* confidence due to the potential of self-selection because participants were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.3.17.2 Animal Evidence

There are 0 studies from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cancer effects. The study quality evaluation for this study is shown in Figure 116.

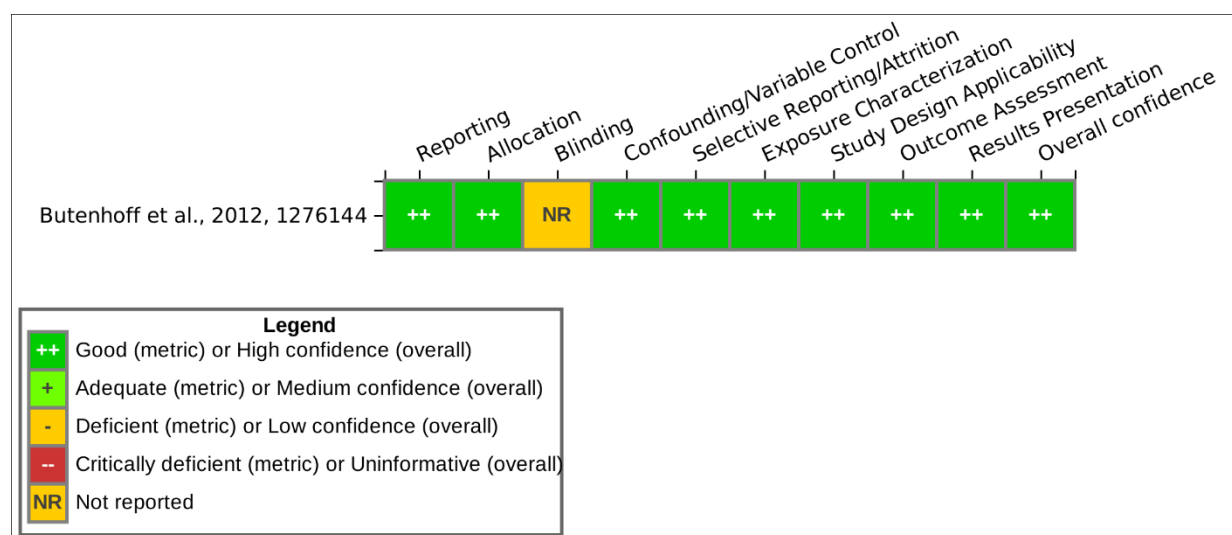


Figure 116. Summary of Study Evaluation for Toxicology Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on [HAWC](#).

A single chronic cancer bioassay in animals was previously identified for PFOS {Thomford, 2002, 5432392; Butenhoff, 2012, 1276144}. In this study, conducted by Thomford (2002, 5432392) and published in Butenhoff et al. (2012, 1276144), male and female Crl:CD®(SD)IGS BR rats were administered diets containing 0, 0.5, 2, 5, or 20 ppm PFOS for 103–104 weeks. Increased incidence of hepatocellular adenomas in the high dose groups for male (7/60; 12%) and female rats (5/60; 8%) and combined adenomas/carcinomas in females (6/60; 10%) were observed, but there was not a clear dose-related response in the lower dose groups. At 105 weeks there was an accompanying increase in eosinophilic clear cell foci, and cystic hepatocellular degeneration in males given 2, 5, and 20 ppm PFOS. Low levels of single cell necrosis in all dose groups for both males and females were identified, though the increase compared to controls was significant only at the highest dose in each sex.

Thyroid and mammary gland tumors were also observed but did not exhibit dose-response {Thomford 2002, 5432392; Butenhoff, 2012, 1276144}. The incidence of thyroid tumors was significantly elevated only in the recovery group males exposed to 20 ppm PFOS for 52 weeks then control diet until termination, but not in the animals receiving 20 ppm for 104 weeks. The most frequent thyroid tumor type in females was C-cell adenomas, but the highest incidence was that for the controls and there was a lack of dose-response among the exposed groups. There was also a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose-response for all tumor classifications.

3.3.17.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cancer outcomes is discussed in Section 3.4.3 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 16 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOS that lead to cancer effects. A summary of these studies is shown in Figure 117. Additional analysis on the mechanistic actions of PFOS on cancer health outcomes is pending and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	1	1	3
Cell Growth, Differentiation, Proliferation, Or Viability	2	0	8	9
Cell Signaling Or Signal Transduction	0	0	5	5
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	0	1	1
Hormone Function	0	1	2	3
Oxidative Stress	1	0	0	1
Xenobiotic Metabolism	0	1	2	3
Other	1	0	1	2
Grand Total	4	2	11	16

Figure 117. Summary of Mechanistic Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on [Tableau](#).

3.3.17.4 Evidence Integration

In summary, the human epidemiological studies identified since the 2016 assessments do not provide additional clarity on the association between PFOS and cancer. No new animal toxicity studies examining the carcinogenicity of PFOS have been published since the 2016 assessment.

There were no new human epidemiological studies identified that evaluated bladder or prostate cancer. A new study supports the association between PFOS and kidney cancer {Shearer, 2021, 7161466}. In addition, 6 new studies were identified that further evaluated breast cancer. The new studies do not provide much additional clarity on possible association with breast cancer as the studies have mixed results. One study did not observe any association between PFOS and breast cancer with serum concentrations up to 99.8 ng/mL {Hurley, 2018, 5080646}, one study observed an association between PFOS and risk for breast cancer {Wielsøe, 2017, 3858479}, three studies reported increased risk only in specific groups of subjects {Ghisari, 2017, 3860243; Mancini, 2019, 5381529; Tsai, 2020, 6833693}, and one suggests that exposure through gestation may decrease risk of breast cancer in the daughters by TC level (Cohn, 2020, 5412451). Overall, while some intriguing findings for PFOS and breast cancer have been observed, the small sample sizes, narrow exposure levels, latency issues and lack of replication of the results for certain subgroups, cancer subtypes or genetic variants, limit the ability to make firm conclusions regarding PFOS and breast cancer.

In the single available chronic animal bioassay examining the potential carcinogenicity of PFOS {Thomford, 2002, 5432392; Butenhoff, 2012, 1276144}, mild increases in the incidence of hepatocellular adenomas were observed in the high dose groups of both male and female rats. However, no clear dose-related response was observed in the lower dose groups. Thyroid and mammary gland tumors were also observed but did not exhibit a dose-dependent response.

Overall, the current assessment supports the findings from the 2016 Health Advisory Health Assessment that the available evidence is not adequate to quantify or make definitive conclusions about the carcinogenicity of PFOS. Therefore, no studies or endpoints from the available epidemiological or animal toxicity literature were considered for the derivation of PODs.

4.0 Dose-Response Assessment

4.1 Non-Cancer

4.1.1 Study and Endpoint Selection

As outlined in Section 3.3, several studies were evaluated further for identification of specific endpoints to carry forward for dose-response (BMD) modeling. The following general considerations were used to prioritize studies for estimating PODs for potential use in toxicity value derivation. Well-conducted (i.e., high or medium confidence) human studies were prioritized for POD derivation when the human data provided an association between PFOS and an adverse effect and were compared to PODs derived from animal data when possible. Such human studies were available for immunotoxicity, developmental, serum lipid, and hepatic effects. For other health effects where the epidemiological data were suggestive of adverse effects (i.e., endocrine and nervous system effects), dose response data from the animal studies were prioritized. Preferred animal studies consisted of medium and high confidence studies of longer exposure duration (e.g., chronic or subchronic studies versus 28-day studies) or with exposure during sensitive windows of development (i.e., perinatal periods) with exposure levels near the lower dose range of doses tested across the evidence base, along with medium or high confidence animal studies evaluating exposure periods relevant to developmental outcomes. These types of animal studies increase the confidence in the RfD because they are based on data

with relatively low risk of bias and do not require low-dose and exposure duration extrapolation. For all other health outcomes (e.g., reproductive, hematological, musculoskeletal, special sense), the human and animal evidence was inconsistent and/or inadequate to assess potential health effects quantitatively. Therefore, these health endpoints were not considered for the derivation of toxicity values.

Table 15 summarizes the studies and endpoints identified for POD derivation.

Table 15. Summary of Endpoints and Studies Considered for Dose-Response Modeling and Derivation of Points of Departure for All Effects in Humans and Rodents

Endpoint	Study Reference and Confidence	Strain/Species/Sex	Notes
Immune Effects			
Reduced antibody concentrations for diphtheria and tetanus	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz-Jørgensen et al. (2018, 5083631); Medium confidence	Human, male and female children	Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects. BMD modeling performed by study authors.
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al., 2016, 3748828; Medium confidence	C57BL/6 Mice, F ₁ males	Indicative of immunosuppression. Effect was consistently observed across multiple studies: Peden-Adams et al. (2008, 1424797), Dong et al. (2009, 1424951), Zheng et al. (2009, 1429960), Keil et al. (2008, 1332422).
Extramedullary Hematopoiesis in the Spleen Developmental Effects	NTP 2019, 5400978; High confidence	Sprague-Dawley Rats, male and female	Blood cell production outside of the bone marrow which occurs when normal cell production is impaired.
Decreased Birth Weight	Chu et al., 2020, 6315711; Sagiv et al., 2018, 4238410; Starling et al., 2017, 3858473; Wikström et al., 2020, 6311677; High confidence	Human, male and female infants	Effect was generally large in magnitude and coherent with epidemiological evidence for other biologically related effects.
Decreased Fetal Body Weight	Lee et al., 2015, 2851075; Medium confidence	CD-1 Mice, F ₁ males and females	Effect was consistently observed across multiple studies and species.
Decreased Pup Body Weight	Luebker et al., 2005, 757857; Medium confidence	Sprague-Dawley Rats, F ₁ male and female	Effect was consistently observed across multiple studies and species.
Increased Number of Dead Fetuses	Lee et al., 2015, 2851075; Medium confidence	CD-1 Mice, females	Effect was consistently observed across multiple studies and species.
Serum Lipid Effects			

Endpoint	Study Reference and Confidence	Strain/Species/Sex	Notes
Increased Total Cholesterol	Dong et al., 2019, 5080195; Medium confidence	Human, male and female	Effect supported by an association in PFOS and blood pressure from the epidemiological studies. BMD modeling performed by study authors.
Hepatic Effects			
Individual Cell Necrosis in the Liver	Butenhoff et al., 2012, 1276144; High confidence	Sprague-Dawley rats, females	Effect was observed in males and females, but was accompanied by inflammatory cell response in females. Effect was qualitatively observed in Xing, 2016, 3981506; Cui, 2009, 757868. Effect is further supported by changes in serum ALT levels in animals and humans.
Endocrine Effects			
Decreased Free T4	NTP, 2019, 5400978; High confidence	Sprague-Dawley rats, male and female	Effect was generally large in magnitude and consistent with hypothyroxinemia in that a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology.
Decreased Total T4	NTP, 2019, 5400978; High confidence	Sprague-Dawley rats, male and female	Effect was generally large in magnitude and consistent with hypothyroxinemia in that a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology.
Decreased Total T3	NTP, 2019, 5400978; High confidence, Seacat et al., 2002, 757853; Medium confidence	Sprague-Dawley rats, male and female; Cynomolgus Monkeys, male and female	Effect was generally large in magnitude and consistent with hypothyroxinemia in that a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology.
Nervous Effects			
Decreased Performance on the Object Location Recognition Memory Test	Mshaty et al., 2020, 6833692; Medium confidence	C57BL/6J, F ₁ males	Accompanied by dose-dependent effects on the object recognition memory test and visual discrimination task and significant increases in hippocampal neurotransmitter concentrations, including glutamate and GABA.

4.1.2 Estimation or Selection of Points of Departure (PODs) for RfD Derivation

Consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR to represent a minimal, biologically significant level of change. The BMD technical guidance (U.S. EPA, 2012) sets up a hierarchy by which BMRs are selected, with the first and preferred approach using a biological or toxicological basis to define what minimal level of response or change is biologically significant. If that biological or toxicological information is lacking, the BMD technical guidance recommends BMRs that can be used instead, specifically a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk

for dichotomous data. The BMRs selected for dose-response modeling of PFOS-induced health effects are listed in Table 16 along with the rationale for their selection.

Table 16. Benchmark Response Levels Selected for BMD Modeling of Health Outcomes

Endpoint	BMR	Rationale
Immune Effects		
Reduced antibody concentrations for diphtheria and tetanus	5%	Diphtheria and tetanus are serious infectious diseases that can lead to medical conditions that range in severity and including the most severe, fatality. Anti-tetanus and anti-diphtheria antibody concentrations can protect against and prevent these diseases. For an endpoint of mortality, a BMR of 1% is recommended. For a developmental effect, a BMR of 5% is recommended. Given the range of health outcomes includes fatality and the effect on children, a BMR of 5% is a reasonable and appropriate choice. The study design of the critical study is of sufficient statistical sensitivity to support this BMR {U.S. EPA, 2012, 1239433}.
Decreased Plaque Forming Cell (PFC) Response to SRBC	1SD	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on 1 standard deviation (SD) for continuous endpoints when biological information is not sufficient to identify the BMR.
Extramedullary Hematopoiesis in the Spleen	10%	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on a 10% for dichotomous endpoints when biological information is not sufficient to identify the BMR.
Developmental Effects		
Decreased Birth Weight	5%	EPA guidance recommends a 5% BMR for developmental effects (reference EPA risk assessment guidelines). A 5% change was used because effects were observed after exposure after developmental exposure (US EPA, 1991, 732120). A 5% change in markers of growth (one aspect of development) in gestational studies (e.g., fetal weight), without leading to death, has generally been considered an appropriate biologically significant response level and has been used as the BMR for benchmark dose modeling in final IRIS assessments for some other chemicals U.S. EPA (2003, 1290574), U.S. EPA (2004, 198783), U.S. EPA (2012, 3114808).

Endpoint	BMR	Rationale
Decreased Fetal Body Weight, Decreased Pup Body Weight, Increased Number of Dead Fetuses	0.5SD	A 0.5SD change was used because the developmental effects were observed after exposure after developmental exposure (US EPA, 1991, 732120). A 0.5SD change in markers of growth/development in gestational studies (e.g., fetal weight), without leading to death, has generally been considered a minimally biologically significant response level and has been used as the BMR for benchmark dose modeling in final IRIS assessments for other chemicals U.S. EPA (2003, 1290574), U.S. EPA (2004, 198783), U.S. EPA (2012, 3114808).
Serum Lipids		
Increased Cholesterol	10%	No information is readily available that allows for determining a minimally biological significant response. Modeling human cholesterol used an adverse level of 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. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on a 10% for dichotomous endpoints when biological information is not sufficient to identify the BMR.
Hepatic Effects		
Individual Cell Necrosis	10%	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on a 10% for dichotomous endpoints when biological information is not sufficient to identify the BMR.
Endocrine Effects		
Decreased free T4, total T4 and total T3	1 SD	No information is readily available that allows for determining a minimally biological significant response. Decreases in thyroid hormones can lead to severe medical conditions. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify the BMR. Additionally, 1 SD is consistent with the BMR used for adult thyroid hormone changes in the perfluorobutane sulfonate (PFBS) toxicity assessment based on similar rationale, as the levels at which there is concern for hypothyroxinemia in adults is unclear.
Neural Effects		
Decreased Performance on the Object Location Recognition Memory Test	1 SD	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance U.S. EPA (2012, 1239433) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify the BMR. Additionally, 1

Endpoint	BMR	Rationale
		SD is consistent with the BMR used for adult thyroid hormone changes in the PFBS toxicity assessment as the levels at which there is concern for hypothyroxinemia in adults is unclear.

4.1.3 Toxicokinetic Modeling Approaches to Convert Administered Dose to Internal Dose in Animals and Humans

4.1.3.1 Toxicokinetic Model for Animal Internal Dosimetry

Following review of the available models in the literature, EPA chose the Wambaugh et al. (2013, 2850932) model based on the following criteria:

1. Availability of model parameters across the species of interest.
2. Agreement with out-of-sample datasets.
3. Flexibility to implement life-stage modeling.

In this case, an oral dosing version of the original model introduced by Andersen et al. (2006, 818501) and summarized in Section 3.2.2 was selected for having the fewest number of parameters that would need to be estimated. In addition, the Wambaugh et al. (2013, 2850932) approach allowed for a single model structure to be used for all species in the toxicological studies allowing for model consistency for the predicted dose metrics associated with LOAELs and NOAELs from 13 animal studies of PFOS.

4.1.3.1.1 Animal Model Parameters

The 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 (Table 17). The predictions were generally similar to the experimental values with the exception of male CD1 mice where model simulations over predicted the calibration time-course concentration dataset. However, the female-specific parameters for CD1 mice predicted the male-specific concentration data well (Appendix E) resulting in female-specific parameters being used for all mouse-specific PFOS modeling. 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 is the focus of Section 4.1.2.

Table 17. PK Parameters from Wambaugh et al., 2013 Meta-Analysis of Literature Data for PFOS

Parameter	Units	CD1 Mouse (F) ^a	CD1 Mouse (M) ^a	Sprague- Dawley Rat (F) ^a	Sprague- Dawley Rat (M) ^a	CynomolgusMonkey (M/F) ^a
Body weight ^b (BW)	kg	0.02	0.02	0.203	0.222	3.42
Cardiac Output ^c (Q _{cc})	L/h/kg ^{0.74}	8.68	8.68	12.39	12.39	19.8
Absorption rate (k _a)	1/h	1.16 (0.617–42,400)	433.4 (0.51–803.8)	4.65 (3.02–1,980)	0.836 (0.522–1.51)	132 (0.225–72,100)
Central Compartment Volume (V _{cc})	L/kg	0.264 (0.24–0.286)	0.292 (0.268–0.317)	0.535 (0.49–0.581)	0.637 (0.593–0.68)	0.303 (0.289–0.314)
Intercompartment transfer rate (k ₁₂)	1/h	0.0093 (2.63×e ⁻¹⁰ –38,900)	2,976 (2.8×e ⁻¹⁰ –4.2×e ⁴)	0.0124 (3.1×e ⁻¹⁰ –46,800)	0.00524 (2.86×e ⁻¹⁰ –43,200)	0.00292 (2.59×e ⁻¹⁰ –34,500)
Intercompartment ratio (R _{V2:V21})	Unitless	1.01 (0.251–4.06)	1.29 (0.24–4.09)	0.957 (0.238–3.62)	1.04 (0.256–4.01)	1.03 (0.256–4.05)
Maximum resorption rate (T _{maxc})	μmol/h	57.9 (0.671–32,000)	1.1×e ⁴ (2.1–7.9x e ⁴)	1,930 (4.11–83,400)	1.34×e ⁻⁶ (1.65×e ⁻¹⁰ –44)	15.5 (0.764–4,680)
Renal resorption affinity (K _T)	μmol	0.0109 (1.44×e ⁻⁵ –1.45)	381 (2.6×e ⁻⁵ –2.9×e ³)	9.49 (0.00626–11,100)	2.45 (4.88×e ⁻¹⁰ –60,300)	0.00594 (2.34×e ⁻⁵ –0.0941)
Free fraction	Unitless	0.00963 (0.00238–0.0372)	0.012 (0.0024–0.038)	0.00807 (0.00203–0.0291)	0.00193 (0.000954–0.00249)	0.0101 (0.00265–0.04)
Filtrate flow rate (Q _{filc})	Unitless	0.439 (0.0125–307)	27.59 (0.012–283)	0.0666 (0.0107–8.95)	0.0122 (0.0101–0.025)	0.198 (0.012–50.5)
Filtrate volume (V _{filc})	L/kg	0.00142 (4.4×e ⁻¹⁰ –6.2)	0.51 (3.5×e ⁻¹⁰ –6.09)	0.0185 (8.2×e ⁻⁷ –7.34)	0.000194 (1.48×e ⁻⁹ –5.51)	0.0534 (1.1×e ⁻⁷ –8.52)

M = male; F = female.

Means and 95% confidence interval (in parentheses) from Bayesian analysis are reported. For some parameters the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).

^a Data sets modeled for the mouse and rat were from Chang et al. (2012, 1289832) and for the monkey from Seacat et al. (2002, 757853) and Chang et al. (2012, 1289832).

^b Average bodyweight for species: individual-specific bodyweights.

^c Cardiac outputs obtained from Davies and Morris (1993, 192570).

While this model provided parameters for mice, rats, and monkeys, there are some limitations that must be acknowledged. First, posterior parameter distributions for each sex/species combination were determined using a single study. Any variability between studies or differences in study design will not be accounted for in the uncertainty of these parameters. Second, issues with parameter identifiability for some sex/species combinations results in large ranges for some parameters. The wide credible intervals of the parameter distributions represent parameters that are not sensitive to the concentration-time datasets on which the model was trained. However, these uncertain model parameters will not impact the median prediction used for BMD modeling and simply demonstrate that the available data are unable to identify all parameters across every species over the range of doses used for model calibration. Finally, the model is only parameterized using adult, single dose, PFOS study designs. Any gestational/lactational PK modeling would require additional parameters to describe the relevant life stages.

Even with these limitations, the Wambaugh et al. (2013, 2850932) model allowed for sex-dependent concentration-time predictions for PFOS across mouse, rats, and monkeys, adequately predicted newer datasets published after publication, and was amendable to adding a life stage component for predicting developmental study designs. For these reasons, we used this model for animal-specific PK predictions.

4.1.3.1.2 Out-of-Sample Comparisons

To evaluate the model's ability to predict PFOS concentration-time data in the species of interest, we compared model fits to in vivo datasets published following the 2016 HESD (Table 18). For rats, the data of Kim et al. (2016, 3749289), and Huang et al. (2019, 7410147) were used. Model simulations demonstrated good agreement with available data for adult time-course PFOS PK predictions in the rat. However, there was no comparable PK dataset for PFOS in mice. Therefore, only the original study used for parameter determination {Chang, 2012, 1289832} was compared to model simulations and this comparison demonstrated agreement with the in vivo data.

Using the Wambaugh et al. (2013, 2850932) model, we predicted the half-life, V_d , and clearance and compared these species-specific predictions to values obtained from in vivo studies using classical PK analysis when data were available.

Table 18. Model Predicted and Literature PK Parameter Comparisons for PFOS

	Male			Female		
	$t_{1/2,\beta}$ (days)	$V_{d,\beta}$ (L/kg)	CL (L/d/kg)	$t_{1/2,\beta}$ (days)	$V_{d,\beta}$ (L/kg)	CL (L/d/kg)
Rat						
Model	44.13	0.638	0.01	282.05	0.538	0.0013
Literature	28.7 ^a , 39.7 ^b	0.382 ^a , 0.681 ^b	0.0092 ^a , 0.013 ^b	24.8 ^a , 32.8 ^b	0.288 ^a , 0.421 ^b	0.008 ^a , 0.009 ^b
Mouse						
Model	134.83	0.472	0.0024	38.4	1.41	0.0255
Literature	—	—	—	—	—	—

PK = pharmacokinetic; PFOS = perfluorooctane sulfonic acid; $t_{1/2,\beta}$ = terminal-phase elimination half-life; $V_{d,\beta}$ = volume of distribution during the terminal phase; CL = clearance.

^a Information obtained from Kim et al. (2016, 3749289).

^b Information obtained from Huang et al. (2019, 5387170).

Following out-of-sample dataset evaluation of the female rat PK parameters (Table 18) and visual inspection of the resulting concentration-time fits, we determined that only male PK model parameters would be used for all rat-specific modeling. This assumption agrees with Kim et al. (2016, 3749289) where they report no PK differences between the sexes for PFOS ADME.

4.1.3.1.3 Life-Stage Modeling

The Wambaugh et al. (2013, 2850932) model was modified to allow for a gestation, lactation, and post-weaning phase (Figure 118). Using the original model structure and published parameters, simulations assumed that dams were dosed prior to conceptions and up to the date of parturition. Following parturition, a lactational phase involved PFOS transfer from the breastmilk to the suckling pup where the pup was modeled as a one-compartment PK model. Finally, a post-weaning phase utilized the body weight scaled Wambaugh model to simulate dosing to the growing pup and accounted for filtrate rate as a constant fraction of cardiac output.

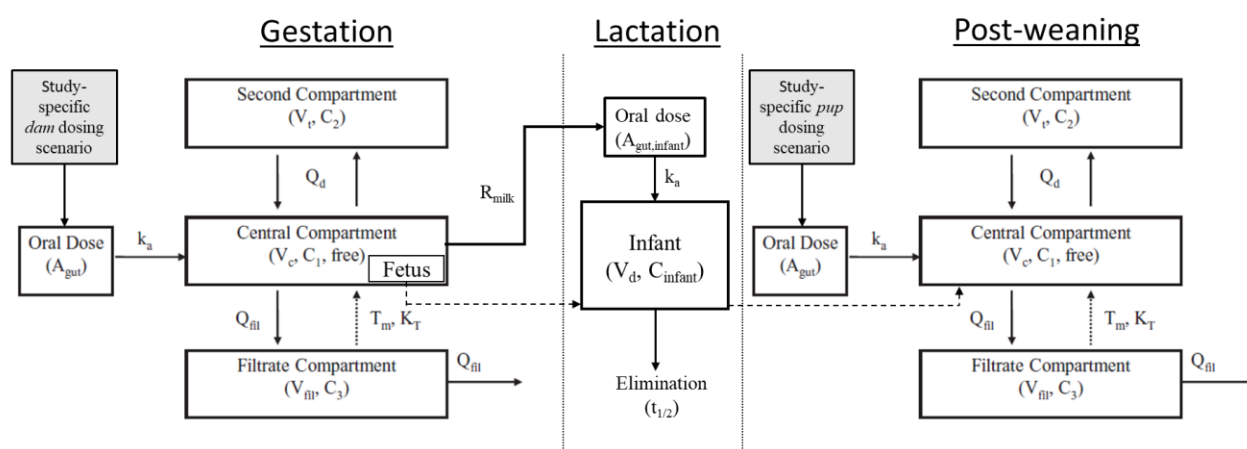


Figure 118. Model Structure for Life Stage Modeling

Model parameters for three-compartment model are the same as those described earlier. Pup-specific parameters include milk consumption in kg_{milk}/day (R_{milk}), infant-specific volume of distribution (V_d), and infant-specific half-life ($t_{1/2}$).

This methodology was adapted from Kapraun et al. (2021 in prep., 9641977) and relies on the following assumptions for gestation/lactation modeling:

- During gestation and up through the instant birth occurs, the ratio of the fetal concentration (mg of substance per ml of tissue) to the maternal concentration is constant.
- All (100%) of the substance in the breast milk ingested by the offspring is absorbed by the offspring.
- The elimination rate of the substance in offspring is proportional to the amount of substance in the body and is characterized by an infant-specific half-life that is a fixed constant for any given animal species as described in Table 19 below.
- Following the lactation period, infant time course concentrations are tracked using the body weight scaled Wambaugh model to model post-weaning exposure and infant growth.

A simple one-compartment model for infant lactational exposure was chosen because of differences in beta-phase V_d s between measured values in the literature and predicted V_d s following extrapolation to low-body weights. In humans, the V_d is assumed to be extracellular water. Goeden et al. (2019, 5080506) adjusts for life stage-specific changes in extracellular water using an adjustment factor where infants have 2.1 times more extracellular water than adults resulting in a larger V_d . However, this large difference in extracellular water is not observed in rats {Johanson, 1979, 9641334}. Johanson et al. (1979, 9641334) demonstrated a 5% decrease in blood water content from early postnatal life (~0.5 weeks) to adulthood (> 7 weeks) in the rat. Therefore, we used the literature reported V_d {Kim, 2016, 3749289; Chang, 2012, 1289832} for the one compartment model to describe infant toxicokinetics (Table 19). Finally, the Wambaugh model was not parametrized for a post-partum infant and it was not possible to evaluate the mechanistic assumptions for renal elimination with postnatal toxicokinetic data. Therefore, the parameters listed in Table 19 in a one-compartment gestation/lactation model were used in conjunction with the parameters published in Wambaugh et al. (2013, 2850932) to predict developmental dose metrics for PFOS.

Table 19. Additional PK Parameters for Gestation/Lactation for PFOS

Parameter	Units	Rat	Mouse
Maternal Milk:Blood Partition Coefficient (P_{milk})	Unitless	0.13 ^a	0.32 ^e
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.83 ^b	0.41 ^f
Elimination Half-Life ($t_{1/2}$)	Days	40 ^c	36.87 ^g
Volume of Distribution (V_d)	L/kg	0.28 ^d	0.26 ^g
Starting Milk Consumption Rate (r^0_{milk})	kg _{milk} /day	0.001 ^h	0.0001 ⁱ
Week 1 Milk Consumption Rate (r^1_{milk})	kg _{milk} /day	0.003 ^h	0.0003 ⁱ
Week 2 Milk Consumption Rate (r^2_{milk})	kg _{milk} /day	0.0054 ^h	0.00054 ⁱ
Week 3 Milk Consumption Rate (r^3_{milk})	kg _{milk} /day	0.0059 ^h	0.00059 ⁱ

PK = pharmacokinetic; PFOS = perfluorooctane sulfonic acid.

^a Information obtained from Loccisano et al. (2013, 1326665) (derived from Kuklenyik et al. (2004, 1598132)).

^b Information obtained from Lau et al. (2003, 757854).

^c Average of male/female half-lives reported in Huang et al. (2019, 5387170), Kim et al. (2016, 3749289), and Chang et al. (2012, 1289832).

^d Information obtained from Kim et al. (2016, 3749289).

^e Assume same P_{milk} as PFOA (lack of mouse data).

^f Information obtained from Wan et al. (2020, 7174720).

^g Information obtained from Chang et al. (2012, 1289832).

^h Information obtained from Kapraun et al. (2021 in prep., 9641977) (adapted from Lehmann et al. (2014, 2447276)).

ⁱ Information obtained from Kapraun et al. (2021 in prep., 9641977) (mouse value is 10% of rat based on assumption that milk ingestion rate is proportional to body mass).

These developmental-specific parameters include the maternal milk: blood PFOS partition coefficient (P_{milk}), the ratio of the concentrations in the fetus(es) and the mother during pregnancy (R_{fm}), the species-specific in vivo determined half-life ($t_{1/2}$) and V_d for PFOS, and the species-specific milk consumption rate during lactation (r^i_{milk}) for the i^{th} week of lactation. Milk rate consumptions are defined as:

- r^0_{milk} , the starting milk consumption rate in kg milk per day (kg/d);
- r^1_{milk} , the (average) milk consumption rate (kg/d) during the first week of lactation (and nursing);
- r^2_{milk} , the (average) milk consumption rate (kg/d) during the second week of lactation; and
- r^3_{milk} , the (average) milk consumption rate (kg/d) during the third week of lactation.

where R_{milk} used in the model is a piecewise linear function comprising each r^i_{milk} depending on the week of lactation.

Using this gestation/lactation model, we fit one study for PFOS exposure in rats to ensure the model predicted the time-course concentration curves for both the dam and the pup. For all gestation/lactation studies, time zero represents conception followed by a gestational window (21 days for the rat, 17 days for the mouse). Dosing prior to day zero represents pre-mating exposure to PFOS.

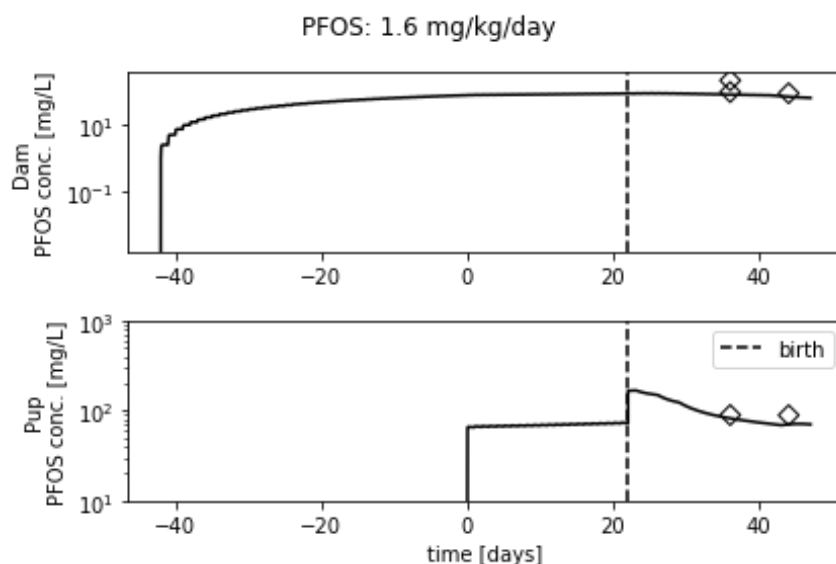


Figure 119. Gestation/Lactation Predictions of PFOS in the Rat

Top panel represents predicted dam concentrations with open diamonds (\diamond) representing the dam concentrations reported in Luebker et al. (2005, 1276160). Bottom panel represents predicted pup concentrations with open diamonds (\diamond) representing the reported pup concentrations in Luebker et al. (2005, 1276160) where the source of PFOS exposure is from the breast milk. Vertical dashed line represents birth.

Figure 119 demonstrates the model's ability to predict gestation/lactation study design in the rat for dams exposed to 1.6 mg/kg/day PFOA giving birth to pups who are exposed through lactation {Luebker, 2005, 1276160}. For developmental PK simulations, the original Wambaugh et al. (2013, 2850932) model with increasing maternal weight predicts dam concentrations in female rats while the one compartmental lactational transfer model predicts infant concentrations for pups exposed both *in utero* and/or through lactation.

While this model fits the selected PFOS developmental study design, there are several limitations to using this method. First, perinatal fetal concentrations assume instantaneous equilibration across the placenta and does not account for the possibility of active transporters mediating distribution to the fetus. In addition, clearance in the infant during lactation is assumed to be a first order process governed by a single half-life. At low doses, this assumption is in line with adult clearance, but it is unclear how physiological changes during development impact the infant half-life. Finally, PFOS concentrations in the breast milk are assumed to partition passively from the maternal blood. This assumption does not account for the presence of active transport in the mammary gland or time-course changes for PFOS uptake to the milk.

The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies or in humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose-metrics across life stages were selected for simulation in a mouse, rat, monkey, or human. Concentrations of PFOA in blood were considered for all the dose-metrics. For studies in adult animals the dose-metric options were generally a maximum blood concentration (C_{max} , mg/L) and a time averaged blood concentration i.e., the AUC over the duration of the study (AUC, mg * day/L) or the blood concentration over the last 7 days (C_{last-7} , mg/L). In developmental studies dose-metrics were developed for the dam, the fetus (during gestation) and the pup (during lactation) for both time C_{max} and averaged blood concentrations. In the dam, the C_{max} and the average blood concentration (C_{avg}) and the AUC were calculated over a range of life stages: during gestation (AUC_{avg_dam_gest}), during lactation (AUC_{avg_dam_lact}) or combined gestation and lactation (AUC_{avg_dam_gest_lact}). In pups for C_{max} for two different life stages were calculated either during gestation or lactation ($C_{max_pup_gest}$, $C_{max_pup_lact}$). In pups for time averaged metrics, a C_{avg} and AUC were calculated for during gestation, lactation or combined gestation and lactation ($C_{avg_pup_gest}$, $C_{avg_pup_lact}$, $C_{avg_pup_gest_lact}$ and AUC_{avg_pup_gest}, AUC_{avg_pup_lact}, AUC_{avg_pup_gest_lact}).

4.1.3.2 Toxicokinetic Model for Human Dosimetry

Our key factors in model determination were to implement a human model from the literature that required minimal new development, that was able to model gestational and lactational exposure to infants, and that was able to describe time course changes in serum concentration due to changes in bodyweight during growth. In addition, previous modeling efforts suggests that limiting model complexity helps to prevent errors and facilitates rapid implementation {Bernstein, 2021, 9639956}. For the human and animal endpoints of interests, serum concentration was identified 9639956 as a suitable internal dosimetry target. This also pointed toward the acceptability of a simpler model that did not have individual tissue dosimetry.

For these reasons, EPA selected the model published by Verner et al. (2016, 3299692), which is a one compartment developmental model for humans. Several adjustments were undertaken to

facilitate the application of the model to our use. The first of which was to implement the model, originally written for acslX, in an R/MCSim framework. This allows for the code to be more accessible to others by updating it to a contemporary modeling language. In addition to the modeling language conversion, a few other modifications were made. A second modification was to the bodyweight curves. Bodyweight curves for non-pregnant adults were based on Centers for Disease Control and Prevention (CDC) growth data for juveniles and values from the EPA Exposure Factors Handbook in adults {Kuczmarski, 2002, 3490881; U.S. EPA, 2011, 786546}. Linear interpolation was used to connect individual timepoints from these two sources to produce a continuous function over time. Bodyweight during pregnancy was defined based on selected studies of maternal body weight changes during pregnancy {Portier, 2007, 192981; Carmichael, 1997, 1060457; Thorsdottir, 1998, 4940407; Dewey, 1993, 1335605; U.S. EPA, 2011, 786546}.

A third modification was the update of two parameters, the ratio of PFOS concentration in cord blood to maternal serum, and the ratio of PFOS concentration in breastmilk and maternal serum. In the original model these parameters were based on an average of values available in the literature; here, we identified literature made available since the original model was published and updated those parameters with the averages of all identified values (Table 20). The values for cord blood to maternal serum ratio are presented in Section D.2.3. One restriction implemented on the measurements of the cord blood to maternal serum ratio was to only include reports where the ratio was reported. This was due to potential bias that could be introduced if more data are below the LOD for measurement in cord blood compared to maternal serum.

This updated model was used to simulate the HED from the animal PODs that were obtained from BMD modeling of the animal studies (Table 21) It was also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling. For PODs resulting from chronic exposure, such as a long-term animal study or an epidemiological study, the steady state approximation was used to calculate an HED that would result in the same dose metric after chronic exposure. For PODs from exposure to developmental animals, the life-stage developmental model was used to calculate an HED that results in the same dose metric during the developmental window selected by the Dose-Response team.

Table 20. Summary of Studies Reporting the Ratio of PFOS Levels in Breastmilk and Maternal Serum or Plasma

Source	HERO ID	Milk: Maternal Ratio	New Study
Haug 2011	2577501	0.014	No
Seung-Kyu Kim 2011	2919258	0.011	No
Liu 2011	2919240	0.020	No
Karrman 2007	1290903	0.010	No
Cariou 2015 ^a	3859840	0.011	Yes
Sunmi Kim 2011 ^b	1424975	0.030	Yes
Verner 2016	3299692	0.014	–
Additional Studies	–	0.016	–

Whether studies were included in the analysis of Verner et al. (2016, 3299692) is noted. The reported values were based on the mean of ratios in the study populations except when noted otherwise.

^a Median result based on the report of Pizzurro et al. (2019, 5387175).

^b Median result as reported by the authors.

Several alternative models to EPA's updated version of the Verner et al. (2016, 3299692) model for the calculation of HED from an internal POD were considered. Typically, PBPK models are preferred over a one-compartment approach because they can provide individual tissue information and have a one-to-one correspondence with the biological system that can be used to incorporate additional features of PK, including tissue specific internal dosimetry and local metabolism. In addition, though PBPK models present a great increase in complexity; many of the additional parameters are chemical-independent and have widely accepted values. Even some of the chemical-dependent values can be extrapolated from animal studies when parameterizing a model for humans, where data are typically scarcer. The decision to not use one of the PBPK models for PFOS was motivated in part by previous issues identified when evaluating the application of PBPK models to other PFAS compounds for the purpose of risk assessment. In general, it is uncommon for a model to pass EPA's extensive internal QA review without some technical errors being identified {Bernstein, 2021, 9639956}. However, while these errors usually don't substantially alter the results of the model, correction of the free-fraction error was judged to result in a significant impact that could not be easily resolved. The correction of any error can be time consuming to verify as biologically appropriate and consistent with available PK data, sometimes requiring extensive consultation with the original model authors. The large majority of PBPK models for PFOS are based on the original publications of Loccisano, et al. {Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665} and it was noted during a review of this model's code that the implementation of protein binding appears to 'double-count' the parameter that corresponds to the free fraction of PFOS in plasma.

A new publication describing a developmental PBPK model in rats and humans was also evaluated for this effort {Chou, 2021, 7542658}. This model used the in vitro extrapolation that was previously developed by Worley et al. (2015, 3981311) for PFOA as an initial point for parameter optimization for PFOS. The complex nature of this renal model, with processes for resorption, secretion, and passive diffusion presented multiple competing options for parameterization. Specifically, the set of available model parameters can take numerous values that fit the human observations equally well. However, when the model is applied within similar conditions to the human observations, predicting the exact values of the parameters may not impact the model's ability to predict the targeted biomarkers (i.e., human milk, fetal serum, and maternal serum). For our purposes, it was not clear, whether the exposure and internal doses we needed to model would be within the bounds of the doses used to parameterize the Chou et al. (2021, 7542658) model.

Due to the previous issues in implementing PBPK models for PFAS, the known issues with the Loccisano model and the models based upon it and the concerns about application of the Chou et al. (2021, 7542658) model outside its original parameterization space, we decided that a one-compartment model was a good approach for this effort. Another justifying factor for the use of a one-compartment model was that a one-compartment model is best suited to predict blood (or serum/plasma) concentrations, and a major proportion of the PFOS in the body is found in serum/plasma due to albumin binding {Forsthuber, 2020, 6311640}. This makes serum/plasma a good biomarker for exposure. Additionally, there were no other specific tissues that were

considered essential to describe the dosimetry of PFOS. A full PBPK model can predict serum concentrations equally well, but with many more parameters, many of which are difficult to predict for PFOS due to parameter identifiability issues. PFOS presents an unusually high barrier in this regard because much of its PK is dependent on the interaction between PFOS and proteins in the form of binding {Frosthuber, 2020, 6311640} and active transport {Zhao, 2017, 3856461}. These protein interactions are more difficult to extrapolate from animal studies to humans than PK that is dependent on blood flow and passive diffusion.

The only one-compartment approach for PFOS was the model of Verner et al. (2016, 3299692). As this was a developmental model, with life stage time-course descriptions of body weight change and the ability to predict serum concentration, this model met all our requirements while presenting a minimum of technical and QA challenges.

There are several limitations associated with our modeling approach. One of them is that the key parameter, clearance, which is a function of the measured values, half-life and V_d , is difficult to estimate in the human general population. The measurement of half-life is hindered by the slow excretion and ongoing exposure. Some of the variability in measured half-life values probably reflects true variability in the population, rather than the uncertainty in the measurement of the value. There is also a lack of reported V_d values in humans, with only one reported value available {Thompson, 2010, 2919278}. In the Verner et al. (2016, 3299692) model these values are assumed to apply across ages and sexes. The excretion of PFOS in children and infants is not well understood but could be different due to the ontogeny of renal transporters, changes in overall renal function, and changes in the amount of protein binding, especially in serum. It is even difficult to predict the overall direction of change (higher or lower than adults) without a clear understanding of all these age-dependent differences. V_d is also expected to be different in children. Children have a higher body water content, which results in a greater distribution of hydrophilic chemicals to tissues compared to blood in neonates and infants compared to adults {Fernandez, 2011, 9641878}. This is well known in drugs, but PFOS is unlike most drugs in that it undergoes extensive protein interaction, such that its distribution in the body is driven primarily by protein binding and active transport. Hence, it is difficult to infer the degree to which increased body water content will impact the distribution of PFOA.

Another limitation of the modeling approach is that our description of breastfeeding was relatively simplistic. Several assumptions of this approach were that breastfeeding took place for 1 year, there was a constant relationship between maternal serum and breastmilk PFOS concentrations, and weaning was an immediate process with the infant switching from a fully breastmilk diet to the background exposure at exactly 1 year.

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOS Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Table 21 displays the POD and estimated internal and POD_{HEDS} for immune, developmental, serum lipids, hepatic, endocrine and neural endpoints from animal and/or human studies selected for the derivation of candidate RfDs. The PODs from human epidemiological studies (immune, developmental and serum lipid endpoints) were derived using benchmark dose modeling (see

Appendix B.1 for details) which provided an internal serum concentration in mg/L. The internal dose PODs were converted to a POD_{HED} using the model described in 4.1.3.1.3 to calculate the dose that results in the same serum concentrations. Additional details are provided in the footnotes for the individual POD_{HEDS} in Table 21.

The PODs from the animal toxicological studies were derived by first converting the administered dose to an internal dose as described in 4.1.3.1.1. Rationale for the internal dosimetric selected for each endpoint is provided in Appendix B.2. The internal doses were then modeled using the Benchmark Dose Software (BMDS) 3.2 program (see appendix B.2. for additional modeling details). The internal dose animal PODs were converted to a POD_{HED} using the model described in 4.1.3.1.3. For animal studies using the average concentration over the final week of the study (Clast7), the POD_{HED} is the human dose that would result in the same steady-state concentration in adults. When an internal dosimetric of AUC pup during lactation and/or gestation was selected, the POD_{HED} is the dose to the mother that results in the same average AUC in a male fetus/infant over that period.

Table 21. POD_{HEDS} Considered for the Derivation of Candidate RfD Values

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/L)/Internal Dose Metric	POD _{HED} (mg/kg-day)
Immune Effects						
Decreased serum anti-tetanus antibody concentration in children	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz-Jørgensen and Grandjean (2018); Medium confidence	Human, male and female	BMDL _{5RD} , piecewise		7.2×10 ⁻⁴ (see appendix B.1.1 for BMD modeling details)	1.05×10 ^{-7a}
Decreased serum anti-diphtheria antibody concentration in children	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz-Jørgensen and Grandjean (2018); Medium confidence	Human, male and female	BMDL _{5RD} , piecewise		5.4×10 ⁻⁴ (see appendix B.1.2 for BMD modeling details)	7.91×10 ^{-8a}
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al., 2016, 3748828; Medium confidence	C57BL/6 Mice, F ₁ males	BMDL _{1SD} , Hill		1.27 (AUCavg_pup_gest_lact; see appendix B.2.12 for BMD modeling details)	2.01×10 ⁻⁴
Extramedullary Hematopoiesis in the Spleen	NTP 2019, 5400978; High confidence	Sprague-Dawley Rats, female	BMDL _{10RD} , Multistage Degree 2		3.61 (Clast7; see appendix B.2.7	4.63×10 ⁻⁴

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/L)/Internal Dose Metric	POD _{HED} (mg/kg-day)
Extramedullary Hematopoiesis in the Spleen	NTP 2019, 5400978; High confidence	Sprague- Dawley Rats, male	BMDL _{10RD} , Logistic		for BMD modeling details) 9.42 (Clast7; see appendix B.2.12 for BMD modeling details)	1.21×10 ⁻³
Developmental Effects						
Decreased Birth Weight	Chu et al., 2020, 6315711, High confidence	Human, male and female	BMDL _{5RD} , Hybrid		7.6×10 ⁻³ (see appendix B.1.3 for BMD modeling details)	1.65×10 ⁻⁶
	Sagiv et al., 2018, 4238410, High confidence	Human, male and female	BMDL _{5RD} , Hybrid		41.2×10 ⁻³ (see appendix B.1.3 for BMD modeling details)	8.95×10 ⁻⁶
	Starling et al., 2017, 3858473, High confidence	Human, male and female	BMDL _{5RD} , Hybrid		5.8×10 ⁻³ (see appendix B.1.3 for BMD modeling details)	1.26×10 ⁻⁶
	Wikström et al., 2020, 6311677, High confidence	Human, male and female	BMDL _{5RD} , Hybrid		7.9×10 ⁻³ (see appendix B.1.3 for BMD modeling details)	1.72×10 ⁻⁶
Decreased Fetal Body Weight	Lee et al., 2015, 2851075; Medium confidence	CD-1 Mice, F ₁ males and females	BMDL _{5RD} , Exponential 5		2.8×10 ⁻¹ (Cavg_pup_gest see appendix B.2.3 for BMD modeling details)	1.05×10 ⁻⁴
Decreased Pup Body Weight	Luebker et al., 2005, 757857; Medium confidence	Sprague- Dawley Rats, F ₁ male and female	BMDL _{0.5SD} , Exponential 4		2.37 (AUCavg_pup_ge st; see appendix B.2.4 for BMD modeling details)	8.74×10 ⁻⁴
Increased Number of Dead Fetuses	Lee et al., 2015, 2851075; Medium confidence	CD-1 Mice, females	LOAEL ^b	0.5 mg/kg/day	2.13 (AUCavg_dam_g est; see appendix B.2.3 for BMD modeling details)	3.32×10 ⁻⁴
Alterations in Serum Lipids						
Increased Total Cholesterol	Dong et al., 2019, 5080195; Medium confidence	Human, male and female	BMDL _{10RD} , Hybrid		2.41×10 ⁻² (see appendix B.1.4 for BMD modeling details)	3.08×10 ^{-6 c}
Hepatic Effects						

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/L)/Internal Dose Metric	POD _{HED} (mg/kg-day)
Individual Cell Necrosis in the Liver	Butenhoff et al., 2012, 1276144; High confidence	Sprague- Dawley rats, females	BMDL _{10RD} , Multistage 3		24.5 (Clast7; see appendix B.2.1 for BMD modeling details)	3.13×10 ⁻³
Endocrine Effects						
Decreased Free T4	NTP, 2019, 5400978; High confidence	Sprague- Dawley rats, male	LOAEL ^b	0.312 mg/kg/day	10.0 (Clast7; see appendix B.2.7 for BMD modeling details)	3.75×10 ⁻³
Decreased Free T4	NTP, 2019, 5400978; High confidence	Sprague- Dawley rats, female	BMDL _{1SD} , Exponential 4		3.89 (Clast7; see appendix B.2.7 for BMD modeling details)	4.98×10 ⁻⁴
Decreased Total T4	NTP, 2019, 5400978; High confidence	Sprague- Dawley rats, female	BMDL _{1SD} , Exponential 4		2.65 (Clast7; see appendix B.2.7 for BMD modeling details)	3.39×10 ⁻⁴
Decreased Total T3	NTP, 2019, 5400978; High confidence	Sprague- Dawley rats, male	BMDL _{1SD} , Hill		6.81 (Clast7; see appendix B.2.7 for BMD modeling details)	8.72×10 ⁻⁴
Decreased Total T3	NTP, 2019, 5400978; High confidence	Sprague- Dawley rats, female	BMDL _{1SD} , Hill		16.1 (Clast7; see appendix B.2.7 for BMD modeling details)	2.06×10 ⁻³
Decreased Total T3	Seacat et al., 2002, 757853; Medium confidence	Cynomolgus Monkeys, male	LOAEL ^b	0.03 mg/kg/day	8.13 (Clast7; see appendix B.2.10 for BMD modeling details)	1.04×10 ⁻³
Decreased Total T3	Seacat et al., 2002, 757853; Medium confidence	Cynomolgus Monkeys, female	BMDL _{1SD} , Exponential 4		7.09 see appendix B.2.10 for BMD modeling details)	9.07×10 ⁻⁴
Nervous System Effects						
Decreased Performance on the Object Location Recognition Memory Test	Mshaty et al., 2020, 6833692; Medium confidence	C57BL/6J, F ₁ males	NOAEL ^b	0.5 mg/kg/day	8.96×10 ⁻¹ (AUCavg_pup_la ct; see appendix B.2.6)	9.97×10 ⁻⁵

^a Calculated as the dose to mothers & children that results in the same serum concentration at 5 years of age. Note that the model predicted slightly different serum concentrations for male and female children, so the lower HED was selected to be more health protective.

^b No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.
^c Calculated as the dose that would result in the serum concentration POD at steady state.

4.1.5 Derivation of Candidate Lifetime Toxicity Values for the RfD

To calculate the candidate RfD values, EPA applied UFs to the POD_{HEDS} derived from the immune and developmental epidemiological studies. Though multiple POD_{HEDS} were derived for multiple health systems, the decreased serum anti-tetanus antibody concentrations in children, decreased serum anti-diphtheria antibody concentrations in children, and decreased BWT in babies were selected for candidate lifetime RfD derivation. These endpoints were chosen as candidate RfDs because of the robust (i.e., high quality) epidemiological and animal toxicity database supporting these effects the concordance between many of the human and animal health outcomes, and because these endpoints represented the most sensitive effects after PFOS exposure in the lower dose range. UFs were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* {EPA, 2002, 88824} (Table 22).

Table 22. Uncertainty Factors for the Development of the Candidate Lifetime RfD Values

UF	Value	Justification
UF _A	1	A UF _A of 1 is applied to developmental and immunological effects observed in epidemiological studies.
UF _H	10	No information was available relative to variability in the human population that supports a factor other than 10.
UF _S	1	The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991, 732120).
UF _L	1	A UF _L of 1 is applied for LOAEL to NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF _D	1	The database for PFOS contains medium and high-quality studies
UF _{TOT}	10	Composite Uncertainty Factor = UF _A × UF _H × UF _S × UF _L × UF _D

An interspecies uncertainty factor (UF_A) of 1 was applied to developmental and immunological effects observed in epidemiological studies because the dose response information from these studies is directly relevant to humans. There is no need to account for uncertainty in extrapolating from laboratory animals to humans.

An intraspecies uncertainty factor (UF_H) of 10 is applied to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (exposure to other chemicals, lifestyle, socioeconomic status, stress, diet) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation uncertainty factor (UF_L) of 1 is applied because a BMDL is used as the basis for the POD_{HED} derivation. When the POD type is a BMDL, the current

approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling.

A UF for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 1 for the developmental endpoints is applied because the developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure {U.S. EPA, 1991, 732120}. A UF_S of 1 is also applied to the immune endpoints in children because the developing immune system is recognized as a susceptible life stage; therefore, exposure during this time window can be considered more relevant than lifetime exposure {U.S. EPA, 1991, 732120}. According to the WHO/IPCS Immunotoxicity Guidance for Risk Assessment, developmental immunotoxicity encompasses the prenatal, neonatal, juvenile and adolescent life stages and should be viewed differently from the immune system of adults from a risk assessment perspective {IPCS, 2012, 1249755}.

A UF_D of one was applied to account for deficiencies in the database for PFOS. In animals, comprehensive oral short term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a robust epidemiological database which was used quantitatively in this assessment (Table 23). Typically, the specific study types lacking in a chemical's database that influence the value of the database uncertainty factor (UF_D) to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. Effects identified in developmental and multigenerational reproductive toxicity studies have been quantitatively considered in this assessment.

Table 23. Candidate Values

Endpoint	Study/ Confidence	Strain/ Species/ Sex	POD _{HED} (mg/kg-d)	UF _A	UF _H	UF _S	UF _L	UF _D	UF _{TOT}	Candidate Value (mg/kg-d)
Immune Effects										
Decreased serum anti-tetanus antibody concentration in children	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz-Jørgensen and Grandjean (2018); Medium confidence	Human, male and female	1.05 x 10 ⁻⁷	1	10	1	1	1	10	1.1 x 10 ⁻⁸
Decreased serum anti-diphtheria antibody concentration in children	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492);	Human, male and female	7.91 x 10 ⁻⁸	1	10	1	1	1	10	7.9 x 10 ⁻⁹

Endpoint	Study/ Confidence	Strain/ Species/ Sex	POD _{HED} (mg/kg-d)	UF _A	UF _H	UF _S	UF _L	UF _D	UF _{TOT}	Candidate Value (mg/kg-d)
	Budtz-Jørgensen and Grandjean (2018); Medium confidence									
Developmental Effects										
Decreased Birth Weight	Chu et al., 2020, 6315711, High confidence	Human, male and female	1.65 x 10 ⁻⁶	1	10	1	1	1	10	1.7 x 10 ⁻⁷
	Sagiv et al., 2018, 4238410, High confidence	Human, male and female	8.95 x 10 ⁻⁶	1	10	1	1	1	10	8.9 x 10 ⁻⁷
	Starling et al., 2017, 3858473, High confidence	Human, male and female	1.26 x 10 ⁻⁶	1	10	1	1	1	10	1.3 x 10 ⁻⁷
	Wikström et al., 2019, 6311677, High confidence	Human, male and female	1.72 x 10 ⁻⁶	1	10	1	1	1	10	1.7 x 10 ⁻⁷

4.1.6 RfD Selection

The RfD selected for PFOS is 7.9 x 10⁻⁹ mg/kg-day based on the critical effect of decreased serum anti-diphtheria antibody concentration in children. The selected critical effect can lead to severe clinical outcomes in a sensitive lifestage (children) and yields the lowest POD_{HED} and therefore, is expected to be protective of all other health effects in humans. It is plausible that the observed associations with PFOS exposure could be explained by confounding exposure from other PFAS. The authors assessed the possibility of confounding in a follow-up paper {Budtz-Jørgensen, 2018, 5083631} where estimates were adjusted for PFOA and concluded there was no notable attenuation of the observed effects. Exposure levels to PFOS were higher than PFOA (PFOS 17 ng/mL, PFOA 4 ng/mL) and there was a moderately high correlation between PFOS and PFOA, PFHxS, and PFNA (0.50, 0.57, 0.48, respectively).

Another potential issue associated with the selection of decreased serum anti-diphtheria antibody concentration in children for the RfD is the use of a 5% BMR and the clinical significance of that response. For diphtheria and tetanus, a clinically significant decrease would be a decrease that brought a person's antibody concentration below the level thought to provide protection. Generally, that would be 0.1 IU/mL {WHO, 2017, 9642150; Cellesi, 1989, 9642154; Galazka, 1989, 9642152}. If a person had a concentration above 0.1 IU/mL but a 5% decrease brought their concentration below 0.1 IU/mL, that would be clinically significant. Depending on the population, there might be a large number of persons (30–40%) with antibody concentrations close to 0.1 IU/mL {Zasada, 2013, 3194760; Hanvatananukul, 2020, 9642158; Yusoff, 2021, 9642157; Khetsuriani, 2013, 9642159}.

Though decreases in anti-diphtheria antibody concentrations are not in themselves an adverse effect, they do prevent against diphtheria infection, which is very rare in the United States due to high vaccination rates but can cause life-threatening airway obstruction or systemic toxin-mediated cardiac and neurologic complications {Collier, 1975, 9642066}. Among 13 cases

reported in the United States during 1996–2016, no deaths were mentioned. However, diphtheria remains a potentially fatal disease and PFOS-related decreases in anti-diphtheria antibody concentrations are concerning given the historic lethality of diphtheria in the absence of vaccination. Diphtheria is also more prevalent internationally, with countries reporting more than 16,000 cases of diphtheria to the WHO in 2018. It is unknown whether PFOS could impact antibody response to other vaccinations. However, evidence from the animal toxicity studies provide evidence that PFOS induces general immune suppression. Children with autoimmune diseases (e.g., juvenile arthritis) or who are taking medications for other diseases that weaken their immune system would be expected to be more likely to mount a low antibody response. Therefore, children with pre-existing immunological conditions represent susceptible populations for PFOS exposure. There are also concerns about declines in vaccination status {Smith, 2011, 9642143; Bramer, 2020, 9642145} for children overall and if considered an eradicated disease, these diseases could return to the U.S. {Hotez, 2019, 9642144}.

In the 2016 HESD, EPA concluded that the available human studies did not provide consistent evidence of a significant association between PFOS exposure and serological vaccine responses in general. However, this updated review indicates an association between increased serum levels of PFOS and decreased antibody production following routine vaccinations, particularly in children. The findings in the Grandjean et al., 2012 study are now supported by several medium confidence follow-up papers indicating changes in antibody levels of 10-20% per doubling of exposure were observed in the Faroe Islands cohorts (Grandjean et al., 2017, 3858518; Grandjean et al., 2017, 4239492). Overall, the Faroe Islands studies (Grandjean et al., 2012, 1248827; Grandjean et al., 2017, 3858518; Grandjean et al., 2017, 4239492; Mogensen, 2015, 3981889) observed associations between elevated levels of PFOS and decreased adjusted levels against tetanus and diphtheria in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. This effect is further supported by evidence from Timmermann et al. (2020, 6833710) which observed non-significant associations between elevated levels of PFOS and decreased adjusted antibody levels against measles across time in the group with no measles vaccination at age 9 months. The same pattern was observed at the 2-year follow-up.

4.2 Cancer

A small set of animal cancer bioassays and epidemiology studies are available. The limited available studies in humans report elevated risk of bladder, prostate, and breast cancers, though the study designs, analyses, and mixed results preclude the ability to make definitive conclusions on the relationship between PFOS and cancer. A single chronic cancer bioassay in rats was identified for PFOS.

An elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study {Alexander, 2007, 7921926} and a subsequent study to ascertain cancer incidence in the cohort observed elevated but statistically insignificant incidence ratios that were 1.7- to 2-fold higher among workers with higher cumulative exposure {Alexander, 2007, 4727072}. The risk estimates lacked precision because the number of cases was small and the authors did not control for the potential confounding of smoking. A second nested case-control study in a Danish cohort did not identify elevated bladder cancer risk with increased PFOS serum levels (Eriksen et al., 2009, 2919344).

Elevated ORs for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007, 4727072) and the Danish population-based cohort examined by Eriksen et al. (2009, 2919344). However, the CIs included the null, and no association was reported by another case-control study in Denmark {Hardell, 2014, 2968084}. No new studies examining prostate cancer risk were identified during the updated literature review.

The 6 new studies examining PFOS and breast cancer have mixed results. One study did not observe any association between PFOS and breast cancer {Hurley, 2018, 5080646}, one study observed a positive association between PFOS and risk for breast cancer {Wielsøe, 2017, 3858479}, and one study suggested gestational exposure may decrease risk of breast cancer {Cohn, 2020, 5412451}. The other three studies reported increased risk only in specific groups of subjects {Ghisari, 2017, 3860243; Mancini, 2019, 5381529; Tsai, 2020, 6833693}. Overall, study design issues and lack of replication of the results limit the ability to make firm conclusions regarding PFOS and breast cancer.

The only chronic toxicity/carcinogenicity study in animals was a rat study {Thomford, 2002, 5432392; Butenhoff, 2012, 1276144}. Increased incidence of hepatocellular adenomas in the male (12% at the high dose) and female rats (8% at the high dose) and combined adenomas/carcinomas in the females (10% at the high dose) were observed, but they did not display a clear dose-related response. Thyroid tumors (adenomas and carcinomas) were seen in males and females. In males, the incidence of thyroid tumors was significantly elevated only in the high-dose, recovery group males exposed for 52 weeks (10/39) but not in the animals receiving the same dose at 105 weeks. There were very few follicular cell adenomas/carcinomas in the females (5 total) with no dose-response. There was a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose-response for all tumor classifications.

4.2.1 Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329} there is *suggestive evidence of carcinogenic potential* of PFOS in humans. Epidemiological studies identified since the publication of the 2016 HESD do not provide clarity on the associations between PFOS and cancer. No new epidemiological studies were identified that strengthen the potential association between PFOS exposure and bladder or prostate cancers as discussed in the 2016 HESD {U.S. EPA, 2016, 3603365}. However, several new epidemiological studies were identified that examine the association between PFOS and breast cancer with mixed results. The small sample sizes, narrow exposure levels, latency issues and lack of replication of the results for certain subgroups, cancer subtypes, or genetic variants limit the ability to make firm conclusions regarding PFOS and breast cancer. Additionally, the animal evidence for PFOS is limited to a single chronic cancer bioassay. Although liver adenomas were significantly increased in male and female rats at the highest dose and a positive trend was observed ($p = 0.03$), a dose-response pattern was not observed. Incidence of thyroid follicular tumors and mammary gland tumors also did not show a direct response to dose. The available epidemiological and animal toxicity data suggest a potential concern for carcinogenic effects in humans but are not sufficient for a stronger conclusion.

4.2.2 CSF Development

Under EPA's *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}, when the evidence from the epidemiology studies and the cancer bioassays is suggestive for carcinogenicity, a quantitative estimate of risk is generally not performed unless there is a well-conducted study that could serve a useful purpose by providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In the case of PFOS, the existing human and animal evidence does not support a strong correlation between the PFOS exposure dose and tumor incidence to justify a quantitative assessment.

5.0 Relative Source Contribution

5.1 Background

EPA applies a RSC when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant (i.e., PFOS) does not exceed the RfD. 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 (directly or indirectly in beverages like coffee tea or soup); the remainder of the exposure equal to the RfD is allocated to other potential sources. In the case of PFOS, other potential sources include diet, ambient and indoor air, incidental soil/dust ingestion, consumer products and others.

The RSC is derived for the general population using the Exposure Decision Tree approach {U.S. EPA, 2000, 19428}. 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.

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 for the general population 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 subpopulations.

In 2016, EPA applied an RSC of 20% for the final Health Advisory for PFOS {U.S. EPA, 2016, 3603365}, based on the physical properties of PFOS and the limited available information indicating that significant potential exposure sources other than drinking water ingestion exist. At that time, information was not available to quantitatively characterize the exposure from each different source.

Several states have derived their own drinking water health guidelines by applying an RSC of 20% based on the following justifications:

- it is consistent with the default value of EPA and a number of other regulatory agencies,

- there is insufficient evidence for a given chemical to develop a chemical specific RSC {CalEPA, 2021, 9416932; ILEPA, 2019, 9417528; MassDEP, 2019, 6983120; TECQ, 2016, 5975349}.

Alternatively, several states have applied an RSC for PFOS of up to 60%. For instance, Michigan, New Hampshire, Minnesota, and Washington have selected infants and/or children as the target population and applied an RSC of 50% (Dewitt, 2019, 6982827; NHDES, 2019, 5949029; MDH, 2020, 5030007; WA DOH, 2020, 9418278}, and New York applied an RSC of 60% {NYSDOH, 2018, 6984171}.

5.2 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).

5.2.1 Systematic Review

Deluca and coworkers (2021 *in prep.*, 9419129) 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 (perfluorooctanoic acid (PFOA), perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), PFDA, perfluorohexanoic acid (PFHxA), perfluorohexanesulfonate (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; Poonthong, 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 *Systematic Review Protocol for IRIS Assessments* {U.S. EPA, 2019, 6572089} and the Navigation Guide {U.S. EPA, 2020, 7006986}. 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 (2021 *in prep.*, 9419129) 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. Of the 97, there were three studies where both drinking water and serum PFAS were evaluated. Hu et al. (2019, 5381562) stood out in providing a direct estimate of the drinking water RSC and being U.S. representative population-based and worthy of a dedicated summary (see below). The other two publications relate to industry impacted communities (Ohio River Valley and Germany) and identify drinking water as a statistical determinant of sera concentration but do not provide an estimate of RSC {Herrick, 2017, 3981338; Wilhelm, 2015, 3164179}.

5.2.2 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 *litstream*TM software was used to conduct title-abstract (TiAb) and full-text screening, and to extract relevant primary data into a comprehensive evidence database. Parameters of interest included: sampling dates and locations (focused on locations in the United States, Canada, and Europe), numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics.

Detailed data on PFAS occurrence in high-priority household and environmental media from 210 studies were extracted, as well as limited data on human matrices from 422 additional papers. 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.

5.3 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, water, and air {U.S. EPA, 2016, 3603365}.

5.3.1 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 gastrointestinal 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 PFASs 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 limit of quantification (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 {Poohong, 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 one hundred and fifty (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 ($p < 0.05$).

5.3.1.1 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}. Schaidt et 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. Fifty six percent 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 perfluorosulfonic acid (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 PFASs, 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.

5.3.1.2 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 24).

Table 24. Summary of EPA national fish tissue monitoring results for PFOS

Reference	Most Commonly Sampled Species	Site Description	Results
2008–2009 National Rivers and Streams Assessment (NRSA)	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.
2013–2014 NRSA	Largemouth bass Smallmouth bass Black crappie White crappie Walleye/sauger Yellow perch White bass Northern pike Lake trout Brown trout Rainbow trout Brook trout	349 urban and nonurban river sites across the United States.	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 99 percent of samples. Maximum detected concentration 283 ng/g.
2010 National Coastal Condition Assessment (NCCA) Great Lakes Human Health Fish Tissue Study	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.
2015 NCCA Great Lakes Human Health Fish Tissue Study	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, 8.3, 27, 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 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 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 to 19.1 ng/g ww) were found in whitefish, walleye, and yellow perch from the Great Lakes region.

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).

5.3.2 Water

5.3.2.1 Ambient Water

PFOS is one of the dominant PFAS compounds detected in aquatic ecosystems, along with PFOA {Ahrens, 2011, 2657780; Benskin, 2012, 1274133; Dinglasan-Panlilio, 2014, 2545254; Nakayama, 2007, 2901973; Remucal, 2019, 5413103; Zareitalabad, 2013, 5080561}. Though it is widely used and highly persistent in aquatic environments, current information on the distribution of PFOS in surface waters of the United States is somewhat limited; most published PFOS ambient water occurrence data focuses on regions with known PFAS use or occurrence. These regions are primarily freshwater systems in eastern states, including the Mississippi River, Great Lakes, Cape Fear Drainage Basin, and waterbodies near Decatur, Alabama and northern Georgia {Jarvis, 2021, 9416544}. Additional monitoring has been conducted in areas of known AFFF use.

In a recent review, Jarvis et al. (2021, 9416544) found that concentrations of PFOS in surface waters range over eight orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels (range: 0.074–8,970,000 ng/L, arithmetic mean: 786.77 ng/L, geometric mean: 5.468 ng/L, median: 3.6 ng/L). Though these calculated concentrations are not necessarily representative of all the measured PFOS concentrations in U.S. surface waters, the majority of PFOS concentrations reported in these studies (approximately 91%) are less than 300 ng/L.

5.3.2.2 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOS. However, inhalation and dermal absorption (e.g., while showering, bathing, etc.) are not expected to be significant sources of exposure from contaminated drinking water {NJDWQI, 2018, 5026035}. Serum PFOS concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges. As documented by the New Jersey Drinking Water Quality Institute {NJDWQI, 2018, 5026035}, estimates of the relative importance of drinking water with respect to total exposure are highly dependent on the assumed concentration of PFOS in drinking water, and have varied from < 1% (Cornelis, 2012, 2569108, assuming 2 ng/L) to 55% (Noorlander, 2011, 2919242, assuming 9 ng/L). Using data curated from scientific literature published between 2011 and 2017 to estimate aggregate exposure to PFOS, East et al. (2021, 9416543) estimated the relative contribution of drinking water total intake to be 11% for adults and 7% for children.

EPA's UCMR3 required PWS monitoring for six PFAS: PFOS, PFOA, PFNA, perfluorohexanesulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), and PFBS. PFOS was found in 1.93% of systems, at a median concentration of 0.06 µg/L and a maximum concentration of 7 µg/L {U.S. EPA, 2021, 9640861}. EPA found that 4.0% of PWS reported results for which one or more of the six UCMR 3 PFAS were measured at or above their respective method reporting limits (MRLs). The 4.0% figure is based on 198 PWSs reporting measurable PFAS results for one or more sampling events from one or more of their sampling locations. Those 198 PWS serve an estimated total population of approximately 16 million {U.S. EPA, 2017, 9419085}.

Glassmeyer et al. (2017, 3454569) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging contaminants, including 11 PFAS. PFOS was reported in source water at 88 percent of systems, with a median concentration of 2.28 ng/L and maximum concentration of 48.30 ng/L. Similarly, in treated drinking water, PFOS was detected in 80 percent of systems, with a median concentration of 1.62 ng/L and maximum concentration of 36.90 ng/L.

5.3.3 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 Ivansson (2017, 9416541) reported that PFASs 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 PFASs. However, PFASs 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/m²), and outdoor materials (median concentration of 9.5 µg/m²). 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 0.01–0.59 µg/m². 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/m²) {van der Veen, 2020, 6316195}.

5.3.4 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; Poonthong, 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; Poonthong, 2020, 6311690; Makey, 2017, 3860102; Byrne, 2017, 4165183; Fraser, 2013, 2325338}. Figure 120 shows a comparison of percent serum PFOA concentrations estimated to result from ingestion or dermal exposure to indoor dust (comparison from Deluca, 2021 *in prep.*, 9419129).

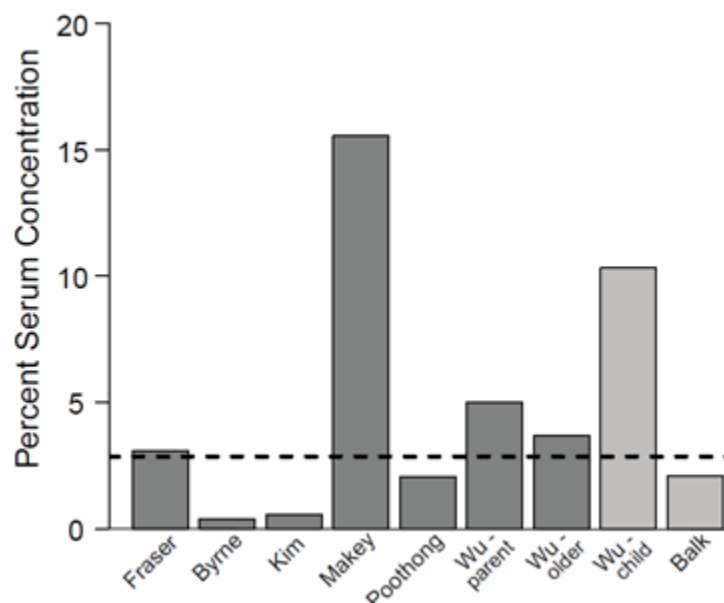


Figure 120. Percent Serum Concentration Attributed to Exposure from House Dust Ingestion and Dermal Contact for PFOS

^a Black dashed line indicates the weighted mean of all studies, weighted by sample size.

^b Dark gray shading indicates adult populations and light gray shading indicates child populations.

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}. PFOS was detected in all dust samples and serum samples from a pool of 50 individuals ranging in age from 2–82 years old; however, the authors reported a weak negative relationship between PFOS concentrations in dust and serum. Similarly, PFOS was measured at the second highest concentrations (geometric mean concentrations ranging from 29.0–34.6 ng/g) and frequencies (ranging from 85–87% detected) in dust sampled from Californian households; concentrations of PFOS in dust were correlated with serum from children, but not with serum from parents with young children or older adults {Wu, 2014, 2533322}. Makey et al. (2017, 3860102) measured PFOS and PFOS precursors in dust and similarly found no significant correlations between concentrations in dust and serum PFOS concentrations in pregnant Canadian participants. Another study of Alaska Natives found no correlation between dust and serum PFOS concentrations, though it was the predominant compound in both media {Byrne, 2017, 4165183}.

Using data curated from scientific literature published between 2011 and 2017 to estimate aggregate exposure to PFOS, East et al. (2021, 9416543) estimated that dust ingestion contributes 13% of total intake for adults and 59% for children. Gebbink et al. (2015, 2850068)

similarly estimated that 0.5–12.8% of direct PFOS uptake by the general adult population could be contributed to dust. However, a Finnish child cohort study provided a much lower estimate for children, with dust ingestion only accounting for up to 5% of the estimated daily intake {Balk, 2019, 5918617}. Poonthong and colleagues (2020, 6311690) looked closely at data from individual participants and found that dust only contributed to about 1% of the total PFOS intake, but total PFAA intake from dust by certain participants reached approximately 95% indicating that for some people, dust may be a significant source of PFAS exposure. Generally, these studies show that although PFOS is often detected in dust, dust concentrations are not generally correlated with serum concentrations, indicating that dust is likely a major source of PFOS only for certain individuals or age groups.

5.3.5 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/m³ (range: 0.94–3.0) and in the particulate phase had a mean concentration of 0.64 pg/m³ (range: 0.35–1.16). 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/m³; with a range of concentrations from detected to 8.1 pg/m³. 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 2.1–7.9 pg/m³ and the gas phase ranged from 1.8–5.0 pg/m³ 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/m³), an agricultural site in Saskatchewan (5 pg/m³), Whistler, British Columbia (4 pg/m³), and Alert, N Nunavut (2 pg/m³) (EC, 2013).

Other reported concentrations of PFOS in air samples from Sydney, Florida (3.4 pg/m³), Tudor Hill, Bermuda (6.1 pg/m³), Malin Head, Ireland (3.3 pg/m³), and Hilo, Hawaii (6.6 pg/m³) 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/m³, respectively {Sasaki, 2003, 5081390}.

Across Europe, PFOS air concentrations were reported to be variable. In the particulate phase PFOS concentrations ranged from < 1.8–46 pg/m³ {Martin, 2004, 1291044}. Most locations had low (~1–2 pg/m³) 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/m³ 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/m³. 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/m³ (range: 0.03–0.50 pg/m³) and 0.18 pg/m³ (range: 0.02–0.97 pg/m³), respectively.

5.3.6 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.

The CDC NHANES has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOS and PFOA have 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 percent between 1999 and 2014, presumably due to restrictions on their commercial usage in the United States. Under EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95 percent by no later than 2010 and elimination of these substances in products by 2015 {U.S. EPA, 2006, 3005012}. However, since the voluntary phase out of some longer-chain PFAS compounds in the United States, manufacturers are shifting to alternative forms of PFAS compounds such as hexafluoropropylene oxide (HFPO) dimer acid and HFPA dimer acid ammonium salt (GenX chemicals). Additionally, other PFAS were found in blood samples from recent (2011–2016) NHANES surveys, for example, PFDA, perfluorododecanoic acid (PFDoDA), PFHpA, PFHxS, PFNA, and 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH or MeFOSAA). There is less publicly available information on the occurrence and health effects of these replacements than for PFOA and PFOS and other members of the carboxylic acid and sulfonate PFAS families.

5.4 Recommended RSC

Findings from studies on populations in the United States, Canada, and Western Europe support the conclusion that diet is the major contributor to total PFOS exposure, typically with drinking water and/or dust as important additional exposure routes, especially for sensitive subpopulations. Estimates of relative exposure from different sources support a 20 percent RSC for drinking water, as described below.

- Hu et al. (2019, 5381562) provides an estimate of the PFOS drinking water RSC for the U.S. general population based on an all-women's prospective national cohort, i.e. the NHS. PFOS was one of 15 PFAS analyzed in archived drinking water collected from 225 homes back in 1989/90. Plasma concentrations were estimated from the drinking water concentrations using a one-compartment model. The modeled serum estimates were compared to matched PFAS concentrations measured in archived serum for a subset of 110 women. For two of the PFAS (PFOA and PFNA) among women consuming ≥ 8 cups of tap water per day, the tap water concentration was a significant predictor of plasma

concentrations. The drinking water RSC was estimated by the ratio of the modeled serum concentration from PFAS measured in drinking water to the median of the actual measured serum concentration. For PFOS, investigators were able to differentiate linear from branched congeners with the former RSC estimated as 2.2% (2.0%–2.5%) and the latter as 3.0% (2.5%–3.2%). The authors conclude that their findings compare well with a default RSC of 20%.

- East et al. (2021, 9416543) applied standard exposure algorithms and exposure factors to data curated from scientific literature published from 2011 to 2017 to estimate exposures for adults and 2-year-olds. Aggregating median route-specific estimated intakes of PFOS, they identified dietary ingestion as the major contributor among adults and incidental dust ingestion among young children. Due to a lack of total diet studies in North America, the authors relied on dietary data from Western Europe. The authors estimated PFOS exposure from drinking water at 4.4 and 1.2 ng/day, or approximately 11 and 7% of total intake, for adults and children, respectively. Estimates of total intake were about a quarter (adults) and a third (children) of that reported in an earlier study using similar methods {Egeghy, 2011, 723765}, and the percent contribution from water was also substantially lower for both age groups.
- Jogsten et al. (2012, 2095418) estimated that for adults, about 93% of the PFOS exposure in Catalonia, Spain was from diet and 6.5% was from drinking water; for toddlers, 97% was from diet and 2.5% was from drinking water.
- Gebbink et al. (2015, 2850068) estimated the relative contributions of the major exposure media to total direct and indirect PFOS exposures under assumptions of low (5th percentile), intermediate (median values), and high (95th percentile) exposures. The authors used a Scenario-Based Risk Assessment modeling approach with data collected in 2007 to estimate the relative contributions to total exposures. The data for direct and indirect contributors to serum PFOS (presented graphically in the published paper) are consistent with the following patterns for exposures in adults:
 - a. Low exposure scenario = diet (~88%) > air (~7%) > water (~3%) > dust (~2%)
 - b. Intermediate exposure scenario = diet (~65%) > dust (14%) ≈ air (14%) > water (~7%)
 - c. High exposure scenario = diet (~43%) > dust (27%) > air (20%) > water (~10%).

The approaches and assumptions used in these studies vary widely; some uncertainties associated with these data include:

- Many of the data are obtained from review papers or individual studies conducted at single locations and are not nationally representative.
- Concentrations range widely in exposure estimates.
- The ambient air and dust exposure estimates are limited, regional, and variable.
- Drinking water exposure varies among age groups and individuals.
- Because of recent reductions in use of PFOS, assessing current relative exposures to the general population is difficult.
- Some of the data are several years old and may not accurately reflect current exposures.

Additionally, data on other routes of exposure are lacking:

- Estimates of dermal exposure to treated fabrics and inhalation exposure associated with contaminated water are not available.

- Drinking water exposure estimates apply only to direct ingestion of tap water and beverages or soups prepared locally. They do not generally include PFOS in water that becomes incorporated in solid foods during home preparation and cooking, or that which is present in commercial beverages.
- Transformation of PFOS precursors that decay or are metabolized to PFOS is a route that is rarely evaluated in dietary studies yet can contribute to total exposure. Air and dust can be vehicles for PFOS derivatives that metabolically degrade to PFOS.

In summary, based on the physical properties, detected levels, and available exposure information for PFOS, drinking water, 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 fish and shellfish from inland and nearshore waters and water ingestion exist (Box 8A in the Decision Tree); however, information is not available to quantitatively characterize exposure from these different sources (Box 8B in the Decision Tree). Therefore, EPA recommends an RSC of 20% (0.20) for PFOS.

6.0 References

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Appendix A. 2019 Literature Search Strategies

A.1 Specific Aims of the Updated Literature Search for PFOA and PFOS

The specific aims of the PFOA and PFOS updated literature search screening were to:

1. Identify health effects information (epidemiological, toxicological studies and PBPK models), published since the 2016 health effect support documents (HESDs) for PFOA and PFOS that could potentially influence future PFOA or PFOS drinking water regulatory actions.
2. Provide a bibliography of these relevant studies containing human, animal (mammalian model), and physiologically-based pharmacokinetic (PBPK) data.

A.2 Database Searches

Following the EPA's 2013 Conflict of Interest Review Process for Contractor-Managed Peer Reviews of EPA HISA and ISI Documents, EPA's Health Effects Support Documents for [PFOA](#) and [PFOS](#) were released for public comment and panel peer review in 2014. The current updated literature search focused on studies published since 2013, under the assumption that any critical studies published previously would have been considered in the public comment and external peer review processes used in developing the HESDs. This updated literature search focused only on the chemical name with no limitations on lines of evidence (i.e., human, animal, in vitro, in silico) or health outcomes. The databases listed below were searched for the date range of January 2013 through March 10–11, 2019 by an EPA information specialist and stored in the Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608). See Table A-1 for the search strings used for each database listed below:

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- ToxLine (National Library of Medicine)

Because the number of studies retrieved was large even after duplicate removal (3,382), studies were imported into SWIFT Review software (<https://www.sciome.com/swift-review/>; see also {Howard, 2016, 4149688} to identify those most likely applicable to human health. In brief, SWIFT Review has pre-set literature search filters to separate studies that likely present a health outcome from those that likely do not (e.g., exposure only, analytical methods, etc.). The SWIFT Review filters applied to the PFOA and PFOS updated literature search focused on the following lines of evidence: human (e.g., epidemiology studies), animal (e.g., toxicity studies), and in vitro. Application of these filters reduced the number of studies for inclusion/exclusion screening to 1,976. The details of the search strategies that underlie the filters are available at https://hawcprd.epa.gov/media/attachment/SWIFT-Review_Search_Strategies.pdf. Details of the manual inclusion/exclusion screening process are described below.

1 Additionally, the National Toxicology Program (NTP) website was searched for study tables and
2 individual animal data from PFOA and PFOS toxicity studies with reports in preparation that
3 could provide relevant health effects information. Three sets of study tables were identified and
4 included as relevant: 1.) 28-day PFOS study table and individual animal data, 2.) 28-day PFOA
5 study table and individual animal data and 3.) technical report pathology tables and curves for a
6 two-year carcinogenicity study for PFOA. Although peer-reviewed NTP technical reports for the
7 28-day toxicity and 2-year carcinogenicity studies are not yet available, this information was
8 included in this literature search because these data have undergone standard NTP quality
9 assurance/control processing, peer review and are publicly available.

Table A-1. Search String for Database Searches

Database	Search String	Results
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="Perfluorooctanoic 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=(2013-2019))	4/10/2019: 3,081 results
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 "Perfluorooctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR	4/10/2019: 2,191 results

Database	Search String	Results
	“perfluorooctanyl sulfonate”[tw] OR "Perfluorooctanoic acid"[tw] OR "Octanoic acid, pentadecafluoro-"[tw] OR "Perfluorooctanoate"[tw] OR “perfluorooctane sulfonate”[tw] OR "A 5717"[tw] OR "EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR “perfluorooctane sulfonate”[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR ("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw])) AND (2013/01/01:3000[pdat] OR 2013/01/01:3000[mhda] OR 2013/01/01:3000[edat] OR 2013/01/01:3000[crdt])	
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"+"perfluorooctanoic 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: 60 results
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: 0 results
Total number of references from all databases from 2013–2019		3,382 results

Appendix B. Benchmark Dose Modeling

B.1 Epidemiology Studies

B.1.1 Modeling Results for Decreased Tetanus Antibody Concentrations

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 examination controlling for sex, exact age at the seven-year examination, and booster type at age five years. Three model shapes of PFOS were evaluated: a linear model, a piecewise-linear model (with the slope allowed to change at the median exposure level), and a logarithmic function. The logarithmic dose-response model did not show a better fit than the piecewise-linear model. There was no evidence that the piecewise-linear model fit better than the linear model for either the PFOS exposure without adjustment for PFOA ($p = 0.60$; see {Budtz-Jørgensen, 2018, 5083631}, Table 3), or for the model that did adjust for PFOA ($p = 0.71$), but the piecewise model tended to show slightly better fit values due to greater flexibility.

Budtz-Jørgensen (2018, 5083631) showed that the $BMD = \log_2(1-BMR)/\beta$ and used a BMR of 5% to estimate the corresponding BMD_5 and $BMDL_5$ of 1.45 and 0.56 ng/mL based on the piecewise linear model (Table 1, {Budtz-Jørgensen, 2018, 5083631}). Budtz-Jørgensen (2018, 5083631) extended this analysis to control for PFOA concentrations and reported the corresponding BMD_{05} and $BMDL_{05}$ of 3.57 and 0.72 ng/mL.

Though we do not know the clinically relevant response level, a BMR of 5% is a reasonable and appropriate 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 8% during 2001–2016 {Martin, 2018, 8632225}. The case-fatality ratio can be as high as 100% for early and late lifestages cases in the absence of high-quality medical care {WHO, 2017, 9642138}. A BMR of 10% is more appropriate for an effect that would be considered ‘minimally adverse’. A BMR of 1% is for severe effects and decreased antibodies offer diminished protection for severe effects but are not themselves severe effects. Developmental effects can have 5% BMR per EPA guidance.

Table B-1. BMDs and BMDLs for effect of PFOS at age five years on anti-tetanus antibody concentrations at age seven years

BMR	Estimated without control for PFOA		Estimated with control for PFOA	
	BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
5%	1.45	0.56	3.57	0.72 ^a

^aValue on which POD is based for immunotoxicity related to tetanus.

The ratios of the BMD:BMDL for each of pair of estimates are all less than 3 which shows that the BMDL estimates are reasonably stable – although the range of BMDLs are below the low end of the distribution of PFOS concentrations in the study population which was reported as

having a geometric mean of 16.7 ng/mL with a 25th–75th percentile range of 13.5 ng/mL to 21.1 ng/mL ({Grandjean, 2012, 1248827}; Table 2). No information was available to judge the fit of the model in the range of the BMDLs.

The BMD₅ estimate from the multi-PFAS models is somewhat higher than the BMD₅ estimate from the models with just PFOS, but the BMDL₅ estimates are much closer. This may, or may not, reflect control for any potential confounding of the BMDs among the PFAS exposures, but the BMDLs were not meaningfully affected in a risk assessment context where the RfD is defined as having an “uncertainty spanning perhaps an order of magnitude”. Including an additional PFAS in the model that is correlated with PFOS (Pearson correlation of 0.50; {Grandjean, 2012, 1248827}; Table 2), can impact the β and the $se(\beta)$ for PFOS in unpredictable ways depending upon the degree of correlation and the accuracy of each of the PFAS measurements. While it is not clear which estimate is ‘better’, the two BMDL₅ estimates which serve as the PODs for the cRfD are similar (0.56 ng/mL vs. 0.72 ng/mL) and EPA advanced the derivation of the cRfD based on results that control for PFOA because this model fit well overall.

For immunotoxicity related to tetanus, associated with PFOS, the POD is based on a BMR of 5% and a BMDL₅ of 0.72 ng/mL.

B.1.2 Modeling Results for Decreased Diphtheria Antibody Concentrations

Budtz-Jørgensen and Grandjean (2018, 5083631) fit multivariate models of PFOS measured at age five years, against log₂-transformed anti-diphtheria antibody concentrations measured at the seven-year examination controlling for sex, exact age at the seven-year examination, and booster type at age five years. Three model shapes were evaluated: a linear model of PFOS, a piecewise-linear model (with the slope allowed to change at the median exposure level), and a logarithmic function. The logarithmic model did not fit better than the piecewise-linear model. The analyses of diphtheria also showed no evidence that the piecewise-linear model fit better than the linear model for either the PFOS exposure without adjustment for PFOA ($p = 0.30$; see {Budtz-Jørgensen, 2018, 5083631}, Table 3), or for the model that did adjust for PFOS and PFOA ($p = 0.34$), but the piecewise model tended to show slightly better fit values due to greater flexibility.

Budtz-Jørgensen and Grandjean (2018, 5083631) showed that the $BMD = \log_2(1-BMR)/\beta$ and used a BMR of 5% to estimate the corresponding BMD₅ and BMDL₅ of 0.98 and 0.49 ng/mL, respectively, based on the piecewise linear model (Table 1 of {Budtz-Jørgensen, 2018, 5083631}). Budtz-Jørgensen and Grandjean (2018, 5083631) extended this to further control for PFOA concentrations and reported corresponding BMD₅ and BMDL₅ of 1.21 ng/mL and 0.54 ng/mL, respectively.

A BMR of 5% 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 systemic toxin-mediated cardiac and neurologic complications (Collier, 1975). Among 13 cases reported in the U.S. during 1996–2016, no deaths were mentioned. However, diphtheria remains a potentially fatal disease and PFOS-related changes in anti-diphtheria antibody concentrations

cannot be considered to be ‘minimally adverse’ given the historic lethality of diphtheria in the absence of vaccination.

Table B-2. BMDs and BMDLs for effect of PFOS at age five years on anti-diphtheria antibody concentrations at age seven years

BMR	Estimated without control for PFOA		Estimated with control for PFOA	
	BMD (ng/mL)	BMDL (ng/mL)	BMD (ng/mL)	BMDL (ng/mL)
5%	0.98	0.49	1.21	0.54^a

^aValue on which POD is based for immunotoxicity related to diphtheria.

The ratios of the BMD:BMDL for each of pair of estimates without control for PFOA are all less than 3 which shows that the BMDL estimates are reasonably stable. Although, the range of BMDLs are well below the low end of the distribution of PFOS concentrations in the study population which was reported as having a geometric mean of 16.7 ng/mL with a 25th–75th percentile range of 13.5 ng/mL to 21.1 ng/mL ({Grandjean, 2012, 1248827}; Table 2). No information was available to judge the fit of the model in the range of the BMDLs. The ratios of the BMD:BMDL for each of pair of estimates with control for PFOA are all less than 5 which shows that these BMDL estimates are somewhat less stable, but not unreasonably so.

The BMD₅ estimate from the multi-PFAS models is somewhat higher than the BMD₅ estimate from the model with just PFOS, but the BMDL₅ is much closer. Including an additional PFAS in the model that is highly correlated with PFOS (Pearson correlation of 50; {Grandjean, 2012, 1248827}; Table 2), can impact the β and the $se(\beta)$ for PFOS in unpredictable ways depending upon the degree of correlation and the accuracy of each of the PFAS measurements. While it is not clear which estimate is ‘better’, the BMDLs which serve as the PODs for the cRfD are similar (0.49 ng/mL vs. 0.54 ng/mL) and EPA advanced the derivation of the cRfD based on results that control for PFOA because this model fit well overall and these results provide the best available POD for PFOS and diphtheria.

For immunotoxicity related to diphtheria, associated with PFOS, the POD is based on a BMR of 5% and a BMDL₅ of 0.54 ng/mL.

B.1.3 Modeling Results for Decreased Birthweight

Four high confidence studies Chu et al. (2020, 6315711); Sagiv et al. (2018, 4238410); Starling et al. (2017, 3858473), and Wikström et al.(2019, 6311677) 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. Sagiv et al. (2018, 4238410) collected maternal samples in trimester 1, while Wikström et al. (2020, 6311677) collected them in trimesters 1 and 2. The samples from Starling et al. (2017, 3858473) were from trimesters 2 and 3, while Chu et al. (2020, 6315711) collected exclusively in trimester 3.

All four 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 (Cis), estimated from linear regression models. 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 of the confidence interval to estimate BMD and BMDL values using the general equation $y = mx + b$, substituting the re-expressed β values from these studies for m . The intercept b represents the baseline value of birth weight in an unexposed population and it can be estimated through $\bar{y} = m\bar{x} + b$ using an average birth weight from an external population as \bar{y} , an average exposure as \bar{x} and re-expressed β from the studies as m .

The CDC Wonder site (<https://wonder.cdc.gov/nativity.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 were $3,261.6 \pm 590.7$ 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 since it is more relevant for deriving toxicity values for the US general public than the study-specific birthweight data. Also, the CDC Wonder database is queryable such that the exact percentage of the population falling below the cut-off value for clinical adversity could be determined. The America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals report (3rd edition) (<https://www.epa.gov/americaschildrenenvironment/ace-biomonitoring-perfluorochemicals-pfcs#B6>) provides the median blood serum levels of PFOS of 3 ng/mL in 2015-2016 in women ages 16 to 49 years. These values, assumed to be representative of women of reproductive age, are subsequently used in the estimation of BMD and BMDL values from the four available epidemiological studies.

Chu et al. (2020, 6315711) reported a β coefficient of -83.3 g (95% CI: $-133.2, 33.4$) per $\ln(\text{ng/mL})$ increase for the association between birth weight and maternal PFOS serum concentrations 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 25th–75th percentile range (4.4–11.9 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:

$$\mu = \ln(q_{50}) = \ln(7.2) = 1.97 \quad (1)$$

$$\sigma = \ln(q_{75}/q_{25})/1.349 = \ln(11.9/4.4)/1.349 = 0.75 \quad (2)$$

Then, EPA estimated the 25th–75th percentiles at 10 percentile intervals of the exposure distribution and corresponding responses of reported β coefficient. The re-expressed β coefficient is determined by minimizing the sum of squared differences between the curves generated by the re-expressed β and the reported β . 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 benchmark response (BMR) is to have a basis for what constitutes a minimal level of change in the endpoint that is biologically significant. For birth weight, there is no accepted percent change that is considered adverse. However, there is a clinical measure for what constitutes an adverse response. Babies born weighing less than 2,500 g are considered to have low birth weight, and further, low birth weight is associated with a wide range of health conditions throughout life {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 approach⁹. Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cut-off for adversity in the tail of the response distribution. Application of the hybrid approach requires the selection of an extra risk value for BMD estimation. In the case of birth weight, an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from toxicology studies, and that low birth weight 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 of 5% extra risk, the BMD would be the dose that results in 12.86% of the responses falling below the 2,500 g cut-off value:

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

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

Based on the mean birth weight for all births in the US in 2018 of 3,261.6 g with a standard deviation of 590.7 g, EPA calculated the mean response that would be associated with the 12.86th percentile of the distribution falling below 2,500 g. In this case, the mean birth weight would be 3,169.2 g. Given the median exposure of 3 ng/mL from ACE Biomonitoring on Perfluorochemicals report as \bar{x} , the mean birth weight in the U.S. as \bar{y} and the re-expressed β as m term, the intercept b can be estimated as:

$$b = \bar{y} - m\bar{x} = 3261.6 \text{ g} - \left(-11 \text{ g}\left(\frac{\text{ng}}{\text{mL}}\right)^{-1}\right) 3.0 \frac{\text{ng}}{\text{mL}} = 3,294.6 \text{ g} \quad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x , using 3,294.6 g for the b term and -11.0 for the m term. Doing so results in a value of 11.4 ng/mL:

$$x = (y - b)/m = (3169.2 \text{ g} - 3294.6 \text{ g})/(-11 \text{ g}\left(\frac{\text{ng}}{\text{mL}}\right)^{-1}) = 11.4 \text{ ng/mL}$$

To calculate the BMDL, the method is essentially the same except that the lower limit (LL) on the β coefficient (-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:

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

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%.

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

$$\begin{aligned} 95\% \text{ one-sided LL} &= \beta - 1.645(SE(\beta)) = -11 \text{ g} \left(\frac{\text{ng}}{\text{mL}}\right)^{-1} - 1.645 \left(3.37 \text{ g} \left(\frac{\text{ng}}{\text{mL}}\right)^{-1}\right) \\ &= -16.5 \text{ g} \left(\frac{\text{ng}}{\text{mL}}\right)^{-1} \end{aligned}$$

Using this value for the m term results in a BMDL value of 7.6 ng/mL maternal serum PFOS concentration.

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 in a US cohort. The intercept b is 3,265.0 g based on the β coefficient of -1.1 g per ng/mL. A BMD of 85.6 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 US.

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 CI of -2.6 g per ng/mL. Using the corresponding lower limit (-2.3 g per ng/mL), a BMDL of 41.2 ng/mL is calculated.

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 in a US cohort. Given the reported study-specific median (2.4 ng/mL) and 25th–75th percentile range (1.5–3.7 ng/mL) of the exposure, EPA estimated the mean (0.88) and standard deviation (0.67) of the log-normally distributed exposure. The re-expressed β coefficient is -5.5 g (95% CI: $-21.4, 10.5$) per ng/mL and the intercept b is 3,278.1 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 19.8 ng/mL and 5.8 ng/mL, respectively.

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 in a Swedish cohort. Given the reported study-specific median (5.4 ng/mL) and 25th–75th percentile range (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 3,286.7 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 14.1 ng/mL and 7.9 ng/mL, respectively.

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 2,500 g as the tail probability to represent the probability of extreme (“adverse”) response at zero dose ($P(0)$). However, this exact percentage of 8.27% was calculated without accounting for the existence of background PFOS exposure in the US 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 falls below the cut-off of 2,500 g. EPA estimated the study-specific tail

probability of live births falling below the public health definition of low birth weight (2,500 g) as:

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

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

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\bar{x} = 0$). By using the median of serum PFOS concentrations (3 ng/mL) from the ACE Biomonitoring on Perfluorochemicals report as background exposure (\bar{x}), the tail probabilities using this alternative approach was study-specific and ranged from 8.93%–9.76%. As such, the results from this alternative approach, presented under the column of “Alternative Tail Probability” in Table B-3, are very similar to the main results, presented under the column of “Exact Percentage” in Table B-3, when background exposure was not accounted for while estimating the tail probability.

Table B-3 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 5.8 ng/mL to 57.7 ng/mL. Assuming all other study quality characteristics are equal, the lowest value was selected for the individual study POD. Therefore, for decreased birth weight associated with PFOS, the individual study POD selected from the available epidemiologic literature is 5.8 ng/mL maternal serum PFOS concentration.

Table B-3. BMDs and BMDLs 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	Sample Time Period ^a	Exposure Median (25th–75th percentile)	Exposure Distribution (μ , σ)	Reported β (95% CI)	Re-expressed β (95% CI)	Intercept b	SE of β	95% one-sided LL of β	Exact Percentage (P(0) = 8.27%)		P(0)	Alternative Tail Probability ^b	
									BMD (ng/mL)	BMDL (ng/mL)		BMD (ng/mL)	BMDL (ng/mL)
Chu et al. (2020, 6315711)	3 rd	7.2 (4.4–11.9)	(1.97, 0.75)	–83.3 (–133.2, –33.4)	–11.0 (–17.6, –4.4)	3294.6	3.37	–16.5	11.4	7.6	8.93%	13.0	8.6
Sagiv et al. (2018, 4238410)	1 st	25.7 (18.9–34.9)	(3.25, 0.45)	–17.9 (–40.9, 5.1)	–1.1 (–2.6, 0.3)	3265.0	0.73	–2.3	85.6	41.2	9.76%	119.9	57.7
Starling et al. (2017, 3858473)	2 nd –3 rd	2.4 (1.5–3.7)	(0.88, 0.67)	–13.8 (–53.8, 26.3)	–5.5 (–21.4, 10.5)	3278.1	8.14	–18.9	19.8	5.8*	9.39%	25.1	7.3
Wikström et al. (2019, 6311677)	1 st –2 nd	5.4 (4.0–7.6)	(1.68, 0.48)	–46.0 (–88.0, –3.0)	–8.4 (–16.0, –0.5)	3286.7	3.94	–14.8	14.1	7.9	9.14%	16.8	9.5

*Smallest BMDL using the four individual studies

^aSample time periods include the maternal serum samples collected during the first trimester (1st), first or second trimester (1st–2nd), second or third trimester (2nd–3rd), third trimester (3rd)^bThe 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 of 24 ng/mL in 1999–2000 in women ages 16 to 49 years. The CDC Fourth National Report on Human Exposure to Environmental Chemicals (<https://www.cdc.gov/exposurereport/index.html>) provides the median of serum PFOS concentrations (5.27 ng/mL) among NHANES females in 2011–2012. EPA performed a sensitivity analysis by estimating BMD and BMDL using these values as background exposures. The results for Starling et al. (2017, 3858473), presented in Table B-4, demonstrate the robustness of EPA’s approaches with alternative assumptions on background exposures.

Table B-4. 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 <i>b</i>	Exact percentage (P(0) = 8.27%)		Alternative Tail Probability ^b		
			BMD (ng/mL)	BMDL (ng/mL)	P(0)	BMD (ng/mL)	BMDL (ng/mL)
Starling et al. (2017, 3858473)	3.00	3278.1	19.8	5.8	9.39%	25.1	7.3
	5.27	3290.6	22.1	6.4	9.04%	25.7	7.5
	24.00	3393.5	40.8	11.9	6.52%	31.9	9.3

^aAssumptions on background exposure for the estimation of intercept using Equation (3).

^bThe tail probability of live births falling below the public health definition of low birth weight based on Normal distribution.

B.1.4 Modeling Results for Increased Cholesterol

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 point 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 BMD₁₀ and BMDL₁₀ of 44.2 ng/mL and 24.1 ng/mL, respectively. Key variables or other key 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 versus non-linear models), the impact of potential confounders, or the role of reverse causality.

B.2 Toxicology Studies

B.2.1 Butenhoff, 2012, 1276144

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Butenhoff (2012, 1276144) study using the Benchmark Dose Software (BMDS) 3.2 program.

This study addresses individual cell necrosis in the liver in female Sprague-Dawley Crl:CD(SD)IGS BR rats.

B.2.1.1 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 benchmark response (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 B-5. The average concentration over the final week of study ($C_{7,avg}$) 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 B-5. 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

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	65	7
0.029	1.4	55	6
0.120	6.0	55	6
0.299	14.9	55	6
1.251	62.3	65	15

The benchmark dose (BMD) modeling results for individual cell necrosis in the liver are summarized in Table B-6 and Figure B-1. The best fitting model was the Multistage Degree 3 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 Multistage Degree 3 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 (BMDL₁₀) from the selected Multistage Degree 3 model is 24.5 mg/L.

Table B-6. 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

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	0.977	236.4	0.00108	−0.02	53.7	15.0	EPA selected the Multistage Degree 3 model. All models had adequate fit (p-values greater than
Gamma	1.000	234.4	0.00002	−0.02	56.3	24.5	
Log-Logistic	1.000	234.4	−0.00043	−0.03	61.1	22.9	
Multistage Degree 4	0.977	236.4	−0.00042	−0.02	56.9	24.5	

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 3	1.000	232.4	0.00042	-0.01	55.7	24.5	0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Multistage Degree 2	0.999	232.4	0.00928	0.03	52.4	24.4	
Multistage Degree 1	0.955	232.7	0.15063	0.21	44.6	23.7	
Weibull	1.000	234.4	2.9×e ⁻⁶	-0.03	61.2	24.5	
Logistic	0.983	232.5	0.05929	0.16	48.1	32.3	
Log-Probit	1.000	234.4	5.3×e ⁻⁷	-0.02	55.0	15.3	
Probit	0.980	232.6	0.06914	0.17	47.5	31.0	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₁₀ = dose level corresponding to a 10% response level; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

^aSelected model in bold.

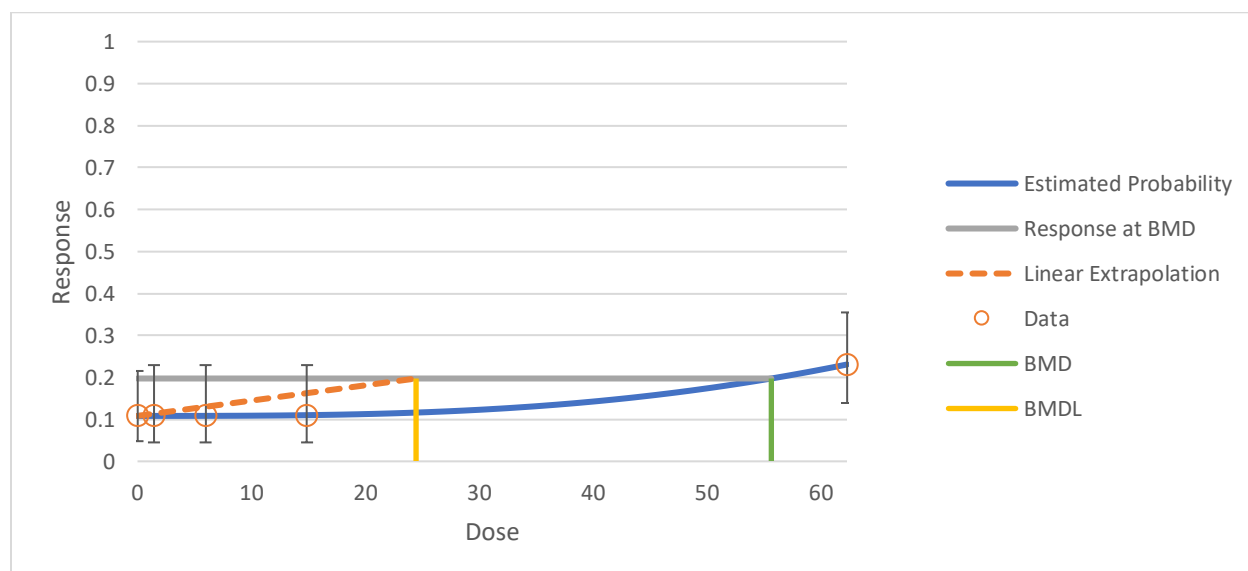


Figure B-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Individual Cell Necrosis in the Liver in Female Sprague-Dawley Crl:CD(SD)IGS BR Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.2 Lau 2003, 757854

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Lau (2003, 757854) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses pup body weight, offspring survival at postnatal day 5 (PND 5) and offspring survival at PND 22 in F₁ male and female Sprague-Dawley rats.

B.2.2.1 Pup Body Weight

Decreased mean response of pup body weight was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of a change in the mean equal to 0.5 standard deviation from the control mean and a 5% change were chosen. The doses and response data used for the modeling are listed in Table B-7. The AUC normalized per day during gestation (AUC_{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 pup body weight.

Table B-7. Dose-Response Modeling Data for Pup Body Weight in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	8	5.9 ± 0.6 ^b
1	10.4	8	5.7 ± 0.3
2	20.8	8	5.4 ± 0.3
3	31.2	8	5.3 ± 0.3
5	52.0	5	5.0 ± 0.2

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for pup body weight are summarized in Table B-8 and Figure B-2. The best fitting model was the Exponential 3 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 Exponential 3 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change (BMDL₅) from the selected Exponential 3 model is 11.8 mg/L.

Table B-8. Summary of Benchmark Dose Modeling Results for Pup Body Weight in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD ₅ (mg/L)	BMDL ₅ (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD ₅	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.869	30.5	−0.7	0.2	0.23	15.6	11.8	8.9	6.3	EPA selected the Exponential 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 3 model had the lowest AIC.
Exponential 3	0.869	30.5	−0.7	0.2	0.23	15.6	11.8	8.9	6.3	
Exponential 4	0.840	32.1	0.3	0.3	−0.09	12.5	6.4	6.8	3.3	
Exponential 5	0.572	34.1	0.2	0.2	−0.04	13.3	6.5	7.7	3.3	
Hill	0.585	34.1	0.2	0.2	−0.03	13.4	5.8	8.1	2.9	
Polynomial Degree 4	0.821	30.7	−0.8	0.2	0.33	16.6	12.8	9.5	7.0	
Polynomial Degree 3	0.821	30.7	−0.8	0.2	0.33	16.6	12.8	9.5	7.0	
Polynomial Degree 2	0.821	30.7	−0.8	0.2	0.33	16.6	12.8	9.5	7.0	
Power	0.821	30.7	−0.8	0.2	0.33	16.6	12.8	9.5	7.0	
Linear	0.821	30.7	−0.8	0.2	0.33	16.6	12.8	9.5	7.0	

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; BMD₅ = dose level corresponding to a 5% change in the response; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the response.

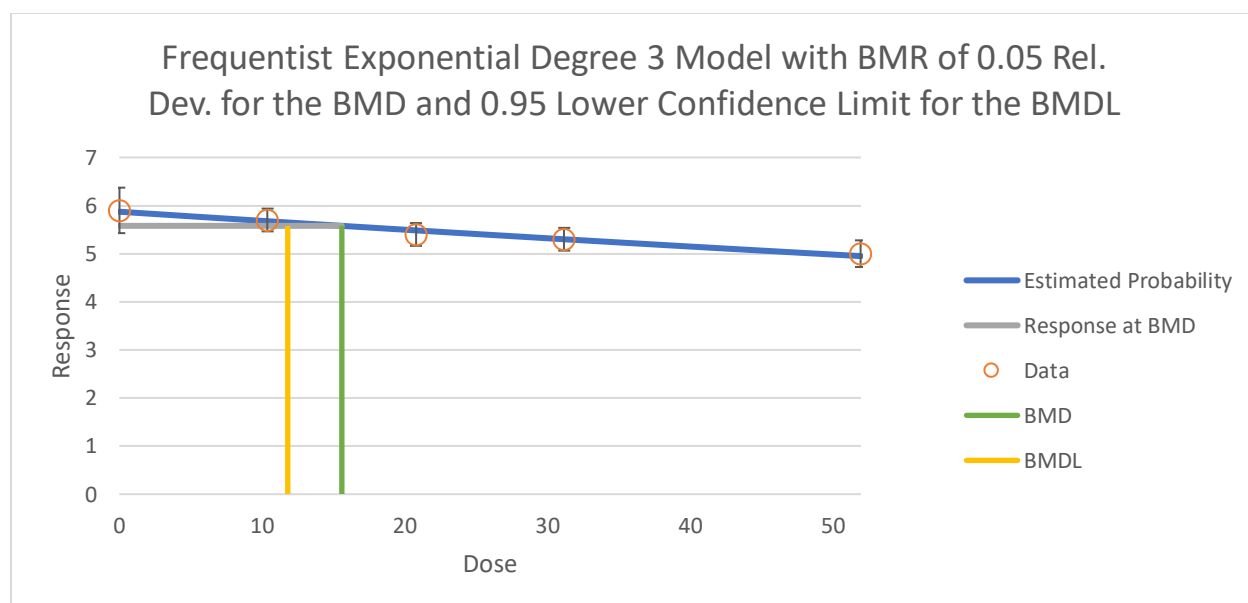


Figure B-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Exponential 3 Model for Pup Body Weight in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.2.2 Offspring Survival at PND 5

Decreased mean response of offspring survival at PND 5 was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. BMRs of a change in the mean equal to 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-9. The AUC normalized per day during gestation ($AUC_{avg,pup,gest}$), AUC normalized per day during lactation ($AUC_{avg,pup,lact}$), AUC normalized per day during gestation/lactation ($AUC_{avg,pup,gest,lact}$), maximum fetal concentration during gestation ($C_{max,pup,gest}$), and maximum pup concentration during lactation ($C_{max,pup,lact}$) were all considered and shown below because survival could be a result of exposure during a sensitive window of development where a C_{max} metric is expected to better correlate with the effect or an accumulation of exposure where an AUC metric is expected to better correlate with the effect and this could occur during the gestation or lactation lifestages.

Table B-9. Dose-Response Modeling Data for Offspring Survival at PND 5 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose					Number per Group	Mean Response (%) ^a
	$AUC_{avg,pup,gest}$ (mg/L)	$AUC_{avg,pup,lact}$ (mg/L)	$AUC_{avg,pup,gest,lact}$ (mg/L)	$C_{max,pup,gest}$ (mg/L)	$C_{max,pup,lact}$ (mg/L)		
0	0	0	0	0	0	9	90 ± 6 ^b
1	10.4	29.5	19.7	19.8	46.6	9	86 ± 12

Administered Dose (mg/kg/day)	Internal Dose					Number per Group	Mean Response (%) ^a
	AUC _{avg, pup,gest} (mg/L)	AUC _{avg, pup,lact} (mg/L)	AUC _{avg,pup,gest,lact} (mg/L)	C _{max, pup,gest} (mg/L)	C _{max, pup,lact} (mg/L)		
2	20.8	59.0	39.4	39.6	93.2	9	79 ± 21
3	31.2	88.5	59.2	59.4	139.7	9	45 ± 27
5	52.0	147.5	98.6	99.0	232.9	9	4 ± 9

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for Offspring Survival at PND 5 are summarized in Table B-10, Table B-11, Table B-12, Table B-13, and Table B-14. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table B-10. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 5 for AUC_{avg,pup,gest} in F1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	410.9	-1.64	-1.64	4.3	3.2	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.717	385.6	0.40	0.16	18.4	13.1	
Exponential 4	<0.0001	410.9	-1.64	-1.64	4.3	3.2	
Exponential 5	0.638	387.2	0.05	0.31	19.9	13.5	
Hill	0.623	387.2	0.04	0.33	20.1	12.5	
Polynomial Degree 4	0.097	389.6	0.27	-0.41	10.9	6.0	
Polynomial Degree 3	0.097	389.6	0.27	-0.41	10.9	6.0	
Polynomial Degree 2	0.097	389.6	0.27	-0.41	10.9	6.2	
Power	0.129	389.1	0.11	-0.41	11.7	7.2	
Linear	0.009	394.6	-1.80	-1.80	5.2	4.2	

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.

Table B-11. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 5 for AUC_{avg,pup,lact} in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	410.9	-1.64	-1.64	12.3	9.1	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.717	385.6	0.40	0.16	52.0	37.1	
Exponential 4	<0.0001	410.9	-1.64	-1.64	12.3	9.1	
Exponential 5	0.638	387.2	0.05	0.31	56.4	38.2	
Hill	0.623	387.2	0.04	0.33	56.9	35.6	
Polynomial Degree 4	0.097	389.6	0.27	-0.41	31.0	16.9	
Polynomial Degree 3	0.097	389.6	0.27	-0.41	31.0	16.9	
Polynomial Degree 2	0.097	389.6	0.27	-0.41	31.0	17.5	
Power	0.129	389.1	0.11	-0.41	33.2	20.4	
Linear	0.009	394.6	-1.80	-1.80	14.7	12.0	

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.

Table B-12. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 5 for AUC_{avg,pup,gest,lact} in F₁ Male and Female Sprague-Dawley Following Exposure to PFOS (constant variance)

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	410.9	-1.64	-1.64	8.2	6.1	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.717	385.6	0.40	0.16	34.9	24.8	
Exponential 4	<0.0001	410.9	-1.64	-1.64	8.2	6.1	
Exponential 5	0.638	387.2	0.05	0.31	37.7	25.5	
Hill	0.623	387.2	0.04	0.33	38.0	23.8	
Polynomial Degree 4	0.097	389.6	0.27	-0.41	20.7	11.3	
Polynomial Degree 3	0.097	389.6	0.27	-0.41	20.7	11.3	
Polynomial Degree 2	0.097	389.6	0.27	-0.41	20.7	11.7	
Power	0.129	389.1	0.11	-0.41	22.2	13.7	
Linear	0.009	394.6	-1.80	-1.80	9.8	8.0	

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.

Table B-13. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 5 for $C_{\text{max,pup,gst}}$ in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	410.9	-1.64	-1.64	8.3	6.1	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.717	385.6	0.40	0.16	34.9	24.9	
Exponential 4	<0.0001	410.9	-1.64	-1.64	8.3	6.1	
Exponential 5	0.638	387.2	0.05	0.31	37.9	25.6	
Hill	0.623	387.2	0.04	0.33	38.2	23.9	
Polynomial Degree 4	0.097	389.6	0.27	-0.41	20.8	11.3	
Polynomial Degree 3	0.097	389.6	0.27	-0.41	20.8	11.3	
Polynomial Degree 2	0.097	389.6	0.27	-0.41	20.8	11.8	
Power	0.129	389.1	0.11	-0.41	22.3	13.7	
Linear	0.009	394.6	-1.80	-1.80	9.9	8.0	

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.

Table B-14. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 5 for $C_{\text{max,pup,lact}}$ in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	410.9	-1.64	-1.64	19.5	14.3	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.717	385.6	0.40	0.16	82.3	58.5	
Exponential 4	<0.0001	410.9	-1.64	-1.64	19.5	14.3	
Exponential 5	0.638	387.2	0.05	0.31	89.1	60.3	
Hill	0.623	387.2	0.04	0.33	89.8	56.2	
Polynomial Degree 4	0.097	389.6	0.27	-0.41	49.0	26.7	
Polynomial Degree 3	0.097	389.6	0.27	-0.41	49.0	26.7	
Polynomial Degree 2	0.097	389.6	0.27	-0.41	49.0	27.7	
Power	0.129	389.1	0.11	-0.41	52.5	32.3	
Linear	0.009	394.6	-1.80	-1.80	23.2	18.9	

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.

B.2.2.3 Offspring Survival at PND 22

Decreased mean response of offspring survival at PND 22 was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen. The doses and response data used for the modeling are listed in Table B-15. The AUC normalized per day during gestation (AUC_{avg,pup,gest}), AUC normalized per day during lactation (AUC_{avg,pup,lact}), AUC normalized per day during gestation/lactation (AUC_{avg,pup,gest,lact}), maximum fetal concentration during gestation (C_{max,pup,gest}), and maximum pup concentration during lactation (C_{max,pup,lact}) were all considered and shown below because survival could be a result of exposure during a sensitive window of development where a C_{max} metric is expected to better correlate with the effect or an accumulation of exposure where an AUC metric is expected to better correlate with the effect and this could occur during the gestation or lactation lifestages.

Table B-15. Dose-Response Modeling Data for Offspring Survival at PND 22 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose					Number per Group	Mean Response (%) ^a
	AUC _{avg,pup,gest} (mg/L)	AUC _{avg,pup,lact} (mg/L)	AUC _{avg,pup,gest,lact} (mg/L)	C _{max,pup,gest} (mg/L)	C _{max,pup,lact} (mg/L)		
0	0	0	0	0	0	9	78 ± 3 ^b
1	10.4	29.5	19.7	19.8	46.6	9	74 ± 18
2	20.8	59.0	39.4	39.6	93.2	9	61 ± 39
3	31.2	88.5	59.2	59.4	139.7	9	34 ± 21
5	52.0	147.5	98.6	99.0	232.9	9	2 ± 6

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for Offspring Survival at PND 22 are summarized in Table B-16, Table B-17, Table B-18, Table B-19, and Table B-20. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table B-16. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 22 for AUC_{avg,pup,gest} in F1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	0.006	417.2	-1.2	-1.2	4.9	3.5	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.983	407.0	0.1	0.1	17.3	10.5	
Exponential 4	0.006	417.2	-1.2	-1.2	4.9	3.5	
Exponential 5	0.852	408.9	0.1	0.1	17.3	10.5	
Hill	0.808	409.0	0.1	0.1	17.6	7.3	
Polynomial Degree 4	0.393	408.8	0.5	-0.4	10.2	5.7	
Polynomial Degree 3	0.393	408.8	0.5	-0.4	10.2	5.7	
Polynomial Degree 2	0.393	408.8	0.5	-0.4	10.2	5.7	
Power	0.477	408.4	0.4	-0.3	11.4	5.9	
Linear	0.354	408.2	0.7	-1.1	6.8	5.4	

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.

Table B-17. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 22 for AUC_{avg,pup,lact} in F1 Male and Female Sprague-Dawley Rats for AUC Normalized per Day During Lactation Following Exposure to PFOS (constant variance)

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	0.006	417.2	-1.2	-1.2	14.0	9.8	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.983	407.0	0.1	0.1	48.9	29.7	
Exponential 4	0.006	417.2	-1.2	-1.2	14.0	9.8	
Exponential 5	0.852	408.9	0.1	0.1	49.1	29.7	
Hill	0.808	409.0	0.1	0.1	50.0	20.6	
Polynomial Degree 4	0.393	408.8	0.5	-0.4	28.8	16.3	
Polynomial Degree 3	0.393	408.8	0.5	-0.4	28.8	16.3	
Polynomial Degree 2	0.393	408.8	0.5	-0.4	28.8	16.3	
Power	0.477	408.4	0.4	-0.3	32.4	16.7	
Linear	0.354	408.2	0.7	-1.1	19.2	15.3	

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.

Table B-18. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 22 for AUC_{avg,pup,gest,lact} in F1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	0.006	417.2	-1.2	-1.2	9.4	6.6	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.983	407.0	0.1	0.1	32.7	19.8	
Exponential 4	0.006	417.2	-1.2	-1.2	9.4	6.6	
Exponential 5	0.852	408.9	0.1	0.1	32.8	19.8	
Hill	0.808	409.0	0.1	0.1	33.4	13.8	
Polynomial Degree 4	0.393	408.8	0.5	-0.4	19.3	10.9	
Polynomial Degree 3	0.393	408.8	0.5	-0.4	19.3	10.9	
Polynomial Degree 2	0.393	408.8	0.5	-0.4	19.3	10.9	
Power	0.477	408.4	0.4	-0.3	21.7	11.2	
Linear	0.354	408.2	0.7	-1.1	12.8	10.2	

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.

Table B-19. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 22 for C_{max,pup,gest} in F1 Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	0.006	417.2	-1.2	-1.2	9.4	6.6	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.983	407.0	0.1	0.1	32.8	19.9	
Exponential 4	0.006	417.2	-1.2	-1.2	9.4	6.6	
Exponential 5	0.852	408.9	0.1	0.1	33.0	19.9	
Hill	0.808	409.0	0.1	0.1	33.5	13.8	
Polynomial Degree 4	0.393	408.8	0.5	-0.4	19.3	10.9	
Polynomial Degree 3	0.393	408.8	0.5	-0.4	19.3	10.9	
Polynomial Degree 2	0.393	408.8	0.5	-0.4	19.3	10.9	
Power	0.477	408.4	0.4	-0.3	21.7	11.2	
Linear	0.354	408.2	0.7	-1.1	12.9	10.3	

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.

Table B-20. Summary of Benchmark Dose Modeling Results for Offspring Survival at PND 22 for C_{max,pup,lact} in F₁ Male and Female Sprague-Dawley Rats for Maximum Fetal Concentration during Lactation Following Exposure to PFOS (constant variance)

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	0.006	417.2	-1.2	-1.2	22.1	15.5	No models had adequate fit for the constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.983	407.0	0.1	0.1	77.3	46.9	
Exponential 4	0.006	417.2	-1.2	-1.2	22.1	15.5	
Exponential 5	0.852	408.9	0.1	0.1	77.6	46.9	
Hill	0.808	409.0	0.1	0.1	78.9	32.7	
Polynomial Degree 4	0.393	408.8	0.5	-0.4	45.5	25.7	
Polynomial Degree 3	0.393	408.8	0.5	-0.4	45.5	25.7	
Polynomial Degree 2	0.393	408.8	0.5	-0.4	45.5	25.7	
Power	0.477	408.4	0.4	-0.3	51.1	26.4	
Linear	0.354	408.2	0.7	-1.1	30.3	24.1	

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.

B.2.3 Lee, 2015, 2851075

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Lee (2015, 2851075) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses fetal body weight in F₁ male and female CD-1 mice and the number of dead fetuses in P₀ female CD-1 mice.

B.2.3.1 Fetal Body Weight

Decreased mean response of fetal body weight was observed in F₁ male and female CD-1 mice. Continuous models were used to fit dose-response data. Benchmark responses (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 B-21. The average pup concentration during gestation (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 B-21. Dose-Response Modeling Data for Fetal Body Weight in F₁ Male and Female CD-1 Mice Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	10	1.7 ± 0.2
0.5	0.9	10	1.5 ± 0.1
2	3.5	10	1.3 ± 0.1
8	14.0	10	1.1 ± 0.2

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for fetal body weight are summarized in Table B-22 and Figure B-3. The best fitting model was the Exponential 5 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 Exponential 5 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change from the control mean (BMDL₅) from the selected Exponential 5 model is 0.3 mg/L.

Table B-22. Summary of Benchmark Dose Modeling Results for Fetal Body Weight in F₁ Male and Female CD-1 Mice Following Exposure to PFOS (constant variance)

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 _{0.5SD}	Dose Group near BMD ₅	Control Dose Group					
Exponential 2	0.001	-19.5	-0.4	-0.4	2.1	2.0	1.5	1.8	1.4	EPA selected the Exponential 5 model. Exponential 4 and 5 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 5 model had the lowest AIC.
Exponential 3	0.001	-19.5	-0.4	-0.4	2.1	2.0	1.5	1.8	1.4	
Exponential 4	0.682	-32.4	-0.3	0.2	0.2	0.4	0.2	0.4	0.3	
Exponential 5	0.682	-32.4	-0.3	0.2	0.2	0.4	0.2	0.4	0.3	
Hill	— ^b	-30.6	2.6×e ⁻⁶	2.6×e ⁻⁶	2.6×e ⁻⁶	0.3	0.2	0.4	0.2	
Polynomial Degree 3	<0.001	-17.5	-2.8	-2.8	2.3	2.5	1.9	2.2	1.8	
Polynomial Degree 2	<0.001	-17.5	-2.8	-2.8	2.3	2.5	1.9	2.2	1.8	
Power	<0.001	-17.5	-2.8	-2.8	2.3	2.5	1.9	2.2	1.8	
Linear	<0.001	-17.5	-2.8	-2.8	2.3	2.5	1.9	2.2	1.8	

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; BMD₅ = dose level corresponding to a 5% change in the mean from the control mean; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the mean from the control mean.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

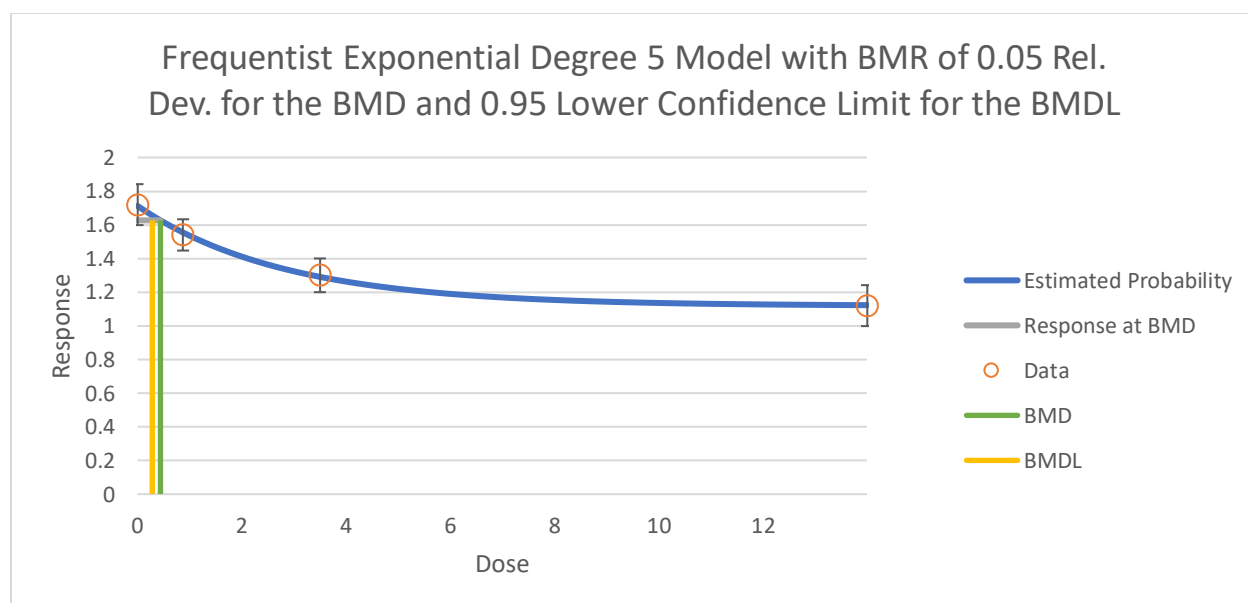


Figure B-3. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 5 Model for Fetal Body Weight in F₁ Male and Female CD-1 Mice Following Exposure to PFOS

B.2.3.2 Number of Dead Fetuses

Increased mean response of fetal body weight was observed in P₀ female CD-1 mice. Continuous models were used to fit dose-response data. A benchmark response (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 B-23. The AUC normalized per day during gestation (AUC_{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 AUC metric is more appropriate.

Table B-23. Dose-Response Modeling Data for Fetal Body Weight in P₀ Female CD-1 Mice Following Exposure to PFOS

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

^aData are presented as mean ± standard deviation.

The BMD modeling results for fetal body weight for AUC normalized per day during gestation (AUC_{avg_dam_gest}) and maximum maternal concentration during gestation (C_{max_dam}) are summarized in Table B-24 and Table B-25, respectively. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint.

Table B-24. Summary of Benchmark Dose Modeling Results for Fetal Body Weight for $AUC_{avg_dam_gest}$ in P₀ Female CD-1 Mice Following Exposure to PFOS (nonconstant variance)

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	149.5	4.2	-3.2	8.2	6.5	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	149.5	4.2	-3.2	8.2	6.5	
Exponential 4	0.045	73.1	0.5	0.5	0.2	0.1	
Exponential 5	— ^a	71.0	2.3×e ⁻⁶	2.3×e ⁻⁶	0.4	0.2	
Hill	— ^a	71.0	1.1×e ⁻⁵	1.1×e ⁻⁵	0.4	0.2	
Polynomial Degree 3	<0.0001	118.4	-1.1	-1.1	0.4	0.2	
Polynomial Degree 2	<0.0001	118.4	-1.1	-1.1	0.4	0.2	
Power	<0.0001	118.4	-1.1	-1.1	0.4	0.2	
Linear	<0.0001	118.4	-1.1	-1.1	0.4	0.2	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

Table B-25. Summary of Benchmark Dose Modeling Results for Fetal Body Weight for C_{max_dam} in P₀ Female CD-1 Mice Following Exposure to PFOS (nonconstant variance)

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	149.5	4.2	-3.2	35.6	28.3	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	149.5	4.2	-3.2	35.6	28.3	
Exponential 4	0.045	73.1	0.5	0.5	0.8	0.6	
Exponential 5	— ^a	71.0	-4.5×e ⁻⁶	-4.5×e ⁻⁶	1.6	0.8	
Hill	— ^a	71.0	-7.5×e ⁻⁷	-7.5×e ⁻⁷	1.9	1.1	
Polynomial Degree 3	<0.0001	118.4	-1.1	-1.1	1.5	1.0	
Polynomial Degree 2	<0.0001	118.4	-1.1	-1.1	1.5	1.0	
Power	<0.0001	118.4	-1.1	-1.1	1.5	1.0	
Linear	<0.0001	118.4	-1.1	-1.1	1.5	1.0	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

B.2.4 Luebker, 2005, 757857

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Luebker (2005, 757857) study using the Benchmark Dose Software (BMDs) 3.2 program. This study addresses pup body weight relative to the litter at PND0 and at LD5 in F₁ male and female Sprague-Dawley rats.

B.2.4.1 Pup Body Weight Relative to Litter at PND0

Decreased mean response of pup body weight relative to the litter at PND0 was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A benchmark response (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 B-26. The AUC normalized per day during gestation (AUC_{avg,pup,gest}) was selected for this model rather than alternate metrics such as C_{max} because the AUC normalized per day during gestation is expected to better correlate with an accumulation of effect resulting in decreased pup body weight..

Table B-26. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter at PND0 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	17	6.4 ± 0.8 ^b
0.4	15.4	17	6.0 ± 1.2
0.8	30.8	17	6.0 ± 1.2
1	38.5	17	5.9 ± 1.6
1.2	46.1	17	5.7 ± 1.2
1.6	61.5	17	5.4 ± 1.0
2	76.9	17	5.4 ± 1.0

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for pup body weight relative to the litter at PND0 are summarized in Table B-27 and Figure B-4. All models had adequate fit (p-values greater than 0.1), and after visual inspection the Exponential 2 model was chosen. The 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 (BMDL_{0.5SD}) from the selected Exponential 2 model is 25.8 mg/L.

Table B-27. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter at PND0 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

Model ^a	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	0.983	373.6	0.28	0.3	41.9	25.8	EPA selected the Exponential 2 model. All models had adequate fit (p-values greater than 0.1), and after visual inspection the Exponential 2 model was chosen.
Exponential 3	0.983	373.6	0.28	0.3	41.9	25.8	
Exponential 4	0.954	375.5	0.32	0.2	39.7	11.1	
Exponential 5	0.952	375.6	0.28	0.3	41.9	11.1	
Hill	0.954	375.5	0.32	0.2	39.5	7.2	
Polynomial Degree 6	0.982	373.6	0.03	0.3	43.6	28.2	
Polynomial Degree 5	0.982	373.6	0.03	0.3	43.6	28.1	
Polynomial Degree 4	0.982	373.6	0.03	0.3	43.6	28.1	
Polynomial Degree 3	0.982	373.6	0.03	0.3	43.6	28.1	
Polynomial Degree 2	0.982	373.6	0.03	0.3	43.6	28.1	
Power	0.982	373.6	0.03	0.3	43.6	28.1	
Linear	0.982	373.6	0.03	0.3	43.6	28.1	

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.

^aSelected model in bold.

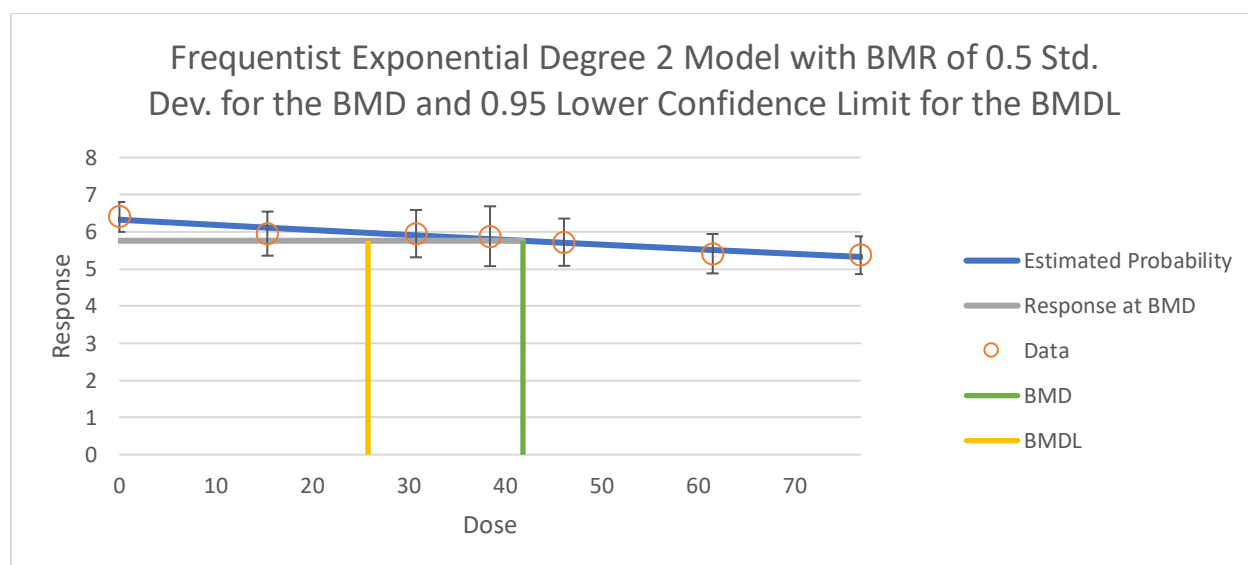


Figure B-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Exponential 2 Model for Pup Body Weight Relative to the Litter at PND0 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.4.2 Pup Body Weight Relative to Litter at LD5

Decreased mean response of pup body weight relative to the litter at LD5 was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations and 5% change from the control mean. The doses and response data used for the modeling are listed in Table B-28. The AUC normalized per day during gestation (AUC_{avg,pup,gest}) was selected for this model rather than alternate metrics such as C_{max} because the AUC normalized per day during gestation is expected to better correlate with an accumulation of effect resulting in decreased pup body weight..

Table B-28. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (LD5) in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

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

^aData are presented as mean ± standard deviation.

^bStandard 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 LD5 are summarized in Table B-29 and Figure B-5. The Exponential 5 model was selected as it had the lowest AIC among the viable models. The BMDL₅ from the selected Exponential 5 model is 2.4 mg/L.

Table B-29. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter (LD5) in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	0.942	474.3	0.4	-0.6	0.2	27.7	17.3	10.7	7.2	EPA selected the Exponential 5 model. The Exponential 5 model was selected as it had the lowest AIC among the viable models.
Exponential 3	0.942	474.3	0.4	-0.6	0.2	27.7	17.3	10.7	7.2	
Exponential 4	0.858	476.3	0.5	-0.6	0.2	26.3	7.2	9.9	2.4	
Exponential 5	0.856	476.3	0.4	-0.6	0.2	27.7	7.2	10.7	2.4	
Hill	0.859	476.3	0.5	-0.6	0.2	26.0	4.2	9.7	1.2	
Polynomial Degree 6	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.7	
Polynomial Degree 5	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.9	
Polynomial Degree 4	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.5	
Polynomial Degree 3	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.5	
Polynomial Degree 2	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.5	
Power	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.5	
Linear	0.935	474.3	0.3	-0.6	0.3	30.0	20.0	11.9	8.5	

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; BMD₅ = dose level corresponding to a 5% change in the mean from the control mean; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in the mean from the control mean.

^aSelected model in bold.

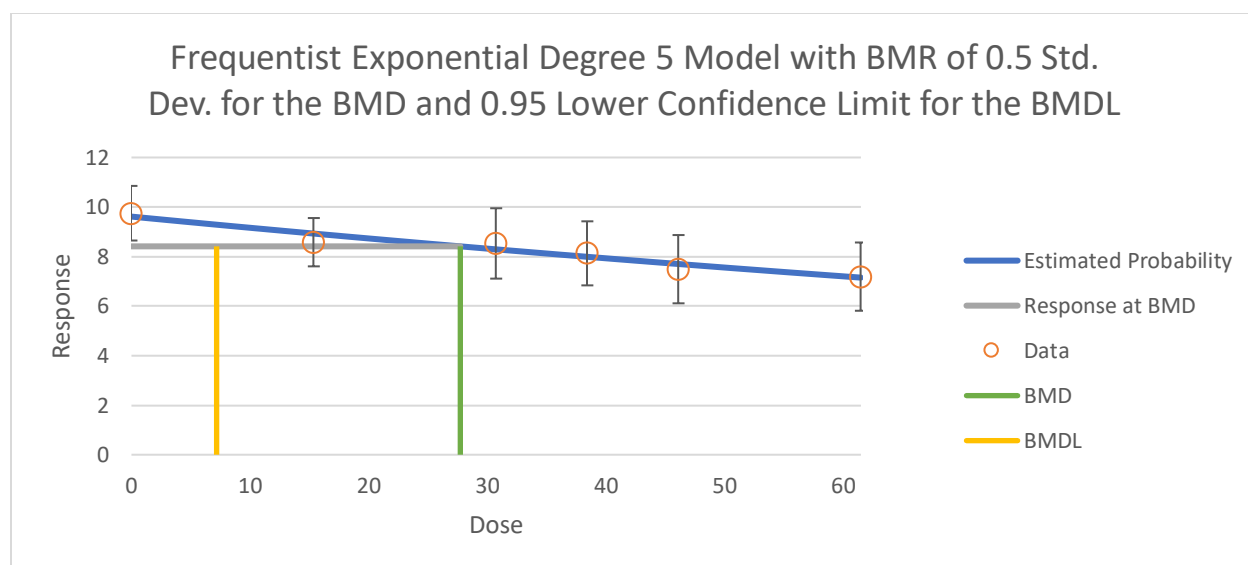


Figure B-5. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 5 Model for Pup Body Weight Relative to the Litter at LD5 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.5 Luebker, 2005, 1276160

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Luebker (2005, 1276160) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses pup body weight relative to the litter observed on postnatal day 1 (PND1) in F₁ male and female Sprague-Dawley rats.

B.2.5.1 Pup Body Weight Relative to Litter (PND1)

Decreased mean response of pup body weight relative to the litter was observed in F₁ male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A benchmark response (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 B-30. The AUC normalized per day during gestation ($AUC_{avg,pup,gest}$) was selected for this model rather than alternate metrics such as C_{max} because the AUC normalized per day during gestation is expected to better correlate with an accumulation of effect resulting in decreased pup body weight.

Table B-30. Dose-Response Modeling Data for Pup Body Weight Relative to the Litter (PND1) in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	23	6.6 ± 0.6 ^b
0.1	3.8	25	6.6 ± 0.5
0.4	15.3	22	6.4 ± 0.7

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
1.6	61.6	20	5.7 ± 0.5
3.2	131.0	20	5.3 ± 0.4

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for pup body weight relative to the litter at PND1 are summarized in Table B-31 and Figure B-6. The best fitting model was the Exponential 4 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 Exponential 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 change in the mean equal to 0.5 standard deviations from the control mean (BMDL_{0.5SD}) from the selected Exponential 4 model is 8.4 mg/L.

Table B-31. Summary of Benchmark Dose Modeling Results for Pup Body Weight Relative to the Litter at PND1 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

Model ^a	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	0.317	185.9	-0.03	0.1	23.6	19.3	EPA selected the Exponential 4 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 4 model had the lowest AIC.
Exponential 3	0.317	185.9	-0.03	0.1	23.6	19.3	
Exponential 4	0.759	184.9	0.39	-0.5	13.8	8.4	
Exponential 5	0.820	186.4	-0.07	-0.1	19.0	8.8	
Hill	0.857	186.4	-0.05	-0.1	18.7	8.0	
Polynomial Degree 4	0.204	186.9	-0.06	0.3	26.3	22.2	
Polynomial Degree 3	0.204	186.9	-0.06	0.3	26.3	21.9	
Polynomial Degree 2	0.204	186.9	-0.06	0.3	26.3	21.9	
Power	0.204	186.9	-0.06	0.3	26.3	21.9	
Linear	0.204	186.9	-0.06	0.3	26.3	21.9	

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.

^aSelected model in bold.

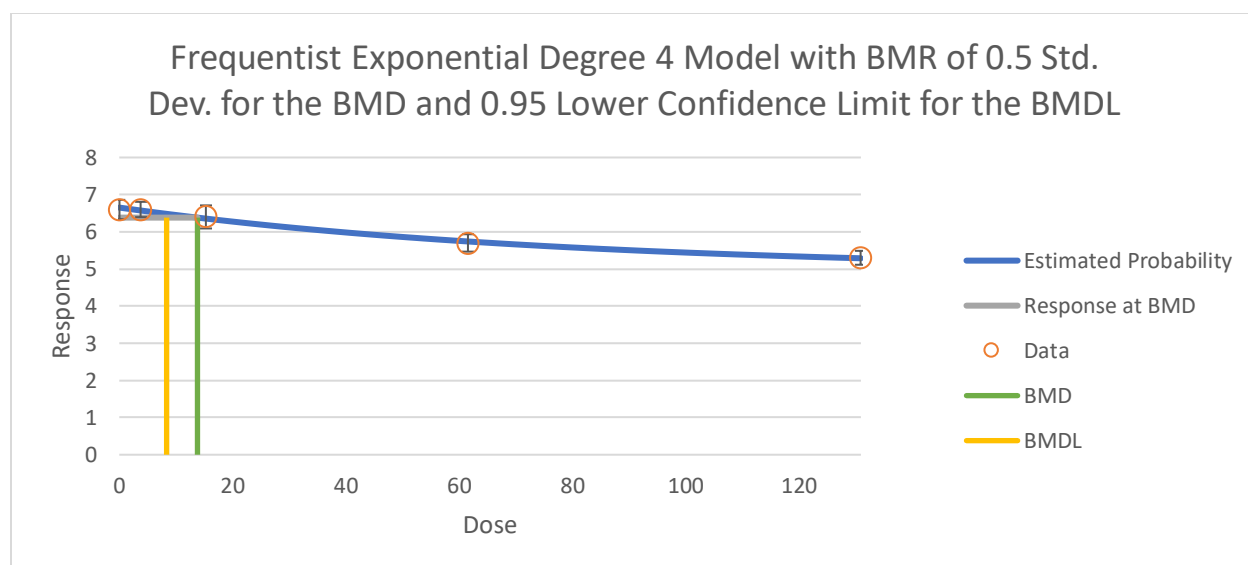


Figure B-6. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Pup Body Weight Relative to the Litter at PND1 in F₁ Male and Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.6 Mshaty, 2020, 6833692

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Mshaty (2020, 6833692) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses object location recognition memory test in F₁ male C57BL/6J mice.

B.2.6.1 Object Location Recognition Memory Test in F₁ Male C57BL/6J

Decreased mean response of object location recognition memory test was observed in F₁ male C57BL/6J. Continuous models were used to fit dose-response data. A benchmark response (BMR) of a change in the mean equal one standard deviations 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 B-32. The AUC normalized per day during lactation (AUC_{avg,pup,lact}) was selected for this model because the average blood concentration is expected to correlate with an accumulation of effects leading to decreased response on the memory test.

Table B-32. Dose-Response Modeling Data for Object Location Recognition Memory Test in F₁ Male C57BL/6J Mice Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (Discrimination index)
0	0	15	0.27 ± 0.2 ^b
0.1	0.4	15	0.19 ± 0.1
0.25	0.9	15	0.14 ± 0.2
1	3.6	15	0.05 ± 0.1

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for object location recognition memory test are summarized in Table B-33. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table B-33. Summary of Benchmark Dose Modeling Results for Object Location Recognition Memory Test in F₁ Male C58BL/6J Following Exposure to PFOS (constant variance)

Model	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	0.752	-53.6	-0.1	0.4	1.5	0.6	No models had adequate fit for constant or non-constant variance (Test 2 p-value < 0.05 and Test 3 p-value < 0.05).
Exponential 3	0.752	-53.6	-0.1	0.4	1.5	0.6	
Exponential 4	0.718	-52.1	0.2	0.1	1.2	0.4	
Exponential 5	0.718	-52.1	0.2	0.1	1.2	0.4	
Hill	0.809	-52.2	0.2	0.1	1.2	0.4	
Polynomial Degree 3	0.314	-51.9	0.3	1.1	2.9	2.0	
Polynomial Degree 2	0.314	-51.9	0.3	1.1	2.9	2.0	
Power	0.314	-51.9	0.3	1.1	2.9	2.0	
Linear	0.314	-51.9	0.3	1.1	2.9	2.0	

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_{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 deviations from the control mean.

B.2.7 NTP, 2019, 5400978

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the NTP (2019, 5400978) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum alanine aminotransferase (ALT) levels, serum triiodothyronine (T3) levels, serum free thyroxine (T4) levels, and extramedullary hematopoiesis in the spleen in male Sprague-Dawley rats and serum T3 levels, serum free T4 levels, serum total T4 levels, and extramedullary hematopoiesis in the spleen in female Sprague-Dawley rats.

B.2.7.1 Serum Alanine Aminotransferase in Male Sprague-Dawley Rats

Increased mean response of serum ALT was observed in male Sprague-Dawley rats. Continuous models were used to fit dose-response data. A benchmark response (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 B-34. The average concentration over the final week of study (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 increased serum ALT.

Table B-34. Dose-Response Modeling Data for Serum Alanine Aminotransferase in Male Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (IU/L) ^a
0	0	10	48 ± 3.2 ^b
0.312	10.0	10	52 ± 6.3
0.625	20.1	10	66 ± 15.8
1.25	40.1	10	56 ± 9.5
2.5	80.2	10	75 ± 25.3
5	160.4	10	71 ± 12.6

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for serum ALT are summarized in Table B-35. The data was non-monotonic, and no models provided an adequate fit, therefore a no-observed-adverse-effect level (NOAEL) approach was taken for this endpoint.

Table B-35. Summary of Benchmark Dose Modeling Results for Serum Alanine Aminotransferase in Male Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance)

Model	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	<0.0001	498.7	1.7	-1.6	71.5	42.0	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	498.7	1.7	-1.6	71.5	42.0	
Exponential 4	0.0002	471.8	0.3	0.3	3.3	1.8	
Exponential 5	0.001	469.3	-0.4	0.2	8.6	5.1	
Hill	0.001	469.1	-0.4	0.2	8.8	5.6	
Polynomial Degree 5	<0.0001	496.2	-0.9	-1.3	50.2	26.9	
Polynomial Degree 4	<0.0001	496.2	-0.9	-1.3	50.2	26.9	
Polynomial Degree 3	<0.0001	496.2	-0.9	-1.3	50.2	26.9	
Polynomial Degree 2	<0.0001	496.2	-0.9	-1.3	50.2	26.9	
Power	<0.0001	496.2	-0.9	-1.3	50.2	26.9	
Linear	<0.0001	496.2	-0.9	-1.3	50.2	26.9	

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.

B.2.7.2 Serum Triiodothyronine Levels in Male Sprague-Dawley Rats

Decreased mean response of serum T3 levels was observed in male Sprague-Dawley rats. 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 B-36. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 effects leading to a decreased serum T3 level.

Table B-36. Dose-Response Modeling Data for Serum Triiodothyronine Levels in Male Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0	0	10	87.4 ± 16.8 ^b
0.312	10.0	10	77.8 ± 17.2
0.625	20.1	10	60.6 ± 14.7
1.25	40.1	10	57.5 ± 8.4
2.5	80.2	10	55.4 ± 8.7

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum T3 levels are summarized in Table B-37 and Figure B-7. 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 AIC. The BMDL_{1SD} from the selected Hill model is 6.8 mg/L.

Table B-37. Summary of Benchmark Dose Modeling Results for Serum Triiodothyronine Levels in Male Sprague-Dawley Rats Following Exposure to PFOS (constant variance)

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	0.007	416.5	-1.1	1.5	32.3	21.8	EPA selected the Hill model. The Exponential 4, Exponential 5, and Hill models all 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 AIC.
Exponential 3	0.007	416.5	-1.1	1.5	32.3	21.8	
Exponential 4	0.317	408.8	1.1	-0.3	8.3	4.7	
Exponential 5	0.712	408.6	0.2×e ⁻³	0.2×e ⁻²	11.8	6.4	
Hill	0.760	408.6	1.4×e⁻²	0.5×e⁻²	11.4	6.8	
Polynomial Degree 4	0.003	418.6	-1.4	1.9	41.7	30.2	
Polynomial Degree 3	0.003	418.6	-1.4	1.9	41.7	30.2	
Polynomial Degree 2	0.003	418.6	-1.4	1.9	41.7	30.2	
Power	0.003	418.6	-1.4	1.9	41.7	30.2	

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			
Linear	0.003	418.6	-1.4	1.9	41.7	30.2	

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.

^aSelected model in bold.

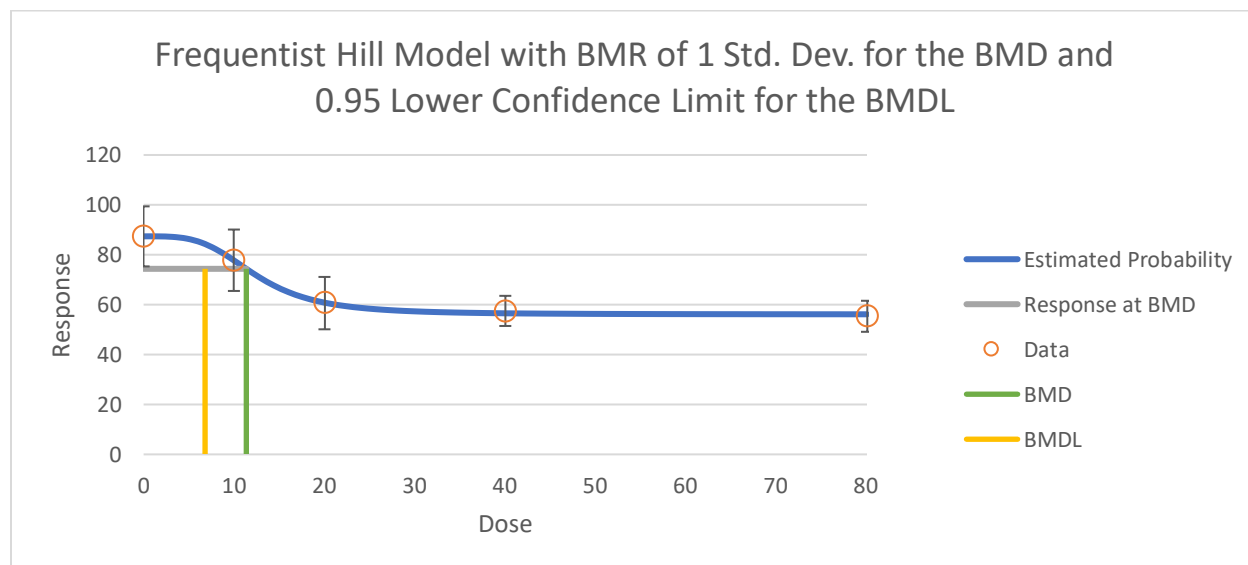


Figure B-7. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for Serum Triiodothyronine Levels in Male Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7.3 Serum Triiodothyronine Levels in Female Sprague-Dawley Rats

Decreased mean response of serum T3 levels was observed in female Sprague-Dawley rats. 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 B-38. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 effects leading to a decreased serum T3 level.

Table B-38. Dose-Response Modeling Data for Serum Triiodothyronine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0	0	10	93.1 ± 15.9 ^b
0.312	10.0	10	81.4 ± 9.6
0.625	20.1	10	72.5 ± 13.5
1.25	40.1	10	69.2 ± 11.5
2.5	80.2	10	62.0 ± 5.6
5	160.4	9	51.6 ± 4.3

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum T3 levels are summarized in Table B-39 and Figure B-8. 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 AIC. The BMDL_{1SD} from the selected Hill model is 16.1 mg/L.

Table B-39. Summary of Benchmark Dose Modeling Results for Serum Triiodothyronine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance)

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	0.059	445.5	-1.0	2.4	70.9	51.8	EPA selected the Hill model. The Exponential 4, Exponential 5, and Hill model all 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 AIC.
Exponential 3	0.059	445.5	-1.0	2.4	70.6	51.8	
Exponential 4	0.451	441.1	-0.4	1.3	38.8	22.6	
Exponential 5	0.451	441.1	-0.4	1.3	39.0	22.6	
Hill	0.575	440.4	-0.2	1.0	33.6	16.1	
Polynomial Degree 5	0.014	448.9	-1.4	2.7	84.3	65.0	
Polynomial Degree 4	0.014	448.9	-1.4	2.7	84.3	65.0	
Polynomial Degree 3	0.014	448.9	-1.4	2.7	84.3	64.9	
Polynomial Degree 2	0.014	448.9	-1.4	2.7	84.3	65.0	
Power	0.014	448.9	-1.4	2.7	84.3	64.9	
Linear	0.014	448.9	-1.4	2.7	84.3	65.1	

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.

^aSelected model in bold.

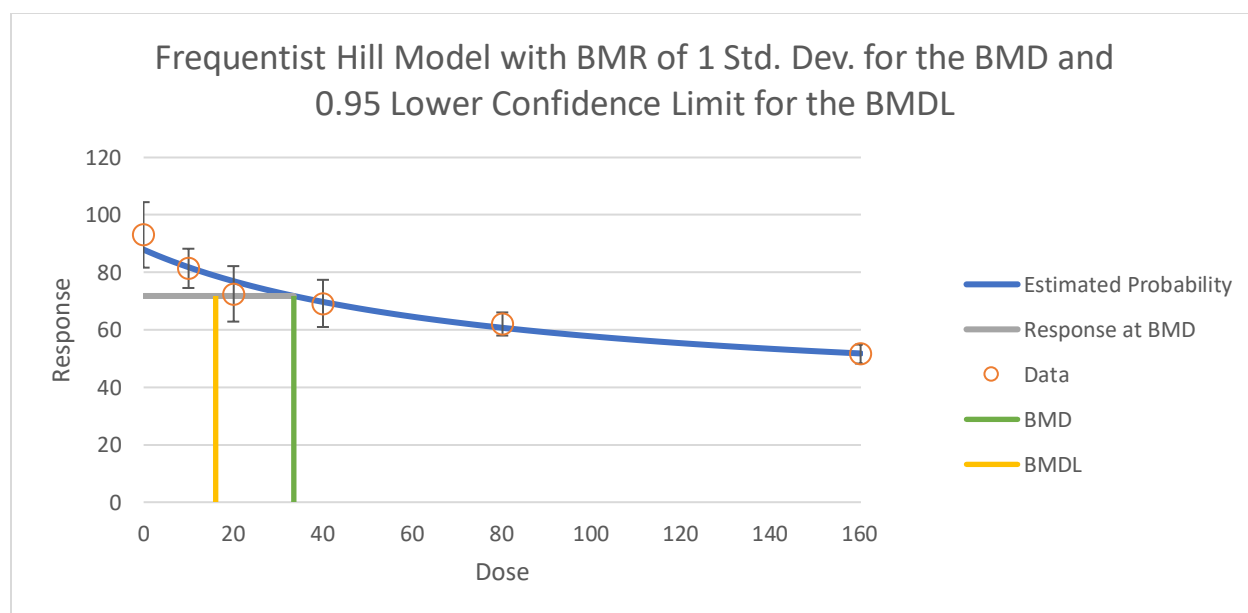


Figure B-8. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for Serum Triiodothyronine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7.4 Serum Free Thyroxine Levels in Male Sprague-Dawley Rats

Decreased mean response of serum free T4 levels was observed in male Sprague-Dawley rats. 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 B-40. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 effects leading to a decreased serum free T4 level.

Table B-40. Dose-Response Modeling Data for Serum Free Thyroxine Levels in Male Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0	0	10	2.5 ± 0.70^b
0.312	10.0	10	0.9 ± 0.32
0.625	20.0	10	0.5 ± 0.16
1.25	40.1	10	0.4 ± 0.06
2.5	80.2	10	0.4 ± 0.16
5	160.4	10	0.3 ± 0.03

^aData are presented as mean \pm standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum free T4 are summarized in Table B-41. No models provided an adequate fit for the constant or nonconstant variance models and an effect was observed at the lowest dose, therefore there is not a NOAEL and instead a LOAEL approach was taken for this endpoint.

Table B-41. Summary of Benchmark Dose Modeling Results for Serum Free Thyroxine in Male Sprague-Dawley Rat Following Exposure to PFOS (constant variance)

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	<0.0001	64.2	0.3	0.3	2.0	1.5	No models had adequate fit for the constant or non-constant variance (Test 2 p-value was less than 0.05).
Exponential 3	<0.0001	64.2	0.3	0.3	2.0	1.5	
Exponential 4	0.600	39.4	0.3	0.3	1.4	1.2	
Exponential 5	0.655	40.4	0.2	0.2	2.4	1.2	
Hill	0.935	39.7	-0.001	-0.001	3.4	0.6	
Polynomial Degree 5	<0.0001	137.0	-1.1	5.6	90.8	65.7	
Polynomial Degree 4	<0.0001	137.0	-1.1	5.6	90.8	65.7	
Polynomial Degree 3	<0.0001	137.0	-1.1	5.6	90.8	65.7	
Polynomial Degree 2	<0.0001	137.0	-1.1	5.6	90.8	65.7	
Power	<0.0001	137.0	-1.1	5.6	90.8	65.7	
Linear	<0.0001	137.0	-1.1	5.6	90.8	65.7	

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.

^aSelected model in bold.

B.2.7.5 Serum Free Thyroxine Levels in Female Sprague-Dawley Rats

Decreased mean response of serum free T4 levels was observed in female Sprague-Dawley rats. 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 B-42. The average concentration over the final week of study (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 effects leading to a decreased serum free T4 level.

Table B-42. Dose-Response Modeling Data for Serum Free Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0	0	10	1.7 ± 0.7 ^b
0.312	10.0	10	1.1 ± 0.3
0.625	20.1	10	0.7 ± 0.1
1.25	40.1	10	0.6 ± 0.2
2.5	80.2	10	0.6 ± 0.2
5	160.4	9	0.5 ± 0.1

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum free T4 levels are summarized in Table B-43, Table B-44, and Figure B-9. The BMD modeling results for serum free T4 levels including all dose groups is summarized in Table B-43. The BMD modeling results for serum free T4 levels excluding the highest dose is summarized in Table B-44 because none of the models adequately fit the entire dataset, the highest dose is far from the level of concern of 1 SD, and the response has plateaued at the highest doses. From Table B-44, the best fitting model was the Exponential 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 Exponential 4 model had the lowest AIC. The BMDL_{1SD} from the selected Exponential 4 model is 3.9 mg/L.

Table B-43. Summary of Benchmark Dose Modeling Results for Serum Free Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance)

Model	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	<0.0001	38.3	0.6	3.9	205.1	113.9	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	38.3	0.6	3.9	205.6	114.3	
Exponential 4	0.020	-8.7	0.6	-0.3	7.0	4.1	
Exponential 5	0.020	-8.7	0.6	-0.3	7.0	4.1	
Hill	0.037	-10.0	0.9	-0.3	6.6	2.9	
Polynomial Degree 5	<0.0001	45.5	0.2	4.3	219.9	148.7	
Polynomial Degree 4	<0.0001	45.5	0.2	4.3	219.9	148.7	
Polynomial Degree 3	<0.0001	45.5	0.2	4.3	219.9	148.7	
Polynomial Degree 2	<0.0001	45.5	0.2	4.3	219.9	148.7	
Power	<0.0001	45.5	0.2	4.3	219.9	148.8	
Linear	<0.0001	45.5	0.2	4.3	219.9	148.7	

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.

Table B-44. Summary of Benchmark Dose Modeling Results for Serum Free Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS, Dropping the Highest Dose Group (nonconstant variance)

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	<0.0001	40.7	0.9	3.0	70.2	37.0	EPA selected the Exponential 4 model. The Exponential 4, Exponential 5, and Hill models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 4 model had the lowest AIC.
Exponential 3	<0.0001	40.7	0.9	3.0	70.2	37.0	
Exponential 4	0.230	3.1	0.9	-0.5	6.7	3.9	
Exponential 5	0.246	3.5	0.2	-0.2	8.8	4.7	
Hill	0.290	3.3	0.3	-0.2	9.0	5.3	
Polynomial Degree 4	<0.0001	46.9	0.3	3.4	91.8	59.7	
Polynomial Degree 3	<0.0001	46.9	0.3	3.4	91.8	59.7	
Polynomial Degree 2	<0.0001	46.9	0.3	3.4	91.8	59.7	
Power	<0.0001	46.9	0.3	3.4	91.8	59.7	
Linear	<0.0001	46.9	0.3	3.4	91.8	59.7	

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.

^aSelected model in bold.

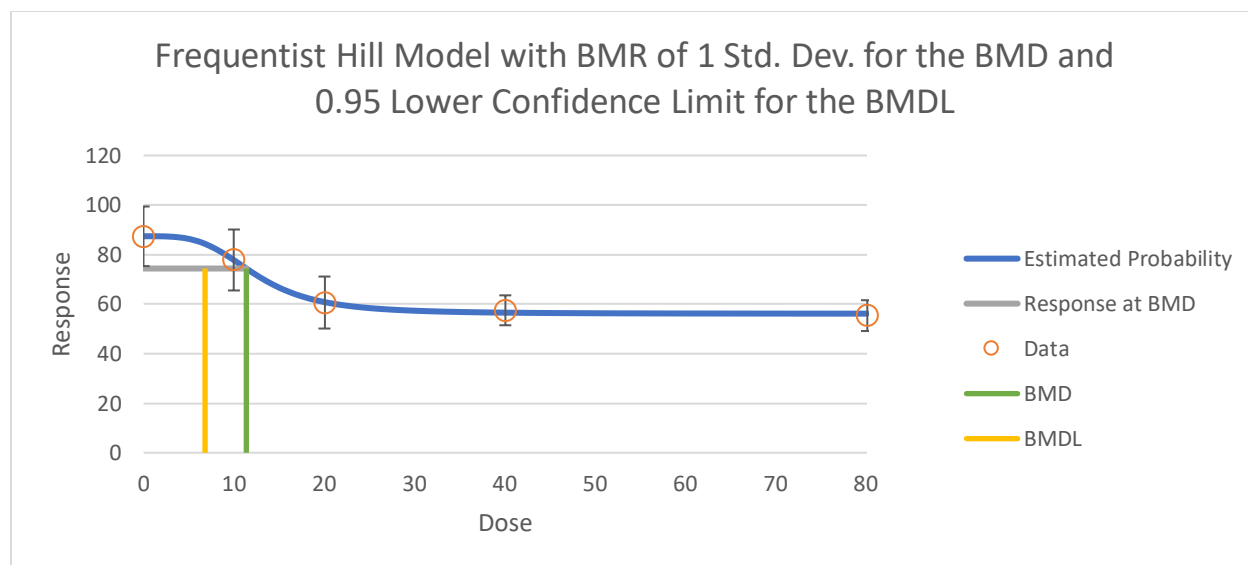


Figure B-9. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Serum Free Thyroxine (T4) Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7.6 Serum Total Thyroxine Levels in Female Sprague-Dawley Rats

Decreased mean response of serum total T4 levels was observed in female Sprague-Dawley rats. 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 B-45. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 effects leading to a decreased serum total T4 level.

Table B-45. Dose-Response Modeling Data for Serum Total Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (µg/dL) ^a
0	0	10	2.2 ± 0.8 ^b
0.312	10.0	10	1.1 ± 0.4
0.625	20.1	10	0.6 ± 0.2
1.25	40.1	10	0.3 ± 0.2
2.5	80.2	10	0.4 ± 0.3
5	160.4	9	0.4 ± 0.2

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum total T4 levels are summarized in Table B-46 and Figure B-10. The best fitting model was the Exponential 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 Exponential 4 model had the lowest AIC. The BMDL_{1SD} from the selected Exponential 4 model is 2.6 mg/L.

Table B-46. Summary of Benchmark Dose Modeling Results for Serum Total Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance)

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	<0.0001	75.5	0.3	0.3	3.9	2.7	EPA selected the Exponential 4 model. The Exponential 4, Exponential 5, and Hill model had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 4 model had the lowest AIC.
Exponential 3	<0.0001	75.5	0.3	0.3	3.9	2.7	
Exponential 4	0.598	33.6	-0.3	-0.3	3.9	2.6	
Exponential 5	0.744	34.3	0.2	-0.1	5.7	2.9	
Hill	0.642	34.6	0.1	-0.1	6.9	4.2	
Polynomial Degree 5	<0.0001	99.6	0.2	4.8	311.5	179.1	
Polynomial Degree 4	<0.0001	99.6	0.2	4.8	311.5	179.1	
Polynomial Degree 3	<0.0001	99.6	0.2	4.8	311.5	276.4	
Polynomial Degree 2	<0.0001	99.6	0.2	4.8	311.5	296.5	
Power	<0.0001	99.6	0.2	4.8	311.5	179.3	
Linear	<0.0001	99.6	0.2	4.8	311.5	296.5	

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.

^aSelected model in bold.

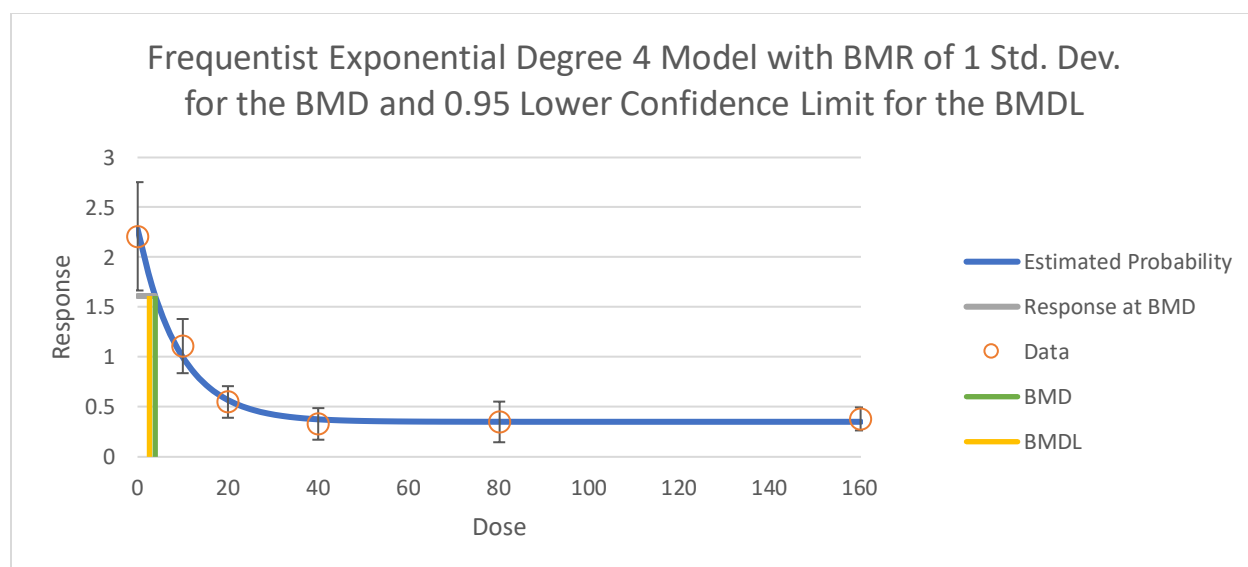


Figure B-10. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Serum Total Thyroxine Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7.7 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 B-47. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 B-47. Dose-Response Modeling Data for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	10	1
0.312	10.0	10	1
0.625	20.1	10	2
1.25	40.1	10	7
2.5	80.2	10	8
5	160.4	10	10

The BMD modeling results for extramedullary hematopoiesis in the spleen are summarized in Table B-48 and Figure B-11. 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 lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level (BMDL₁₀) from the selected Logistics model is 9.4 mg/L.

Table B-48. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in Male Sprague-Dawley Rats Following Exposure to PFOS

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	0.437	55.0	-0.3	0.2	15.4	7.0	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	0.595	53.2	-0.3	0.2	13.6	4.5	
Log-Logistic	0.647	53.0	-0.3	0.2	15.4	7.0	
Multistage Degree 5	0.488	53.7	-0.5	0.3	10.7	4.1	
Multistage Degree 4	0.488	53.7	-0.5	0.3	10.7	4.1	
Multistage Degree 3	0.488	53.7	-0.5	0.3	10.7	4.2	
Multistage Degree 2	0.488	53.7	-0.5	0.3	10.7	4.2	
Multistage Degree 1	0.476	53.4	-1.0	0.6	5.3	3.6	
Weibull	0.550	53.4	-0.4	0.3	11.9	4.4	
Logistic	0.559	52.2	-0.6	-0.1	13.7	9.4	
Log-Probit	0.678	52.8	-0.4	0.2	15.7	7.3	
Probit	0.559	52.3	-0.6	0.0	13.2	9.4	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₁₀ = dose level corresponding to a 10% response level; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

^aSelected model in bold.

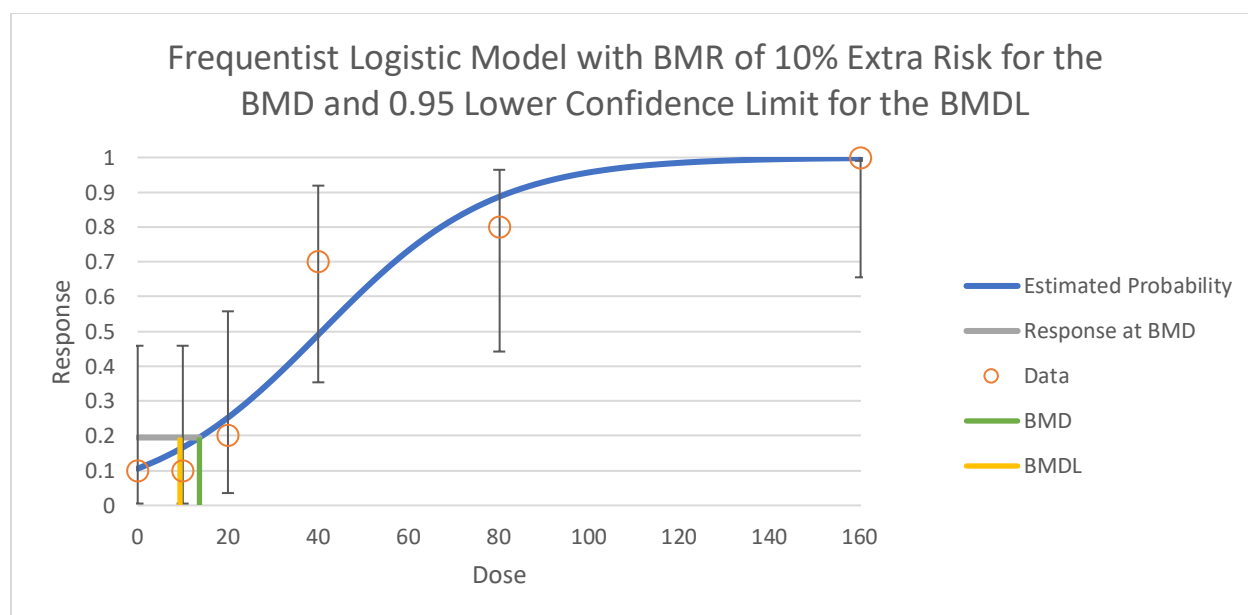


Figure B-11. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Logistic Model for Extramedullary Hematopoiesis in the Spleen in Male Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7.8 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 B-49. The average concentration over the final week of study ($C_{7, \text{avg}}$) 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 B-49. Dose-Response Modeling Data for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS

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	20.1	10	3
1.25	40.1	10	8
2.5	80.2	10	10
5	160.4	10	10

The BMD modeling results for extramedullary hematopoiesis in the spleen are summarized in Table B-50 and Figure B-12. The best fitting model was the Multistage Degree 2 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 2 model had the lowest AIC. The BMDL₁₀ from the selected Multistage Degree 2 model is 3.6 mg/L.

Table B-50. Summary of Benchmark Dose Modeling Results for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS

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	0.844	52.8	0.2	-0.5	29.6	9.0	EPA selected the Multistage Degree 2 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 2 model had the lowest AIC.
Gamma	0.966	50.7	0.0	-0.4	21.8	5.7	
Log-Logistic	0.955	50.8	0.2	-0.4	26.3	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	16.6	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	11.6	3.6	
Multistage Degree 1	0.449	53.0	0.6	0.6	3.5	2.3	
Weibull	0.960	50.7	-0.3	-0.2	17.8	5.0	
Logistic	0.878	49.8	0.3	0.5	7.6	5.2	
Log-Probit	0.963	50.8	0.1	-0.4	22.5	8.8	
Probit	0.889	49.7	0.2	0.5	7.2	5.0	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₁₀ = dose level corresponding to a 10% response level; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

^aSelected model in bold.

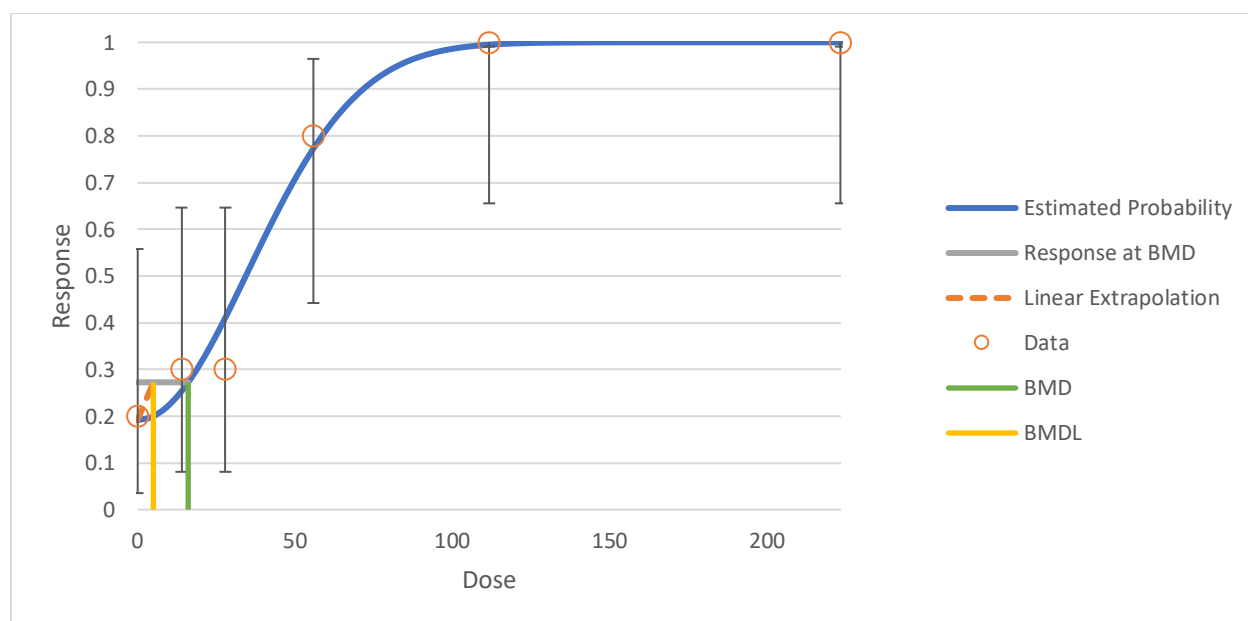


Figure B-12. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Extramedullary Hematopoiesis in the Spleen in Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.8 Qui, 2013, 2850956

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Qui et al. (2013, 2850956) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses epididymis sperm counts in male ICR mice.

B.2.8.1 Epididymis Sperm Count

Decreased mean response of epididymis sperm count was observed in male ICR mice. Continuous models were used to fit dose-response data. A benchmark response (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 B-51. The average concentration over the final week of study ($C_{7,avg}$) 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 effect leading to decreased sperm count in the epididymis.

Table B-51. Dose-Response Modeling Data for Epididymis Sperm Count in Male ICR Mice Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (10 ⁶ /epididymis) ^a
0	0	20	47.7 ± 8.3
0.25	18.2	20	38.6 ± 8.8

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (10 ⁶ /epididymis) ^a
2.5	180.9	20	33.5 ± 5.3
25	1,726.8	20	18.0 ± 3.2
50	3,137.1	20	14.2 ± 7.3

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for epididymis sperm count are summarized in Table B-52. No models provided an adequate fit, therefore a no-observed-adverse-effect level (NOAEL) approach was taken for this endpoint.

Table B-52. Summary of Benchmark Dose Modeling Results for Epididymis Sperm Count in Male ICR Mice Following Exposure to PFOS (constant variance)

Model	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	<0.0001	702.3	-2.6	3.8	512.9	415.0	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	702.3	-2.6	3.8	512.9	415.0	
Exponential 4	0.001	687.9	0.2	2.2	109.2	72.5	
Exponential 5	0.001	687.9	0.2	2.2	109.2	72.5	
Hill	0.002	685.2	-2.5	2.1	92.4	55.4	
Polynomial Degree 4	<0.0001	716.5	-2.4	4.2	912.6	781.0	
Polynomial Degree 3	<0.0001	716.5	-2.4	4.2	912.6	781.0	
Polynomial Degree 2	<0.0001	716.5	-2.4	4.2	912.6	781.0	
Power	<0.0001	716.5	-2.4	4.2	912.6	780.9	
Linear	<0.0001	716.5	-2.4	4.2	912.6	781.0	

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.

B.2.9 Salgado-Freiría, 2018, 5079767

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Salgado-Freiría et al. (2018, 5079767) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum adrenocorticotrophic hormone (ACTH) levels in male Sprague-Dawley rats.

B.2.9.1 Serum Adrenocorticotrophic Hormone Levels in Male Sprague-Dawley Rats

Decreased mean response of serum ACTH levels was observed in male Sprague-Dawley rats. Continuous models were used to fit dose-response data. A benchmark response (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 B-53. The average concentration over the final week of study ($C_{7,avg}$) was selected for this model because rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects leading to decreased mean response of serum ACTH levels.

Table B-53. Dose-Response Modeling Data for Serum Adrenocorticotrophic Hormone Levels in Male Sprague-Dawley Rats Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (pg/mL) ^a
0	0	8	4.0 ± 0.3 ^b
0.5	16.0	8	3.5 ± 0.3
1	32.1	8	3.5 ± 0.5
3	96.2	8	3.5 ± 0.1
6	192.5	8	3.0 ± 0.3

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for serum ACTH levels are summarized in Table B-54. No models provided an adequate fit, therefore a lowest-observed-adverse-effect level (LOAEL) approach was taken for this endpoint.

Table B-54. Summary of Benchmark Dose Modeling Results for Serum Adrenocorticotrophic Hormone Levels in Male Sprague-Dawley Rats Following Exposure to PFOS (nonconstant variance)

Model	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	0.094	29.5	1.1	1.6	91.7	64.2	No models had adequate fit (p-values were less than 0.1).
Exponential 3	0.094	29.5	1.1	1.6	91.8	64.2	
Exponential 4	0.041	31.5	1.1	1.6	91.8	37.8	
Exponential 5	0.094	29.5	1.1	1.6	92.0	64.2	
Hill	0.041	31.5	1.1	1.6	90.8	6.2	
Polynomial Degree 4	0.093	29.5	0.9	1.8	99.6	70.5	
Polynomial Degree 3	0.093	29.5	0.9	1.7	97.7	70.9	
Polynomial Degree 2	0.093	29.5	0.9	1.7	97.4	70.4	

Model	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			
Power	0.093	29.5	0.9	1.7	97.4	70.4	
Linear	0.093	29.5	0.9	1.7	97.5	70.4	

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.

B.2.10 Seacat, 2002, 757853

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Seacat et al. (2002, 757853) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum triiodothyronine (T3) levels in male Cynomolgus monkeys and serum T3 levels in female Cynomolgus monkeys.

B.2.10.1 Serum Triiodothyronine Levels in Male Cynomolgus Monkeys

Decreased mean response of serum T3 levels was observed in male Cynomolgus monkeys. Continuous models were used to fit dose-response data. A benchmark response (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 B-55. The average concentration over the final week of study ($C_{7,avg}$) 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 effects leading to a decreased serum T3 level.

Table B-55. Dose-Response Modeling Data for Serum Triiodothyronine Levels in Male Cynomolgus Monkeys Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (mg/dL) ^a
0	0	6	160 ± 7
0.03	8.1	4	119 ± 31
0.15	35.6	6	125 ± 15
0.75	93.9	4	66 ± 27

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for serum T3 levels are summarized in Table B-56. The data was non-monotonic, and no models provided an adequate fit, therefore a lowest-observed-adverse-effect level (LOAEL) was taken for this endpoint.

Table B-56. Summary of Benchmark Dose Modeling Results for Serum Triiodothyronine Levels in Male Cynomolgus Monkeys Following Exposure to PFOS (constant variance)

Model	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	0.014	186.7	-2.1	0.9	19.8	13.2	No models had adequate fit (p-values were less than 0.1).
Exponential 3	0.014	186.7	-2.1	0.9	19.8	12.1	
Exponential 4	0.014	186.7	-2.1	0.9	19.8	12.1	
Exponential 5	0.014	186.7	-2.1	0.9	19.8	12.1	
Hill	0.002	190.0	2.8×e ⁻⁶	1.7	36.2	18.0	
Polynomial Degree 3	0.005	188.0	0.46	1.3	28.5	18.3	
Polynomial Degree 2	0.005	188.1	0.59	1.2	26.9	18.2	
Power	0.019	186.2	0.72	1.2	25.0	18.2	
Linear	0.019	186.2	0.72	1.2	25.0	18.2	

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.

B.2.10.2 Serum Triiodothyronine Levels in Female Cynomolgus Monkeys

Decreased mean response of serum T3 levels was observed in female Cynomolgus monkeys. 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 B-57. The average concentration over the final week of study (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 effects leading to a decreased serum T3 level.

Table B-57. Dose-Response Modeling Data for Serum Triiodothyronine Levels in Female Cynomolgus Monkeys Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (mg/dL) ^a
0	0	6	135 ± 31
0.03	8.1	4	120 ± 24
0.15	35.6	6	97 ± 8
0.75	93.9	6	87 ± 12

^aData are presented as mean ± standard deviation.

The BMD modeling results for serum T3 levels are summarized in Table B-58 and Figure B-13. The best fitting model was the Exponential 4 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 Exponential 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 change in the mean equal to one standard deviation from the control mean (BMDL_{1SD}) from the selected Exponential 4 model is 7.1 mg/L.

Table B-58. Summary of Benchmark Dose Modeling Results for Serum Triiodothyronine (T3) Levels in Female Cynomolgus Monkeys Following Exposure to PFOS (nonconstant variance)

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	0.039	196.5	-1.4	1.0	53.2	30.5	EPA selected the Exponential 4 model. No other models had adequate fit (p-values greater than 0.1), and the Exponential 4 model had the lowest AIC.
Exponential 3	0.039	196.5	-1.4	1.0	53.2	30.5	
Exponential 4	0.984	192.1	-0.03	-0.1	16.3	7.1	
Exponential 5	— ^b	194.1	-0.04	-0.1	16.5	7.1	
Hill	— ^b	194.1	-0.04	-0.1	15.5	5.0	
Polynomial Degree 3	0.024	197.5	-1.4	1.2	61.5	38.2	
Polynomial Degree 2	0.024	197.5	-1.4	1.2	61.3	38.2	
Power	0.024	197.5	-1.4	1.2	61.5	38.2	
Linear	0.024	197.5	-1.4	1.2	61.5	38.2	

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.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

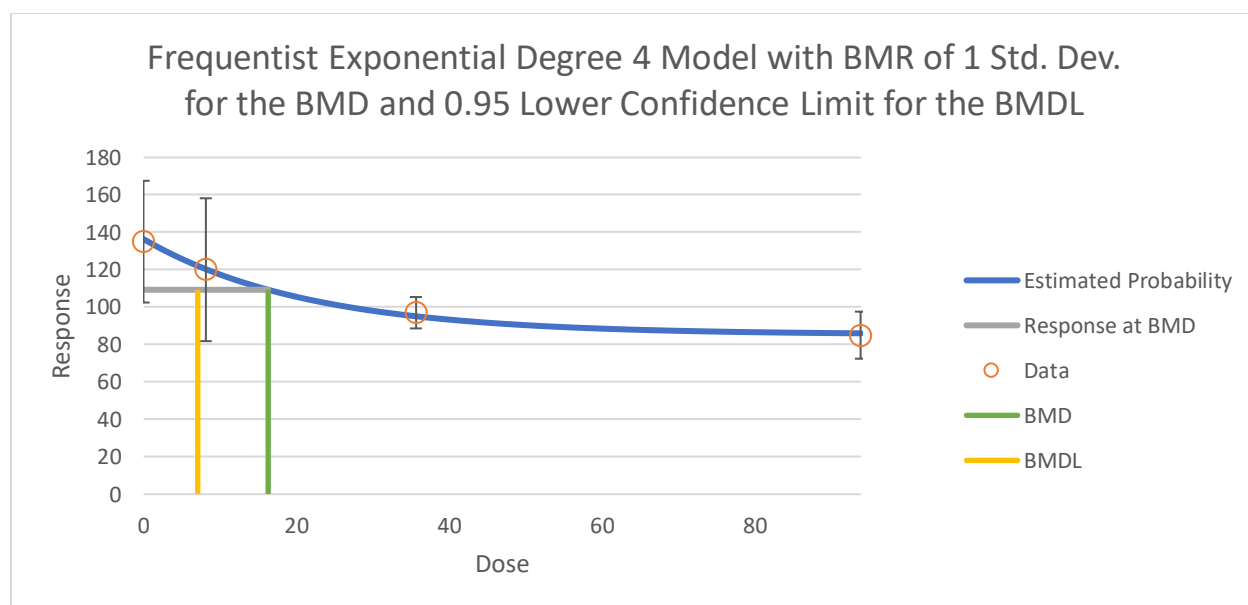


Figure B-13. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Serum Triiodothyronine Levels in Female Cynomolgus Monkeys Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11 Xing, 2016, 3981506

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Xing et al. (2016, 3981506) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum alanine aminotransferase (ALT) levels in male C57BL/6J mice.

B.2.11.1 Serum Alanine Aminotransferase levels

Increased mean response of serum ALT levels was observed in male C57BL/6J mice. Continuous models were used to fit dose-response data. A benchmark response (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 B-59. The average concentration over the final week of study ($C_{7,avg}$) 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 increased serum ALT.

Table B-59. Dose-Response Modeling Data for Serum Alanine Aminotransferase Levels in Male C57BL/6J Mice Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (U/L serum) ^a
0	0	10	31.5 ± 0.4
2.5	223.0	10	38.7 ± 1.1
5	445.9	10	47.3 ± 1.9

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (U/L serum) ^a
10	891.4	10	59.3 ± 2.3

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for serum ALT levels are summarized in Table B-60. No models provided an adequate fit, therefore a no-observed-adverse-effect level (NOAEL) approach was taken for this endpoint.

Table B-60. Summary of Benchmark Dose Modeling Results for Serum Alanine Aminotransferase Levels in Male C57BL/6J Mice Following Exposure to PFOS (nonconstant variance)

Model	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	<0.0001	163.8	-0.6	-0.6	15.3	11.2	No models have adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	163.8	-0.6	-0.6	15.3	11.2	
Exponential 4	0.011	142.5	0.4	0.4	14.8	10.6	
Exponential 5	— ^a	138.0	0.1	0.1	29.9	18.7	
Hill	— ^a	138.0	0.1	0.1	33.1	19.6	
Polynomial Degree 3	0.012	142.9	-0.9×e ⁻³	-0.9×e ⁻³	15.0	10.7	
Polynomial Degree 2	0.012	142.9	-0.9×e ⁻³	-0.9×e ⁻³	15.0	10.7	
Power	0.012	142.9	-0.9×e ⁻³	-0.9×e ⁻³	15.0	10.7	
Linear	0.012	142.9	-0.9×e ⁻³	-0.9×e ⁻³	15.0	10.7	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

B.2.12 Zhong, 2016, 3748828

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Zhong et al. (2016, 3748828) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses plaque forming cell (PFC) response of splenic cells in F₁ male C57BL/6 mice.

B.2.12.1 Plaque Forming Cell Response of Splenic Cells in F₁ Male C57BL/6 Mice

Decreased mean response of PFC response of splenic cells was observed in F₁ male C57BL/6 mice. Continuous models were used to fit dose-response data. A benchmark response (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 B-61. The AUC normalized per day during gestation/lactation ($AUC_{avg,pup,gest,lact}$) 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 B-61. Dose-Response Modeling Data for PFC Response of Splenic Cells in F₁ male C57BL/6 mice Following Exposure to PFOS

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (# cells per 10 ⁶ spleen cells) ^a
0	0	12	465.7 ± 78.5 ^b
0.1	1.2	12	423.0 ± 60.4
1	12.0	12	398.7 ± 72.5
5	59.9	12	340.1 ± 54.4

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for PFC response of splenic cells are summarized in Table B-62 and Figure B-14. The best fitting model was the Hill 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 Hill model had the lowest BMDL. The 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 ($BMDL_{1SD}$) from the selected Hill model is 1.3 mg/L.

Table B-62. Summary of Benchmark Dose Modeling Results for Plaque Forming Cell Response of Splenic Cells in F₁ Male C57BL/6 Mice Following Exposure to PFOS (constant variance)

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	36.6	24.5	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	36.6	24.5	
Exponential 4	0.174	545.7	0.2	0.9	15.8	4.7	
Exponential 5	0.174	545.7	0.2	0.9	15.8	4.7	
Hill	0.189	545.6	0.3	0.8	14.7	1.3	
Polynomial Degree 3	0.160	545.5	0.2	1.4	39.3	27.7	
Polynomial Degree 2	0.160	545.5	0.2	1.4	39.3	27.7	
Power	0.160	545.5	0.2	1.4	39.3	27.7	
Linear	0.160	545.5	0.2	1.4	39.3	27.7	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMDL_{1SD}$ = 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.
^aSelected model in bold.

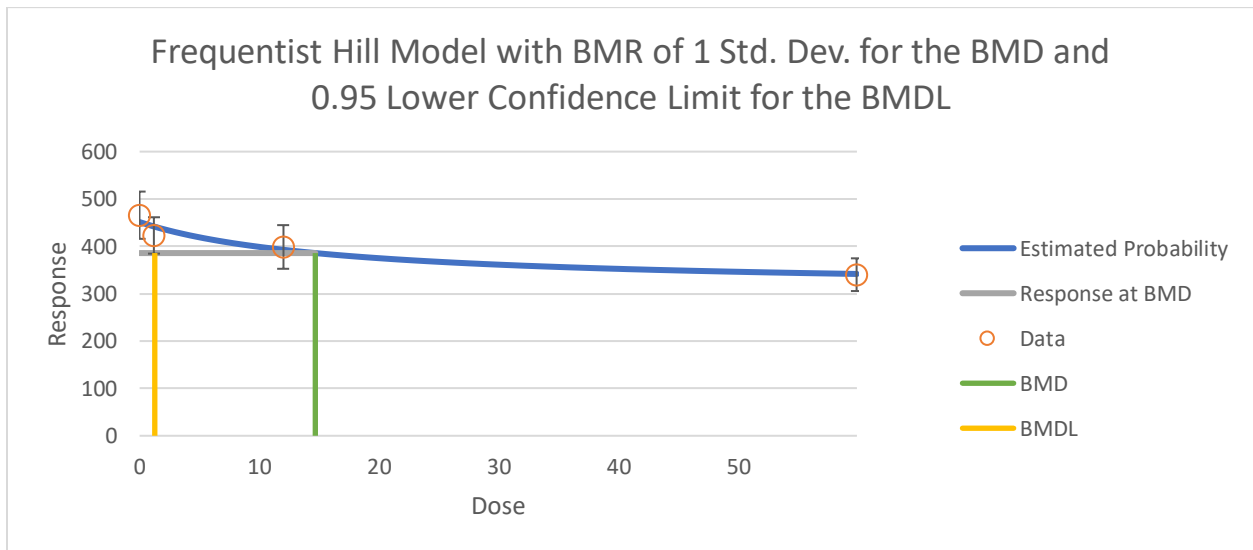


Figure B-14. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for PFC Response of Splenic Cells in F₁ Male C57BL/6 Mice Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

Appendix C. Detailed Information from Epidemiology Studies

C.1 Developmental

Table C-1. Associations Between PFOS Exposure and Developmental Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 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 8.3 (6.0–10.8)	BL (cm), BW (g, z-score), gestational length (weeks), HC (cm), preterm birth	Regression coefficient or OR (preterm birth) per IQR increase 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 Method, 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)
							Preterm birth: 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 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 Method, 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)
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 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 Luis 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 5.133 (3.39– 7.891)	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							
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 Method, 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 1.538 (0.957–2.635) Females: 1.497 (0.920–2.642) Males: 1.558 (0.988–2.628)	BW (g), GA (weeks), low birth weight, preterm birth	Regression coefficient (BW, GA) or OR (low BW, preterm birth) per ln-unit change 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 Low BW: 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 Preterm birth: 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: Low birth weight 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 low BW; 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 Method, 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 Method, 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							
Govarts et al., 2016, 3230364 High	Belgium, 2008– 2009	Cohort	Mother- newborn pairs from FLEHS II N = 213	Cord blood BW (g) 2.63 µL (1.70– 3.90 µL)		Regression coefficient per IQR change 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 Method, 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 9.33 (6.54– 13.65)	GA (weeks), preterm birth (indicated, non- spontaneous, spontaneous, and overall)	Regression coefficient (GA) per ln-unit increase in PFOS and per tertile OR (preterm birth) 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) Preterm birth, 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) Preterm birth, indicated: 1.13 (0.64, 2.01) T2: 0.79 (0.35, 1.78) T3: 0.99 (0.46, 2.12) Preterm birth, non-spontaneous Females: 1.35 (0.56, 3.26) Males: 0.98 (0.46, 2.09) Preterm birth, 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.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: Maternal age, pre-pregnancy BMI, parity, parental education levels, pregnancy complicated with chronic disease, infant sex, GA at blood drawing							
Lauritzen et al., 2017, 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 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 birth weight below the 10 th percentile for GA, sex, and parity.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Results: NO = Norway; SE = Sweden Confounding: Maternal age, height, pre-pregnancy BMI, education, parity, smoking status at conception, interpregnancy interval, offspring sex							
Lind et al., 2017, 3858512 High	Denmark 2010–2012	Cross-sectional	Infants prenatally exposed to PFAS from the Odense Child Cohort N = 212 girls, 299 boys	Maternal serum 8.1 (6.0–11.1)	Anogenital distance (AGD) (mm); clitoral (AGDac), fourchette (AGDaf), penile (AGDap), scrotal (AGDas)	Regression coefficient per In-unit increase in PFOS or by quartiles	AGDac –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 –0.4 (–1.6, 0.8) No statistically significant associations by quartiles, p-trend by quartiles = 0.31 AGDap 0.5 (–1.2, 2.2) No statistically significant associations by quartiles, p-trend by quartiles = 0.55 AGDas 1.2 (–0.4, 2.7) Q2: 0.9 (–0.9, 2.8) Q3: 0.9 (–0.8, 2.7) Q4: 1.9 (0.04, 3.7) p-trend by quartiles = 0.06
Results: Lowest quartile used as reference. Confounding: Age at examination, weight for age z-score, pre-pregnancy BMI, parity, smoking							
Manzano-Salgado et al., 2017, 4238465 High	Spain, 2003–2008	Cohort	Mother (aged ≥16 years)-child pairs from INMA N = 1,202	Maternal plasma Mean = 6.05 (SD = 2.74)	BL (cm), BW (g), GA (weeks), HC (cm), low BW, low BW at term,	Regression coefficient per doubling of PFOS or by quartiles	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					preterm birth, SGA	Low BW, low BW at term, preterm birth, SGA: OR per log2-unit increase in PFOS	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 Low BW: 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 Low BW at term: 0.91 (0.55, 1.50) p-value for sex interaction = 0.15 Preterm birth: 1.10 (0.70, 1.74) p-value for sex interaction = 0.35 SGA: 0.92 (0.70, 1.22) p-value for sex interaction = 0.57 BL, BW, HC: No statistically significant associations by quartiles All outcomes: No statistically significant associations by sex
INMA = Infancia y Medio Ambiente [Environment and Childhood Project]							
Outcome: SGA defined as newborns weighing below the 10 th 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	Maternal serum 5.1 (3.7–6.7)	BW (g), ponderal index (kg/m ²)	Regression coefficient per log10-unit	BW -29 (-289, 232); p-value = 0.828 Females: -251 (-645, 143)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			from the Sapporo Cohort (Hokkaido Study) N = 168 (90 girls, 78 boys)	Female mean: 5.04 (SD = 2.33) Male mean: 5.85 (SD = 2.63)		increase in PFOS and LSM by tertiles	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, gestational age							
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)
Confounding: Maternal age, race/ethnicity, education, pre-pregnancy BMI, and parity, black carbon, prenatal smoking							
Sagiv et al., 2017, 4238410 High	United States, 1999–2002	Cohort	Pregnant women and infants from Project Viva N = 1,644	Maternal blood 25.7 (IQR = 16.0)	Birth weight-for-GA (z-score), gestational length (weeks), preterm birth	Regression coefficient per IQR increase in PFOS and by quartiles Preterm birth: 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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 Preterm birth 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: Preterm birth 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 14 (9.6–18)	BW (z-score), length-for-age (z-score), rapid weight gain, weight-for-age (z-score), weight-for- length (z-score)	Regression coefficient by tertile (per doubling in PFOS) Rapid weight gain: RR by tertile	BW z-score T2: -0.05 (-0.29, 0.19) T3: -0.12 (-0.36, 0.13) p-value for trend = 0.36 Length-for-age z-score T2: 0.05 (-0.33, 0.44) T3: -0.24 (-0.64, 0.15) p-value for trend = 0.08 Weight-for-age z-score T2: 0.01 (-0.31, 0.32) T3: -0.33 (-0.65, -0.01) p-value for trend = 0.07 Weight-for-length z-score T2: -0.16 (-0.41, 0.09) T3: -0.31 (-0.56, -0.05) p-value for trend = 0.66 Rapid weight gain

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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 N = 415 (202 girls, 213 boys)	Maternal serum 2.2 (1.4–3.4)	Adiposity (%), weight-for-age z-score (WAZ), weight-for-length z-score (WLZ), WAZ and WLZ growth from birth to 5 months, rapid growth in WAZ or WLZ	Regression coefficient per ln-unit increase in PFOS and by tertiles Rapid growth: OR per ln-unit increase in PFOS	Adiposity at 5 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 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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							
Wikström et al., 2019, 6311677 High	Sweden 2007–2010	Cross-sectional	Infants exposed prenatally to PFAS from the SELMA study N = 1533 (732 girls, 801 boys)	Maternal serum 5.38 (3.97–7.60)	BW (g), SGA	Regression coefficient (BW) or OR (SGA) per ln-unit increase in PFOS or by quartiles	BW Per increase: -46 (-88, -3) Q2: -27 (-89, 35) Q3: -22 (-84, 41) Q4: -80 (-144, -16) Girls Per increase: -85 (-145, -25) Q2: -32 (-115, 52) Q3: -51 (-137, 34) Q4: -142 (-231, -54) Boys Per increase: -13 (-73, 47) Q2: -28 (-118, 63) Q3: 5 (-86, 96) Q4: -28 (-119, 63) SGA Per increase: 1.19 (-0.87, 1.64) Q2: 0.69 (0.43, 1.08) Q3: 0.79 (0.53, 1.18) Q4: 1.56 (1.09, 2.22) Girls Per increase: 1.4 (0.83, 2.35) Q2: 0.89 (0.39, 2.03) Q3: 0.82 (0.36, 2.03)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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: 1.3 (0.7, 2.4)
							SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy Outcomes: SGA defined as birth weight below the 10 th percentile for GA and sex. Results: Lowest quartile used as reference. Confounding: Sex, GA, maternal weight, parity, cotinine levels
Xiao et al., 2019, 5918609 High	Denmark 1994–1995	Cohort	Pregnant women and their children N = 171	Maternal blood GM = 20.8 µg/g (range: 6.9–47.6 µg/g)	Z-scores for BL, birth weight, 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) Birth weight z-score –0.47 (–0.85, –0.09) Girls: –0.56 (–1.12, 0.00) Boys: –0.40 (–0.89, 0.08) Cranial circumference z-score –0.26 (–0.68, 0.16) Girls: –0.42 (–1.05, 0.21) Boys: –0.15 (–0.68, 0.39)
							Confounding: Child sex, parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury
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-	Regression coefficient or OR (rapid weight gain, obesity) per log-SD increase in PFOS or by quartiles	BMI S: –0.11 (–0.17, –0.05); p-value < 0.05 S-girls: –0.16 (–0.24, –0.08); p-value < 0.05 S-boys: –0.06 (–0.15, 0.02) T: –0.06 (–0.16, 0.04) BMI z-score

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					length (WFL) z-score		<p>S: -0.08 (-0.12, -0.04); p-value < 0.05 Q2: 0.03 (-0.09, 0.15) Q3: -0.06 (-0.18, 0.06) Q4: -0.20 (-0.32, -0.09); p-value < 0.05 S-girls: -0.11 (-0.17, -0.05); p-value < 0.05 Q2: 0.07 (-0.10, 0.24) Q3: 0.03 (-0.16, 0.17) Q4: -0.26 (-0.26, -0.10); p-value < 0.05 S-boys: -0.05 (-0.11, 0.01) Q2: -0.01 (-0.16, 0.15) Q3: -0.11 (-0.27, 0.06) Q4: -0.15 (-0.32, 0.02) T: -0.03 (-0.10, 0.05) Q2: 0.11 (-0.09, 0.32) Q3: 0.07 (-0.14, 0.28) Q4: 0.0005 (-0.2, 0.2)</p> <p>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)</p> <p>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)</p> <p>Weight S: -21.99 (-59.52, 15.55)</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							<p>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)</p> <p>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)</p> <p>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)</p> <p>Rapid weight gain, obesity: not statistically significant for all children</p>
							<p>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.</p> <p>Results: Lowest quartile used as reference.</p> <p>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</p>
Arbuckle et al., 2013, 2152344 Medium	Canada, 2005– 2008	Cross-sectional	Pregnant women (age range = 19–45 years) and their infants N = 100	Cord blood 5.0 (Range = <0.548–21.7)	GA	Regression coefficient per ln-PFOS for preterm vs. term GA	-0.966 (SE = 0.35), p-value = 0.008
							Confounding: Gravida, smoking during pregnancy, mode of delivery
Arbuckle et al., 2020, 6356900	Canada, 2008– 2011	Cohort	Pregnant women (age	Maternal blood	Anoclititoris distance	Regression coefficient per	ACD: 0.07 (-1.03, 1.18) Q2: -0.06 (-1.7, 1.58)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Medium			range = 17–42 years) and their infants from MIREC N = 205	4.50 µg/L (3.30–6.10 µg/L)	(ACD, mm), anofourchette distance (AFD, mm), anopenile distance (APD, mm), anoscrotal distance (ASD, mm)	In-unit change in PFOS and by quartiles	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) 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							
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 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Height Birth: -0.16 (-0.31, -0.02), p-value < 0.05 0–6 mo: -0.04 (-0.23, 0.16) 6–12 mo: -0.02 (-0.23, 0.18) 12–24 mo: 0.04 (-0.17, 0.26) 24–60 mo: 0.09 (-0.12, 0.3) Boys 24–60 mo: 0.18 (0.03, 0.33); p-value < 0.05 60–108 mo: 0.06 (-0.19, 0.31) Boys 60–80 mo: 0.19 (0.01, 0.38); p-value < 0.05 Weight Birth: -0.14 (-0.26, -0.01), p-value < 0.05 0–6 mo: -0.008 (-0.17, 0.16) 6–12 mo: -0.13 (-0.32, 0.07) Girls 6–12 mo: -0.25 (-0.47, -0.04); p-value < 0.05 12–24 mo: -0.05 (-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, In-cord blood cotinine, infant sex, preterm birth, postnatal ETS exposure, breastfeeding							
de Cock et al., 2014, 2713590 Medium	The Netherlands Recruitment: 2011–2013	Cohort	Mother-child pairs N = 89	Cord blood	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
	Follow-up at 1, 2, 4, 6, 9, and 11 months after birth			1,600.0 ng/L (Range = 570–3,200 ng/L)			
	Confounding: Birth weight, 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 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						
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 of 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 10 th percentile for the norms defined by GA, country, and infant's sex. Confounding: Maternal education, maternal age at delivery, maternal height, maternal pre-pregnancy BMI, smoking during pregnancy, parity, child's sex						
Gyllenhammar et al., 2018, 4238300	Sweden, 1996–2011 and	Cohort	Mother-infant pairs of singleton births	Maternal serum 13 (7.4–19)	BL (SD scores), BW (SD scores),	Regression coefficient per IQR increase	BL: 0.1377(–0.0971, 0.3725) BW: 0.0167 (–0.1878, 0.2225)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Medium	follow-up at 5 years of age		from POPUP study N = 377		gestational length (days), HC (SD scores), length (SD scores), weight (SD scores)	in maternal PFOS	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							
Hjermitslev et al., 2020, 5880849 Medium	Greenland, Recruitment: 2010–2011, 2013–2015	Cross-sectional	Pregnant women (≥18 years of age) and their children from ACCEPT N = 256	Maternal serum 8.99 (Range = 1.50–61.3)	BW (g), GA at birth (weeks)	Regression coefficient per 1 ln-ng/mL 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) 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 1-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							
Kashino et al., 2020, 6311632 Medium	Japan, 2003–2009	Cohort	Mother-infant pairs from the Hokkaido Study on Environment	Plasma 3.4 (2.6–4.7)	Birth HC (cm), BL (cm), BW (g)	Regression coefficient per log10 change 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			and Children's Health N = 1,949				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 Hokkaido Study on Environment and Children's Health N = 177	Maternal serum 5.3 (3.9–7.2)	BL (cm), BW (g)	Regression coefficient per 1-ln ng/mL change in PFOS	Length: 0.32 (–0.19, 0.82) BW: –56 (–162.8, 50.8) Length and BW: no statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, GA, infant sex, maternal blood sampling period							
Kwon et al., 2016, Medium 3858531	Korea, 2006–2010	Cohort	Pregnant women and infants from EBGRN N = 268	Cord blood 0.64 (0.29–1.09)	BW (g)	Regression coefficient per 1 log-unit change in PFOS	–49.41 (–95.57, –3.25), p-value = 0.04
EBGRN = Ewha Birth & Growth Retrospective Cohort							
Confounding: Mother's age, pre-pregnancy BMI, past history of alcohol consumption and child's GA, gender, parity							
Lenters et al., 2016, Medium 5617416	Greenland, Poland, and Ukraine 2002–2004	Cohort	Pregnant women and singleton infants from INUENDO N = 1,250	Maternal serum Geometric mean = 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							
Manzano-Salgado et al., 2017, Medium 4238509	Spain, 2003–2008	Cohort	Mother (aged ≥16 years)-child pairs from INMA assessed at birth and 6 months N = 1,154 (568 girls, 586 boys)	Maternal blood Geometric mean = 5.80 (4.52–7.84)	Weight gain z-score, rapid growth	Regression coefficient or RR per log2-unit increase in PFOS	Weight gain z-score –0.02 (–0.11, 0.07) Girls: –0.09 (–0.21, 0.04) Boys: –0.05 (–0.08, 0.19) p-value for sex interaction = 0.54 Rapid growth 0.92 (0.80, 1.06)
INMA = Infancia y Medio Ambiente [Environment and Childhood Project]							
Outcome: Rapid growth defined as a z-score >0.67 standard deviation for weight gain from birth until 6 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, Medium 4829851	Denmark, 1996–2002	Cohort	Pregnant women and their infants from DNBC	Maternal serum	BW (g), GA (days), low BW, preterm birth	Regression coefficient (BW, GA) or OR (low BW, preterm birth) per doubling of	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 3,522 (1,533 girls, 1,969 boys)			PFOS and by quartiles	<p>Males: -24.3 (-67.1, 18.6) p-value for sex interaction = 0.31</p> <p>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</p> <p>Low BW 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)</p> <p>Preterm birth 1.5 (1.1, 2.2) Q2: 2.0 (1.1, 3.6) Q3: 3.3 (1.8, 5.8) Q4: 1.9 (1.0, 3.5)</p>
<p>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</p>							
Robledo et al., 2015, 2851197 Medium	United States, 2005–2009	Cohort	Couples and their children from the LIFE study N = 234	Serum Girls: Geometric mean = 12.44 (95% CI = 11.50, 13.44)	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)	<p>Maternal PFOS</p> <p>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)</p> <p>Boys: BW: 37.51 (-73.45, 148.46) HC: 0.07 (-0.45, 0.60)</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
				Boys: Geometric mean = 21.6 (95% CI = 19.97, 23.39)			BL: 0.22 (–0.43, 0.86) Ponderal Index: 0.00 (–0.07, 0.08) Paternal PFOS Girls: BW: 38.58 (–59.29, 136.45) HC: 0.29 (–0.14, 0.71) BL: –0.05 (–0.62, 0.52) Ponderal Index: 0.05 (–0.02, 0.11) Boys: BW: 36.85 (–73.14, 146.84) HC: 0.16 (–0.37, 0.68) BL: –0.20 (–0.84, 0.43) Ponderal Index: 0.06 (–0.02, 0.13)
LIFE = Longitudinal Investigation of Fertility and the Environment							
Confounding: Maternal and paternal serum lipids, serum cotinine, BMI, maternal age, difference in paternal age, infant gender, individual and partner sum of remaining chemical concentrations in each chemical's respective class							
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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							
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 -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 12 mo Q3: 5.17 (1.53, 8.81); p-value < 0.05 AGDas -0.83 (-1.71, 0.06); p-value = 0.067 Birth: -0.65 (-1.27, -0.02); p-value = 0.043 Birth Q4: -1.46 (-2.44, -0.49); p-value < 0.05 6 mo: -2.21 (-4.28, -0.14); p-value = 0.037

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							12 mo: 0.47 (–1.63, 2.58); p-value = 0.659
							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, gestational age, 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: Gestational age of amniocentesis, maternal age, smoking (cotinine groups), and case or control status							
Valvi et al., 2017, 3983872 Medium	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							BW –81 (–173, 11) Girls: 5 (–124, 135) Boys: –150 (–282, –17) p-value for sex interaction = 0.08
							Confounding: Maternal age at delivery, education, parity, pre-pregnancy BMI, smoking during pregnancy, child sex
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). 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 0.65 (0.40–1.19)	BL (mm), BW (g), HC (mm), 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 [below are details if we want them] BL –0.05 (–3.97, 3.88); p-value = 0.982 Girls: –0.12 (–6.00, 5.76); p-value = 0.968 Boys: –1.70 (–7.05, 3.66); p-value = 0.535 p-value for interaction by sex = 0.557 BW 54.5 (–149.07, 40.06); p-value = 0.259

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							<p>Girls: -57.3 (-201.38, 86.78); p-value = 0.436</p> <p>Boys: -61.6 (-184.61, 61.42); p-value = 0.326</p> <p>p-value for interaction by sex = 0.844</p> <p>HC</p> <p>0.15 (-2.56, 2.86); p-value = 0.915</p> <p>Girls: -0.14 (-4.22, 3.94); p-value = 0.947</p> <p>Boys: -0.42 (-4.07, 3.23); p-value = 0.821</p> <p>p-value for interaction by sex = 0.709</p> <p>Ponderal index</p> <p>-0.04 (-0.09, 0.001); p-value = 0.054</p> <p>Girls: -0.04 (-0.11, 0.02); p-value = 0.198</p> <p>Boys: -0.02 (-0.08, 0.03); p-value = 0.427</p> <p>p-value for interaction by sex = 0.637</p>
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 BW (g) 14.4 µg/L (10– 17.0 µg/L)		Regression coefficient per log10- µg/L 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, gestational age							

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; SD = Standard Deviation; SE = Standard Error; OR = Odds Ratio; 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.

C.2 Reproductive

C.2.1 Male

Table C-2. Associations Between PFOS Exposure and Male Reproductive Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 N = 649 (296 boys)	Maternal serum Total cohort: 8.1	Penile width (mm), anogenital distance (scrotal, as; penile, ap) (mm)	Regression coefficient per ln-unit increase in PFOS, or by quartiles	AGD as Q2: 0.9 (–0.9, 2.8) Q3: 0.9 (–0.8, 2.7) Q4: 1.9 (0.04, 3.7) p-trend by quartiles = 0.06 AGD ap, penile width: no statistically significant associations; p-trend by quartiles = 0.67

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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)	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 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 ratio –0.399 (–0.643, –0.156) p-value = 0.002 Q1: 20.3 (15.2, 26.8) Q2: 19.5 (15.2, 24.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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 75 th and 25 th 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 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	Regression coefficient per log2-unit increase in first trimester maternal serum PFOS	No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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					or pubic hair growth; combined sex-specific puberty indicator	Puberty indicator: mean difference in age at puberty by tertiles	
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 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 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	Cord blood Total cohort: 0.65 (0.40–1.19)	Levels (log10-ng/mL) of estrone (E1), E2, estriol (E3)	Regression coefficient per	E1: 0.071 (-0.05, 0.18); p-value = 0.247

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 340 (169 boys)			log10-unit increase in PFOS	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 PFOA or by quartiles	AGDap Per ln increase: 0.13 (–1.13, 1.38) Q2: –0.97 (–2.81, 0.87) Q3: –1.28 (–3.22, 0.66) Q4: 0.22 (–1.68, 2.13) p-value for trend = 0.908 AGDas Per ln increase: 1.05 (–0.24, 2.35) Q2: –0.87 (–2.78, 1.04) Q3: 0.33 (–1.67, 2.33) Q4: 0.49 (–1.47, 2.46) p-value for trend = 0.3936
			Results: Lowest quartile used as reference. Confounding: AGDap: recruitment site, education, active smoking status, gestational age; AGDas: household income, active smoking status, gestational age				
Zhou et al., 2016, 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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			without (control) asthma N = 231 cases, 225 controls				
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 (%), progressive motility (%), total sperm count (10 ⁶), semen volume (mL), sperm concentration (10 ⁶ /mL), viability (%), FSH (U/L), testosterone (nmol/L)	Mann-Whitney test (Exposed vs. Controls)	AGD Controls: 4.50 (4.0, 5.2) Exposed: 4.00 (3.5, 5.0) Adjusted p-value for comparison of medians = 0.114 Pubis-to-floor distance Controls: 97.0 (93.0, 101.1) Exposed: 95.0 (90.3, 99.0) Adjusted p-value for comparison of medians = 0.320 Penis circumference Controls: 10.10 (9.9, 11.0) Exposed: 9.50 (9.0, 10.0) Adjusted p-value for comparison of medians <0.001 Penis length Controls: 10.0 (9.0, 11.0) Exposed: 9.00 (8.0, 10.0) Adjusted p-value for comparison of medians <0.001 Testicular volume Controls: 16.13 (14.8, 19.0) Exposed: 14.00 (12.6, 16.0) Adjusted p-value for comparison of medians <0.001

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							<p>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</p> <p>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</p> <p>Testosterone Controls: 18.98 (12.9, 17.9) Exposed: 18.98 (16.3, 21.8) Adjusted p-value for comparison of medians < 0.001</p> <p>Crown-to-pubis, Crown-to-pubis/pubis-to-floor, sperm motility, sperm count, semen volume, sperm concentration, viability, FSH: No statistically significant associations after adjusting for comparison of medians</p>
<p>Results: Values for each outcome are reported as median (25th–75th percentile). Confounding: Age</p>							
General Population							
Cui et al., 2020, 6833614 Medium	China 2015–2016	Cross- sectional	Adult men N = 651	<p>Serum 9.94</p> <p>Semen 0.15</p>	<p>Serum levels (ln-transformed) of E2 (pmol/L), FSH (IU/L), LH (IU/L), SHBG (nmol/L), free testosterone,</p>	Percent change per ln-unit increase in serum or semen PFOS, or by quartiles	<p>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</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					total testosterone (nmol/L); free androgen index, total testosterone/LH ratio		<p>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</p> <p>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</p> <p>Total testosterone/LH, Serum PFOS: -4.53 (-8.99, 0.15); p-value = 0.058 p-trend by quartiles = 0.044 Semen PFOS: -5.00 (-9.32, -0.48); p-value = 0.031 p-trend by quartiles = 0.042 No statistically significant associations by age groups</p> <p>E2, FSH, free androgen, LH, free testosterone: No statistically significant associations or trends</p>
Confounding: Age, BMI, smoking status, blood sampling time, fasting status							
Petersen, 2018, 5080277 Medium	Denmark 2007–2009	Cross-sectional	Faroese men born between 1981 and 1984	Serum 19.5	Levels (log-transformed) of E2 (nmol/L), FSH	Regression coefficient per log-	LH: 0.35 (0.02, 0.68); p-value = 0.04

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 263		(IU/L), free testosterone (pmol/L), inhibin B (pg/mL), LH, (IU/L), SHBG (nmol/L), testosterone (nmol/L)	unit increase in PFOS	SHBG: 0.31 (0.02, 0.60); p-value = 0.04 No other statistically significant associations
					Ratios of free testosterone/E2, free testosterone/LH, Inhibin B/FSH, testosterone/E2, testosterone/LH		
					Normal morphology (%), motile sperm (logit-%), total sperm count ((10 ⁶) ^{1/3}), semen volume (mL ^{1/3}), sperm concentration ((10 ⁶ /mL) ^{1/3})		
					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;	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: Site, age (ln-transformed), smoking status					global DNA methylation level (FCM DGML channel no.)		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
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 velocity (VSL) (µm/s), DNA fragmentation index (DFI) (ln-%), high DNA stainability (HDS) (ln-%); semen volume (ln- mL)	Regression coefficient per ln- unit increase in PFOS in serum or in semen, or by quartiles	No statistically significant associations by serum PFOS levels; following results are by semen PFOS Progressive motility: -1.700 (-2.867, -0.532); p-value = 0.03 Q2: -2.30 (-5.27, 0.68) Q3: -1.53 (-4.61, 1.56) Q4: -5.54 (-8.72, -2.36) p-trend = 0.01 VCL: -0.767 (-1.447, -0.087); p-value = 0.1 Q2: -1.60 (-1.50, 2.01) Q3: -2.78 (-2.40, 1.10) ^d Q4: -4.8 (-2.97, -0.72) p-trend = 0.1 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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

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; 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.

^aExposure levels reported as median in ng/mL unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

^dValues are reproduced as reported in publication.

C.2.2 Female

Table C-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,	Maternal serum 8.07 (5 th –95 th percentile = 4.21, 15.50)	Levels of 17-OHP (nM), androstenedione (nM), DHEA (nM),	Percent change per doubling in PFOS	17-OHP 2.1 (–11.9, 18.2) Androstenedione 0.6 (–14.3, 18.2)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
			Age 4 months, N = 165		DHEAS (nM), FSH (IU/L), LH (IU/L)		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							
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							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
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 (10 th –90 th percentiles = 19.3, 50.8)	Breast development, pubic hair development, age at attainment of axillary hair (months), age at menarche, age at attainment of combined puberty indicator	Combined puberty indicator: Mean difference by tertiles of PFOS All other outcomes: Regression coefficient per log2- unit increase in PFOS	Combined puberty indicator T2: –3.73 (–6.59, –0.87) T3: –0.17 (–2.83, 2.49) Breast development –3.01 (–7.96, 1.95), p-value = 0.03 Pubic hair development 1.81 (–2.42, 6.04) Axillary hair 0.50 (–2.79, 3.79), p-value = 0.02 Menarche –0.68 (–3.13, 1.77)
<p>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.</p> <p>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.</p> <p>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</p>							
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)
<p>Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period</p>							
Itoh et al., 2016, 3981465 Medium	Japan, 2002–2005	Cohort	Female infants from the Sapporo Cohort of the Hokkaido Study,	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	Regression coefficient per log10- unit increase in PFOS	Estradiol 0.08 (–0.15, 0.31) Testosterone 0.07 (–0.26, 0.40) Prolactin

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
			N = 106		(log10-ng/mL), SHBG (nmol/L); testosterone to SHBG ratio, testosterone to estradiol ratio		-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)
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 75 th vs. 25 th percentiles, or by quartiles	Estradiol 75 th vs. 25 th 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 75 th vs. 25 th 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							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
Maisonnet 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 maternal serum 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) 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							

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.

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

1 **Table C-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- years postpartum	Cohort	Females from Project Viva, N = 812	Plasma 24.7 (18.1, 33.9)	Sex hormone binding globulin (nmol/L)	Percent difference per log2-unit increase in PFOS	Sex hormone binding globulin: –0.6 (–7.6, 6.9) Ages ≤35: –0.8 (–11.9, 11.7) Ages ≥35: –1.5 (–10.0, 7.8)
Confounding: Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity							
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							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
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							
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)
Confounding: Age, BMI, prior pregnancy loss conditional on previous pregnancy, any alcohol consumption during pregnancy, any cigarette smoking during pregnancy							
Lyngsø et al., 2014, 2850920 Medium	Greenland, 2002–2004	Cross- sectional	Pregnant women from the INUENDO cohort, N = 1,623	Serum, 8.0 (10 th –90 th percentile = 3.6, 25.6)	Menstrual cycle length (long), irregularity	OR per log-unit increase in PFOS or by tertiles	Length 1.1 (0.8, 1.6) T2: 1.3 (0.8, 2.2) T3: 1.2 (0.6, 2.5) Irregularity 1.2 (0.9, 1.8) T2: 1.1 (0.6, 2.1) T3: 1.7 (0.8, 3.5)
Results: Lowest tertile used as the reference group. 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,	Serum 13.9 (9.6, 18.2)	Breastfeeding termination (by 3 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)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
			Ages >18, N = 336		Breastfeeding termination (by 6 months postpartum)		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 (ln-nmol/L), testosterone (ln-nmol/L)	Percent difference in median level per 1% increase in PFOS or by tertiles	17- OHP 0.15 (0.11, 0.20) T2: 7 (–1, 13) T3: 18 (11, 26) p-value for trend <0.001 Androstenedione 0.15 (0.10, 0.21) T2: 8 (0, 17) T3: 17 (8, 25) p-value for trend = 0.001 DHEAS

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
							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

17-OHP = 17-hydroxyprogesterone; BMI= Body Mass Index; DBP = diastolic blood pressure; DHEAS= dehydroepiandrosterone sulfate; GM = Geometric Mean; OR= Odds Ratio; HR= Hazard Ratio; RR= Relative Risk Ratio; SBP = systolic blood pressure; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; LIFE = Longitudinal Investigation of Fertility and the Environment Study; MIREC = Maternal Infant Research on Environmental Chemicals; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; HOME = Health Outcomes and Measures of the Environment; SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study.

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

1 **Table C-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							

AMH = anti-Mullerian hormone; BMI = body mass index; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

1 C.3 Hepatic

2 Table C-6. Associations Between PFOS Exposure and Hepatic Effects in Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Adults							
Jain, 2019, 5381541 Medium	United States 2003–2014	Cross- sectional	Adults from NHANES, Ages >20, N = 108–3,562	Serum	Levels of ALT (log ₁₀ -IU/L),, AST (log ₁₀ - IU/L)	Regression coefficient per log ₁₀ -unit increase	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 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							
Salihovic et al., 2018, 5083555 Medium	Sweden 2001–2014	Cohort	Elderly adults in Sweden, Ages 70 N = 1002 Ages 75 N = 817 Age 80	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	0.03 (0.02, 0.04), p-value <0.0016

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
N = 603							
Confounding: Sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, smoking							
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	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, gilet, seafood consumption, exercise, BMI							
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	ALT 0.01 (–0.32, 0.34) AST 0.19 (–0.17, 0.55)
Confounding: Age, waist-to-hip ratio							
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: –101.3ng/mL; Contractors: 1	Levels of ALT (IU/L), AST (IU/L)	Adjusted regression coefficient per unit increase	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 (5 th –95 th percentile: 0.89, 10.3)	Lobular inflammation	OR per log unit increase by level of lobular inflammation	<2 foci: 0.52 (0.13, 2.09) 2–4 foci: 0.14 (0.01, 1.66)
Results: No foci used as the reference group. Foci measured per 200x field. Confounding: Age, sex, BMI, serum lipids, fasting insulin							
Children and Adolescents							
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	ALT 0.16 (–1.84, 2.15) AST

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							-0.28 (-1.22, 0.65)
Confounding: Age, sex, race							
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) 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) 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							
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	Prenatal exposure: -0.4 (-1.1, 0.2) Mid-childhood exposure: -0.3 (-0.9, 0) Liver fibrosi.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Confounding: Maternal education, prenatal smoking, gestational age at blood draw, and child's sex, race/ethnicity, age at lipids/ALT measurements							
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	<p>Ballooning</p> <p>Few balloon cells: 1.11 (0.52, 2.37)</p> <p>Many cells/prominent ballooning: 1.12 (0.26, 4.95)</p> <p>Grade of steatosis</p> <p>34–66% steatosis: 1.37 (0.54, 3.51)</p> <p>>66% steatosis: 0.88 (0.39, 1.97)</p> <p>Liver fibrosis</p> <p>Mild (stage 1): 1.71 (0.73, 4.03)</p> <p>Significant (stages 2–4): 1.51 (0.53, 4.35)</p> <p>Lobular inflammation</p> <p><2 foci: 0.50 (0.21, 1.22)</p> <p>2–4 foci: 2.92 (0.92, 9.23)</p> <p>Nonalcoholic steatohepatitis</p> <p>3.32 (1.40, 7.87), p-value <0.05</p> <p>Portal inflammation</p> <p>Mild: 1.85 (0.82, 4.21)</p> <p>Moderate-to-severe: 2.26 (0.75, 6.79)</p>
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							

BMI = body mass index; GF = glomerular filtration; GM = geometric mean; IQR = interquartile range; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; SD = standard deviation; SE = standard error.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.^cConfounding indicates factors the models presented adjusted for.

C.4 Immune

Table C-7. Associations between PFOS Exposure and Vaccine Response in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Children							
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 log2-unit 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 PFOA	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 =	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:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			275, 5 years: N = 349				Birth: -14 (-31.59, 8.11) p-value = 0.20 18 mo: 17.55 (-0.84, 39.34) p-value = 0.062 5 yr: 17.17 (-8.66, 50.31) p-value = 0.21 Combined cohort 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Timmermann et al., 2020, 6833710 Medium	Guinea-Bissau 2012–2015	Cohort	Infants enrolled at 4–7 months old (inclusion), followed up at 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	Maternal blood 0.77 (0.53–1.02)	Measles antibody concentration (mIU/mL)	Percent difference per doubling of PFOA	Inclusion (no measles vaccination): –13 (–26, 4) 9-month visit 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							
Zeng et al., 2019, 5081554 Low	China 2013	Cohort	Infants from Guangzhou Birth Cohort Study at birth and 3 months Birth N = 194 (91 girls, 103 boys) 3-month N = 180 (89 girls, 91 boys)	Cord blood 3.17 (1.88–4.94)	HFMD antibody titers (CA16 or EV71) in serum of cord blood or at 3 months	Percent change or OR (below clinical protection) per doubling of PFOS	CA16 Cord blood: –20.6 (–30.0, –9.9) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							<p>p-value for interaction by sex = 0.311</p> <p>3 months: 1.71 (1.12, 2.60);</p> <p>p-value = 0.013</p> <p>Girls: 0.97 (0.88, 1.08)</p> <p>Boys: 2.29 (1.20, 4.36)</p> <p>p-value for interaction by sex = 0.318</p> <p>EV71</p> <p>Cord blood: -23.6 (-33.9, -11.8)</p> <p>Girls: -23.5 (-37.9, -5.8)</p> <p>Boys: -23.4 (-37.2, -6.6)</p> <p>3 months: -10.6 (-16.9, -3.9)</p> <p>Girls: -8.6 (-17.1, 0.9)</p> <p>Boys: -12.2 (-21.3, -1.9)</p> <p>EV71 below clinical protection</p> <p>Cord blood: 1.66 (1.12, 2.45);</p> <p>p-value = 0.011</p> <p>Girls: 1.48 (0.92, 2.37)</p> <p>Boys: 2.01 (1.03, 3.90)</p> <p>p-value for interaction by sex = 0.265</p> <p>3 months: 2.25 (1.44, 3.51);</p> <p>p-value <0.05</p> <p>Girls: 2.05 (1.11, 3.79)</p> <p>Boys: 2.35 (1.19, 4.65)</p> <p>p-value for interaction by sex = 0.579</p>
							<p>Outcome: Clinical protection threshold defined as titers $\geq 1:8$ in modified cytopathogenic effect assay.</p> <p>Confounding: Sex, age, parental education, parental occupation, family income, parity, and birth weight</p>
Adults and Adolescents							
Pilkerton et al., 2018, 5080265	United States 1999–2000	Cross-sectional	Adults and adolescents 12 years and older	Serum	Rubella IgA titers (log-IU)	Regression coefficient by	Adolescents: Per quartile increase: F-value = 1.44, p-value = 0.251

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Medium for youth 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		quartiles or per quartile increase	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
Results: Lowest quartile used as reference group.							
Confounding: Women: age, ethnicity, BMI, educational level, number of live births; men: age, ethnicity, BMI, educational level							
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 baseline Ab: N = 29	Serum GM = 5.22 (95% CI: 4.52–6.02)	Anti-A-H1N1 antibody response measured by HAI or by IHC	RR by tertiles	HAI anti-A-H1N1 antibody Total population T2: 2.6 (0.4, 15.1) T3: 1.3 (0.2, 7.3) p-value for trend = 0.81 Low baseline Ab T2: 6.7 (1.2, 37.9) T3: 1.6 (0.3, 9.7) p-value for trend = 0.81 IHC anti-A-H1N1 antibody Total population T2: 2.6 (0.9, 7.4) T3: 2.4 (0.9, 6.6) p-value for trend = 0.12

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 log-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							

RR = risk ratio; CI = confidence interval; SE = standard error; BMI = body mass index; HAI = hemagglutinin inhibition; ICH = immunohistochemistry; Ab = antibody; HFMD = hand, foot, and mouth disease; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; T2 = tertile 2; T3 = tertile 3; GM = geometric mean.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

1 **Table C-8. Associations between PFOS Exposure and Infectious Disease in Recent Epidemiological Studies**

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Children							
Gourdazi et al., 2017, 3859808 Medium	Hokkaido, Japan 2003–2009	Cohort	Children, early pregnancy followed up at 4 years N = ,1558 (793 boys, 765 girls)	Maternal blood 4.93 (3.67–6.65)	Infectious diseases, total (including Otitis media, Pneumonia, RS virus, Varicella)	OR by quartiles	Girls Q2: 1.42 (0.91, 2.23) Q3: 1.32 (0.86, 2.06) Q4: 1.71 (1.08, 2.72) p-value for trend = 0.036 Boys 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 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							
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 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 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy							Diarrhea/gastric flu 0–3 years: 0.98 (0.93, 1.03) 6–7 years: 1.12 (1.01, 1.24)
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	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	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)
			Children, 10–16 years, all: 375, boys: 191, girls: 184 Children, 16 years, all: 330, boys: 170, girls: 160				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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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							
1	IQR = interquartile range; OR = odds ratio; RR = risk ratio; CI = confidence interval; SE = standard error; BMI = body mass index; LRTI = lower respiratory tract infection; RSV						
2	= respiratory syncytial virus; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; T2 = tertile 2; T3 = tertile 3.						
3	^a Exposure levels reported as median (25 th –75 th percentile) unless otherwise noted.						
4	^b Results reported as effect estimate (95% confidence interval) unless otherwise noted.						
5	^c Confounding indicates factors the models presented adjusted for.						

6 **Table C-9. Associations Between PFOS Exposure and Asthma in Recent Epidemiologic Studies**

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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) Greenland: GM = 20.6 (P5–P95: 10.2–49.6)	Asthma	OR per SD increase in PFOS	Asthma ever (combined): 0.86 (0.67, 1.10) Ukraine: 0.75 (0.39, 1.42) Greenland: 0.88 (0.67, 1.15)
Confounding: Maternal allergy, smoking during pregnancy, education level, maternal age, child sex, child age at follow-up, gestational age at blood sample, parity, breastfeeding, and birthweight ^c							
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 (10y): 1.14 (0.84, 1.54); p-value = 0.392 Asthma ever (10y): 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	Maternal blood 7.73 (5.68–10.44)	Wheeze, self-reported asthma, doctor-	OR per doubling in	Wheeze All: 1.01 (0.79, 1.30) Boys: 1.02 (0.74, 1.39)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			N = 970 (507 boys, 363 girls)		diagnosed asthma	maternal serum PFOS	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)
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 = 0.0631 Boys: 0.94 (0.63, 1.40); p-value = 0.744 Girls: 0.92 (0.57, 1.49); p-value = 0.745

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 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 Method, 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 transformed 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 N = 375 (191 boys, 184 girls)	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 Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							
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 Control boys: 102 Control girls: 123	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
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 exposure	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)	Serum Cases: 33.94 (19.59–61.10)	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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			asthma ages 10-15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123 Sexes evenly divided into high/low hormone classifications	Controls: 28.91 (14.06–42.02)			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 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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 N = 675	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 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							

GM = geometric mean; IQR = interquartile range, OR = odds ratio, RR = risk ratio, CI = confidence interval, SD = standard deviation, BMI = body mass index, MMR = measles, mumps, rubella; TFF1 = Tromsø Fit Futures; GBCA = Genetic and Biomarker study for Childhood Asthma; NHANES = National Health and Nutrition Examination Survey.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

1 **Table C-10. Associations Between PFOS Exposure and Allergies in Recent Epidemiologic Studies**

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Children							
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 exposure	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 exposure	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							
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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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, rhinoconjunctivitis, SPT	OR per log2-unit increase in PFOS	<p>Rhinitis, current, 10 y 1.00 (0.72, 1.40); p-value = 0.983</p> <p>Rhinitis, ever, 10 y 1.05 (0.74, 1.48); p-value = 0.775</p> <p>Rhinoconjunctivitis, ever, 10 y 1.02 (0.72, 1.45); p-value = 0.905</p> <p>Rhinoconjunctivitis, ever, spes IgE >0.35, 10 y 1.02 (0.71, 1.47); p-value = 0.905</p> <p>SPT, any pos, 10 y 0.87 (0.65, 1.17); p-value = 0.359</p> <p>SPT + and/pr sIgE > 0.35, 10 y 0.91 (0.69, 1.19); p-value = 0.476</p>
Confounding: Sex							
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	<p>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</p> <p>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</p>

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Girls: 0.91 (0.58, 1.42); p-value = 0.676
							Allergy, inhaled, current All: 1.11 (0.072, 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 In-unit increase in PFOS, from birth to 7 years old	0.96 (0.79, 1.15); p-value = 0.626
Confounding: Sex, parity, maternal age at delivery, maternal smoking during pregnancy, pre-pregnancy BMI, and annual household income during pregnancy							
Kvalem et al., 2020, 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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 = 0.086
							16 years All: 1.09 (1.03, 1.15); p-value = 0.001 Boys: 1.07 (0.97, 1.17); p-value = 0.18 Girls: 0.99 (0.80, 1.23); p-value = 0.93
Confounding: 10 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							

1 IQR = interquartile range, OR = odds ratio, RR = risk ratio, CI = confidence interval, SD = standard deviation, BMI = body mass index, MMR = measles, mumps, rubella, SPT =
2 skin prick test, IgE = immunoglobulin E; NHANES = National Health and Nutrition Examination Survey.

3 ^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

4 ^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

5 ^cConfounding indicates factors the models presented adjusted for.

1 **Table C-11. Associations Between PFOS Exposure and Eczema in Recent Epidemiologic Studies**

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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)
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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 PFOA	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 N = 1,188 at 1.5, N = 1,184 at 4 years, N = 1,066 at 7 years	Maternal plasma 6.06 (4.52–7.82)	Eczema	OR or RR per log2-unit increase in PFOS	Age 1.5: 1.02 (0.83, 1.27) Age 4: 0.8 (0.65, 0.99) Age 7: 0.82 (0.68, 0.99) Boys at ages 1.5, 4, and 7: 0.91 (0.75, 1.11) Girls at ages 1.5, 4, and 7: 0.77 (0.64, 0.94) From ages 1.5 to 7 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	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			followed from birth up to 5 years of age N = 863				
Confounding: Sex, parental education, parental atopy, breast feeding, and maternal age at childbirth							

Q2 = Quartile 2, Q3 = Quartile 3, Q4 = Quartile 4; ECA: Environment and Childhood Asthma; CHEF: Children's Health and Environment in the Faroe Islands; INMA = Spanish Environment and Childhood (Infancia y Medio Ambiente).

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

6 Table C-12. Associations Between PFOS Exposure and Autoimmune Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Gaylord et al., 2020, 6833754 Medium	United States	Case-control	Children and adolescents younger than 21 years with (cases) and without (controls) celiac disease N = 88 (42 girls, 46 boys)	Serum Cases: 2.02 (IQR = 1.85) Controls: 1.59 (IQR = 1.64)	Celiac disease	OR per ln-unit change in PFOS	2.20 (0.78, 6.18) Girls: 12.8 (1.17, 141); p-value <0.05 Boys: 1.02 (0.24, 4.21)
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
Results: Lowest quintile used as reference.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Confounding: Age, sex, ethnic group (white or non-white), year of sample							
Ammitzbøll et al., 2019, 5080379 Low	Denmark 2019	Case-control	Adults with (cases) or without (controls) RRMS or CIS N = 162 (92 women, 70 men)	Serum Cases: 7.14 (5.76–9.93) Controls: 9.41 (6.41–13.0)	Relapsing remitting multiple sclerosis (RRMS)	Percent change in PFOS comparing MS cases vs. healthy controls	–17 (–27, –6); p-value = 0.004 Females: –14 (–28, 3); p-value = 0.093 Males: –19 (–32, –3); p-value = 0.023
Confounding: Age, sex, breastfeeding							

PFOS = perfluorooctane sulfonic acid; OR = odds ratio; UC = ulcerative colitis; CD = Crohn's disease; RRMS = relapsing remitting multiple sclerosis; CIS = clinically isolated serum syndrome.

^aExposure levels are reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

C.5 Cardiovascular

C.5.1 Cardiovascular Endpoints

Table C-13. Associations Between PFOS Exposure and Cardiovascular Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Children and Adolescents							
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 log ₁₀ -unit increase in PFOA	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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 log ₂ -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							
Lin et al., 2013, 2850967 Medium for CINT 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, CINT	Mean by quartiles	SBP: No associations across quartiles; p-trend = 0.177 CINT: 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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							Ages 20–30: no associations across exposure groups; p-trend = 0.084 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
Lin et al., 2016, 3981457 Medium	Taiwan 1992–2000	Cross-sectional	Adolescents and young adults ages 12–30 N = 848	Serum Geometric Mean = 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	
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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 and Mother-Child Pairs							
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 Age 7 N = 1,086 (535 girls, 551 boys)	Maternal blood Geometric Mean = 5.80 (4.52–7.84)	Blood Pressure (BP) (z-score) Cardiometabolic Risk Score (CMR)	Regression coefficient per log2-unit increase in PFOS	BP All age 4: -0.05 (-0.15, 0.06) Girls: -0.06 (-0.22, 0.09) Boys: -0.02 (-0.18, 0.14) All age 7: 0.06 (-0.04, 0.15) Girls: 0.06 (-0.09, 0.20) Boys: 0.04 (-0.08, 0.17) CMR All age 4: 0.28 (-0.33, 0.89) Girls: 0.10 (-0.73, 0.93) Boys: 0.47 (-0.44, 1.37)
Confounding: Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child							
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.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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) 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)	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
				Controls: 22.0 (IQR = 10.1)			
				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 (LVMI) (g/m ^{2.7}) Relative Wall Thickness (RWT)	Regression coefficient per log-unit increase in PFOS	LVEDD: 0.47 (0.08, 0.87) LVMI: 0.12 (−0.73, 0.97) RWT: −0.01 (−0.01, −0.001)
				Confounding: Sex, systolic blood pressure, antihypertensive medication, 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							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 N = 621 (384 females, 237 males)	Plasma Females: 22.3 (14.3–34.9) Males: 27.2 (19.9–45.2)	DBP, SBP	Partial Spearman correlation coefficient	DBP: 0.15; p-value <0.05 SBP: 0.07
Confounding: Age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), dietary intervention groups							
Lin et al., 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	Plasma 24.7 (18.1–33.9)	DBP, SBP, CRP (mg/L)	Regression coefficient per log2-unit increase in PFOS	SBP: $\beta = 1.2$ (0.3, 2.2); p-value <0.01 Ages <35: 0.6% ($-0.7, 1.8$)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 761 mothers (496 ages <35, 265 ages ≥35)			Percent difference (%) per log ₂ -unit increase PFOS	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							
Liu et al., 2018, 4238514 Medium	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1,871	Serum Geometric Mean (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
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.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: Gender, age, education, sample year, body mass index, smoking habit, alcohol consumption, physical activity, healthy diet score							
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 N = 1,016 (509 females and 507 males)	Plasma 13.23 (9.95–17.77)	CIMT, carotid artery intima-media complex grey scale median (CIM-GSM), carotid artery atherosclerotic plaque	CIMT, CIM-GSM: Regression coefficient per ln-unit increase in PFOS Plaque: OR per ln-unit increase in PFOS	CIMT, CIM-GSM, atherosclerotic plaque: no statistically significant associations
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							<p>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</p> <p>Angina pectoris: No association by quartiles, no significant trend; p-trend = 0.4211</p> <p>Congestive heart disease: No association by quartiles, no significant trend; p-trend = 0.9462</p> <p>CHD: No association by quartiles, no significant trend; p-trend = 0.0910</p> <p>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</p> <p>Stroke: No association by quartiles, no significant trend; p-trend = 0.3084</p> <p>CRP: -0.006; p-value = 0.6062</p>
<p>Comparison: Age groups were defined as <50 years and ≥ 50 years. Results: Lowest quartile used as the reference group.</p>							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) N = 877	Plasma Geometric mean (IQR) = 26.38 (22.8)	Microvascular disease (MVD), nephropathy, neuropathy, retinopathy	OR per log2-unit increase baseline PFOS	MVD: lifestyle arm: 1.37 (1.04, 1.84) Nephropathy, neuropathy, retinopathy: No statistically significant associations
Confounding: Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment; baseline fasting glucose and HbA1c levels for microvascular disease only							
Hutcheson et al., 2020, 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							
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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Results: Lowest quartile used as the reference group. Interquartile ratio = 75 th /25 th percentiles of serum PFOS: 3.08. Confounding: None listed							Males: No statistically significant associations; p-trend = 0.171
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	DBP, SBP, hypertension: no statistically significant associations
Outcome: Hypertension evaluated by individual BP components Confounding: Age							Hypertension: OR comparing above or below median
Chen et al., 2019, 5387400 Low	Croatia 2007–2008	Cross-sectional	Adults aged 44–56 N = 122	Plasma Geometric mean = 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 log-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							

PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoic acid; PFDE = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; MPAH = 2-(N-methyl-PFOA) acetate; PFNA = perfluorononanoic acid; PFUnDA = perfluoroundecanoic acid; BMI = body mass index; DBP = diastolic blood pressure (mmHg); SBP = systolic blood pressure (mmHg); CIMT = carotid artery intima-media thickness (mm); CIM-GSM = carotid artery intima-media complex grey scale median; CRP = C-reactive protein; CHD = coronary heart disease; CVD = cardiovascular disease; CMR = cardiometabolic risk score; HDL = high density lipoprotein cholesterol; LDL = low-density lipoprotein-cholesterol; MVD = microvascular disease; LVEDD = left ventricular end-diastolic diameter (mm); LVMI = left ventricular mass index (g/m²); RWT = relative wall thickness; AI = augmentation index; BAD = brachial artery distensibility; PWV = pulse wave velocity; CI = confidence interval; OR = odds ratio; SE = standard error; NHANES = National Health and Nutrition Examination Survey; IQR = Interquartile range.

^aExposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

C.5.2 Serum Lipids

Table C-14. Associations Between PFOS Exposure and Serum Lipid Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
Children							
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 in 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 <i>low</i> 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 European Youth Study (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)	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
				Median at 21 = 11.9 (male) or 9.1 (female)			
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 1 st trimester Geometric mean = 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 <i>low</i> 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 Geometric mean = 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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
			Children and Adolescents (KorEHS-C) N = 147				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 N = 260 at 3 months, 83 at 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
			Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI2, education, smoking, sex, and lipid outcome at 3 months				

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
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							
Pregnant Women							
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 per unit increase in PFOS 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: Each quintile is compared to the lowest quintile for reference.							
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 <i>low</i> 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 in 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
General Population							
Liu et al., 2018, 4238514 Medium	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1871	Serum Geometric mean = 5.28	Levels of TC (mg/dL), LDL (mg/dL), HDL (mg/dL), TG (ln- mg/dL)	Regression coefficient 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)
Results: Coefficients are presented with standard error in parentheses. 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 Geometric means: 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
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							
Donat-Vargas et al., 2019, 5080588 Medium	Sweden 1990–2013	Cohort	Non-diabetic adults ages 30–60 at baseline in Västerbotten Intervention Programme (VIP) = 15 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 comparing 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 Prevention Program (DPP)	Plasma Median = 27.2	Levels (mg/dL) of TC, LDL, HDL, triglycerides, non- HDL, and very low density lipids (VLDL);	Regression coefficient per doubling PFOS Hazard ratio (HR) or OR for hypercholesterolemia	<u>Cross-sectional</u> TC: 2.53 (-0.10, 5.16) LDL: 1.38 (-1.02, 3.77) HDL: -0.40 (-1.19, 0.39)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
			and Outcomes Study (DPPOS) N = 940 (888 not on metformin)		hypercholesterolemia, hypertriglyceridemia	or hypertriglyceridemia per doubling of PFOS	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 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							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 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							
Liu et al., 2020, 6318644 Medium	United States 2004–2007	Randomized clinical trial	Adults from POUNDS Lost study ages 20+ N = 326	Plasma Median = 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							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							

TC = total cholesterol; LDL = low density lipids; HDL = high density lipids; ApoB = Apolipoprotein B; ApoE = Apolipoprotein E; ApoC-III = Apolipoprotein C-III

^aExposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

C.6 Endocrine

Table C-15. Associations Between PFOS Exposure and Endocrine Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
General Population							
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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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/1.73m ² ; GF-2: GFR between 60 and 90 mL/min/1.73m ² ; GF- 3A: GFR between 45 and 60 mL/min/1.73m ² ; GF- 3B/4: GFR between 15 and 45 mL/min/1.73m 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							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% with thyroid condition N = 202	Serum 1.3	Levels of TSH (μIU/mL), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per log-unit IQR increase in PFOS	TSH: 0.41 (0.05, 0.76), p-value = 0.024 FT3: −0.14 (−0.24, −0.04), p-value = 0.007 FT4: −0.13 (−0.22, −0.04), p-value = 0.004
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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
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	Regression coefficient per ln-unit 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
					Subclinical hypothyroidism	Subclinical hypothyroidism: OR per increase in PFOS	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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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) N = 118 (64 boys, 54 girls)	Cord blood Mean = 7.24	Levels of TSH (μIU/mL), T3 (ln-μg/dL), T4 (μg/dL)	Regression coefficient by quartiles or per ln-unit increase in PFOS	TSH, all newborns: Q2: 0.21 (-0.20, 0.63) Q3: 0.19 (-0.22, 0.61) Q4: 0.65 (0.02, 1.28) Per increase: 0.35 (0.10, 0.59) TSH, boys: Q2: 0.63 (0.04, 1.22) Q3: 0.30 (-0.33, 0.94) Q4: 0.75 (0.13, 1.62) Per increase: 0.33 (0.01, 0.68) 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							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							
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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 to 18 weeks of gestation N = 478	Maternal blood Total PFOS: 4.77 Linear PFOS: 2.49 ΣBr-PFOS: 1.08	Levels of TSH (log-mIU/mL), FT3 (log-pmol/L), FT4 (log-pmol/L) by gestation status and 3 months post-partum	Regression coefficient per unit increase in total, linear, or 1m-PFOS	TSH, linear PFOS Main effect: 0.01 (–0.03, 0.04) 3 months post-partum: 0.06 (0.01, 0.12) TSH, ΣBr-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 in 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							

1 TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; FT3 = free triiodothyronine; FT4 = free thyroxine; TT3 = total triiodothyronine; TT4 = total thyroxine;
2 TGN = thyroglobulin; TPOAb = thyroid peroxidase antibody; TgAb = thyroglobulin antibody; GF = glomerular filtration; GFR = glomerular filtration rate; FCC = Fernald
3 Community Cohort; BMI = body mass index; POI = premature ovarian insufficiency.

^aExposure levels are reported as median unless otherwise noted.^bResults reported as effect estimate (95% confidence interval), unless otherwise noted.^cConfounding indicates factors the models presented adjusted for.

C.7 Metabolic/Systemic

Table C-16. Associations Between PFOS Exposure and Metabolic Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Children and Adolescents							
Ashley-Martin et al., 2018, 3981371 High	Canada, Recruitment 2008–2011	Cohort	Pregnant women and their children, from the MIREC Study N = 1,175	Maternal blood 4.6	Adiponectin, leptin	Regression coefficient per log10-unit increase in PFOS	Adiponectin, leptin: No statistically significant associations
Confounding: Maternal age, pre-pregnancy 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,	Maternal serum 8.04	BMI z-score, WC	Regression coefficient per unit increase in PFOS	BMI z-score, WC: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			Odense Child Cohort N = 593				
	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						
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.	Serum Mean = 86.5	Type 1 Diabetes	OR per ln-unit increase in PFOS	Children with T1D: 0.52 (0.54, 0.87)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			C8 Health Project N = 47				
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 Age 9 Males: 44.5 Females: 39.9	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: 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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), Total body fat (%) per high, medium, and low educational status	Regression coefficient per unit increase in PFOS	WC: -0.12 (-0.20, -0.04), p-value = 0.005 Trunk fat: -0.06 (-0.12, 0.01), p-value = 0.02 BMI: -0.04 (-0.07, 0.0), p-value = 0.03 Total body fat (%), WC, Trunk fat, and BMI for overall, low, and medium education status: No statistically significant associations
Confounding: Sampling design, pre-pregnancy BMI (kg/m ²) 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	Serum, Maternal serum 5 years: 4.7 18 months: 8.25	BMI z-score, Overweight	Risk Ratio (OW), or Regression coefficient per log ₁₀ -unit increase in	BMI z-score 18 months: 0.2 (0.1, 0.4), p-value <0.05 OW

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			Children, 5 years (overweight) N = 371			maternal PFOS, or by tertiles (BMI)	18 months: 1.29 (1.01, 1.64), p-value < 0.05
			Children, 18 months (overweight) N = 444				
			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 and Children's Health N = 176	Maternal serum 5.3	Ponderal index	Regression coefficient per ln-unit increase in PFOS	–1.07 (–1.79, –0.36), p-value = 0.004
			Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, gestational age, infant sex, and maternal blood sampling period				
Lauritzen et al., 2018, 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	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	IGF-1 Girls: –5.6 (–8.2, –2.9) Q4: –11.4 (–16.5, –6.0)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			N = 1123 girls and 1169 boys			ln(PFOS), or by quartiles	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				
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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					index, total fat-free mass index		<p>Early childhood: BMI, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association</p> <p>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</p>
Confounding: Maternal age, race/ethnicity, education, parity, pre-pregnancy BMI, timing of blood draw, household income, child sex, age at outcome assessment							
Fleisch et al., 2017, 3858513 Medium for metabolic function Low for HOMA-IR	United States, Pregnant women recruited 1999–2002, outcome assessed at mid-childhood follow-up	Cohort	Pregnant women and their children from Project Viva N = 584 Median age at follow-up = 7.7 years	Plasma GM = 6.2	Leptin, Adiponectin, HOMA-IR	Percent change per IQR increase in PFOS, or by quartiles	<p>HOMA-IR: Per IQR increase –10.1% (–16.4, –3.3) Q4: –24.7 (–37.8, –8.8) Females: –16.7 (–25.7, –6.7) Q4: –30.7 (–47.5, –8.4)</p> <p>Leptin, adiponectin: No statistically significant associations</p>
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	Cohort	Pregnant women, Odense Child Cohort N = 158	Serum 8.37	Blood glucose, insulin, c-peptide, 2-hour glucose, insulin resistance, beta	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
	assessed 12–20 weeks later				cell function, insulin sensitivity		
	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, glucose blood level	Regression coefficient by quartiles	Glucose blood level, All Q4: 4.3 (0.5, 8.0) <35 years Q4: 6.5 (2.1, 10.9) Q3: 5.2 (0.8, 9.7) Q2: 5.2 (0.8, 9.6) Gestational diabetes, glucose tolerance, hyperglycemia: No statistically significant association
	Results: Lowest quartile used as reference; Q1 (0.1–18.8 ng/mL), Q2 (18.9–25.7 ng/mL), Q3 (25.8–34.9 ng/mL), Q4 (35.0–185.0 ng/mL). Confounding: Pre-pregnancy BMI, prior history of gestational diabetes/parity, race/ethnicity, smoking, and education, maternal age (Full group only)						
Starling et al., 2017, 3858473 High	United States, 2009–2014	Cohort	Pregnant women and their children in the Healthy Start study	Maternal serum 2.4	Maternal glucose	Regression coefficient per unit increase in PFOS and by tertile	Maternal glucose: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
N = 628							
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 N = 189	Serum 3.13	Gestational diabetes (GDM), glucose homeostasis	Regression coefficient per ln-unit increase or by tertiles sum m-PFOS or L-PFOS	GDM: m-PFOS Per ln-unit increase: 1.36 (0.88, 2.11) T2: 1.53 (0.7, 3.34) T3: 1.23 (0.56, 2.72) L-PFOS Per ln-unit increase: 1.58 (0.89, 2.79) T2: 1.34 (0.62, 2.93) T3: 1.37 (0.62, 3.02) Glucose homeostasis: No statistically significant association
Results: Lowest tertile used as reference.							
Confounding: Maternal age, BMI in early pregnancy, fetal sex, serum triglyceride, total cholesterol							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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
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
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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							
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,	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					glucose (30 mins), glucose (2 hours), BMI		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
							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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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). 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	Nested case-control studies	Pregnant mothers and their 17-year-old daughters, ALSPAC	Maternal serum 20.2	Fat mass	Regression coefficient per unit increase in PFOS	Fat mass: No statistically significant association

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
	assessed at age 17		N = 221				
	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	<p>Spearman correlations Body weight: 0.8, p-value <0.05</p> <p>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</p> <p>Per log10-unit increase in PFOS 0.8, p-value <0.05</p> <p>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</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							<p>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</p> <p>HbA1c, glucose, fat mass, WC, leptin: No statistically significant association</p> <p>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.</p> <p>Confounding: Age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups.</p>
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, metabolic syndrome, WC	Regression coefficient per ln-unit increase in PFOS	<p>Fasting blood glucose: 1.96 (SE = 0.79)</p> <p>2-hour glucose, HbA1c, insulin levels, HOMA-IR, beta cell function, metabolic syndrome, WC: No statistically significant associations</p> <p>Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, WC, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)</p>
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),	OR and GM ratio (GMR) per doubling of PFOS, or by quartiles	<p>Diabetes: OR: 2.39 (1.52, 3.76) OR Q4: 3.37 (1.18, 9.56)</p> <p>Glucose (Fasting): GMR: 1.03 (1.01, 1.04) GMR Q4: 1.05 (1.02, 1.09)</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					glucose AUC (ng/mL), HbA1c (ln) (%)		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 assessed during biennial follow up through June 2011	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 OR by tertiles	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) 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),	Metabolic syndrome: OR per ln-unit increase in PFOS All other outcomes: regression coefficient per	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 Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					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 circumference (cm)	In-unit increase in PFOS	
Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity							

1 PFOS = Perfluorooctane sulfonate; HOMA = Homeostatic model assessment; IGF = Insulin-like growth factor; IR = Insulin resistance; BMI = Body mass index; WC = Waist
2 circumference; T1D = Type 1 Diabetes; OR = Odds ratio; RR = Risk ratio; IQR = Interquartile range; OW = Overweight; GM = Geometric mean; GDM = Gestational Diabetes
3 Mellitus; DM = Diabetes Mellitus; GWG = Gestational Weight Gain; HbA1c = Hemoglobin A1c; LSM = Least square mean; AUC = Area under the curve; SD = Standard
4 deviation; KorEHS-C: Korea Environmental Health Survey in Children and Adolescents; EYHS = European Youth Heart Study; SOLAR = Study of Latino Adolescents at Risk of
5 Type 2 Diabetes; HOME = Health Outcomes and Measures of the Environment; MIREC = Maternal Infant Research on Environmental Chemicals; POPUP = Persistent Organic
6 Pollutants in Uppsala Primiparas; ALSPAC = Avon Longitudinal Study of Parents and Children.

7 ^aExposure levels are reported as median in ng/mL unless otherwise noted.

8 ^bResults are reported as effect estimate (95% confidence interval) unless otherwise noted.

9 ^cConfounding indicates factors the models presented adjusted for.

1 C.8 Nervous

2 Table C-17. Associations Between PFOS Exposure and Neurological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 Mid-childhood only: Kaufman Brief Intelligence Test Second Edition (KBIT-2) non- verbal and verbal IQ, (WRAML2) design memory and picture memory	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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							<p>Gross Motor 1.22 (0.79, 1.89) No statistically significant associations, trends, or interactions by sex</p> <p>Fine Motor Overall: 1.25 (0.79, 1.96) No statistically significant associations, trends, or interactions by sex</p> <p>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) T3: 0.66 (0.29, 1.48) p-value for interaction by sex = 0.010</p> <p>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</p>
<p>Outcome: Neuropsychological problems defined as scores $\leq 10^{\text{th}}$ percentile. Results: Lowest tertile used as reference</p>							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 problem, externalizing problems, autism screening (peer-problems minus pro-social)	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 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 Birth Panel Study N = 239	Cord blood Mean = 7.0 (SD = 5.8)	Comprehensive Developmental Inventory (CDI) skill quotients: cognitive, fine-motor, gross-motor, language, self-help, social, whole test	Regression coefficient per IQR increase in ln-transformed PFOS	Cognitive: –0.8 (–2.8, 1.1) Fine-Motor: –1.8 (–3.8, 0.1) Gross-Motor: –3.7 (–6.0, –1.5) Language: –0.9 (–2.9, 1.2) Self-Help: –2.2 (–4.8, 0.3) Social: –1.0 (–3.7, 1.6) Whole Test: –2.1 (–4.1, –0.2)
							Confounding: Maternal education, family income, infant sex and gestational age, breastfeeding, HOME score at 24 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;	Regression coefficient (total behavioral difficulties, problem scores) and OR (borderline behavioral difficulties, problem scores,	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					difficulties in prosocial behavior	difficulties in prosocial behavior) per log-SD increase in PFOS and by quartiles	<p>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)</p> <p>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)</p> <p>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)</p> <p>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)</p> <p>Hyperactivity problems, peer problems: No statistically significant associations</p>
Results: Lowest quartile used as reference.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							
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	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 Medium	Denmark, Recruitment: 1996–2002; Follow-up at average age 10.7 years	Case-control	Mother-child pairs from Danish National Birth Cohort 215 Cases (39 Females; 176 Males) 545 Controls (33 Females; 180 Males)	Maternal plasma Cases: 25.40 (18.73–32.40) Controls: 27.40 (20.40–35.60)	ADHD, ASD	RR and OR (stratified by quartile or by sex) per ln-unit increase in PFOS or by quartiles	ADHD: 0.87 (0.74, 1.02) Q4: 0.79 (0.64, 0.98) ASD: 0.92 (0.69, 1.22) No other statistically significant associations by quartiles or by sex
							Results: Lowest quartile used as reference Confounding: Maternal age at delivery, SES, parity, smoking and drinking during pregnancy, psychiatric illnesses, gestational week of blood drawn, child's sex, birth year
Liew et al., 2018, 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,	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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					performance IQ, 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 37 Cases (7 Females; 29 Males) 50 Controls (15 Females; 35 Males)	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
							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.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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; Follow-up: 2009–2017	Case-Control	Mother-child pairs from CHARGE with children aged 2–5 years N = 453 (239 Cases; 214 Controls; 88 Females; 365 Males)	Maternal serum 5.81 (3.86–9.11)	ASD measured by Autism Diagnostic Interview-Revised (ADI-R)	OR per increase (ln-transformed or linear scale) in modeled, maternal, prenatal PFOS or measured, maternal, postnatal PFOS and by quartiles	By modeled prenatal exposure ln-transformed: 1.18 (0.77, 1.80) 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;	Cohort	Mother-child pairs from MoBa N = 943	Maternal plasma 11.51 (8.77–14.84)	Nonverbal and Verbal Working Memory measured by	Regression coefficient per unit increase in	Nonverbal Working Memory Q2: 0.06 (-0.14, 0.26) Q3: -0.10 (-0.30, 0.10) Q4: -0.02 (-0.22, 0.18)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
	Follow-up: 2007–2011				Stanford Binet Intelligence Scales	PFOS and by quintiles	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 age 1, 2, and 3 years	Cohort	Pregnant women and their children from the Columbia University Birth Cohort N = 302 (150 Females; 152 Males)	Cord blood GM = (Range:)	BSID-II scores: Mental and Psychomotor Development Index (MDI and PDI), Full IQ, Performance IQ, Verbal IQ	Regression coefficient of mean difference per log-unit increase in maternal PFOS	MDI Year 1: –0.61 (–3.17, 1.95) Year 2: 2.36 (–1.23, 5.94) Females: 5.52 (0.64, 10.4) Males: –1.35 (–7.09, 4.39) Interaction p-value = 0.04 Year 3: 1.96 (–1.24, 5.16) PDI Year 1: –0.07 (–4.56, 4.43) Year 2: –1.34 (–4.26, 1.57) Year 3: –0.55 (–5.34, 4.23) Full IQ Year 4: –0.41 (–4.25, 3.43) Year 6: 2.81 (–1.84, 7.46) Performance IQ Year 4: –0.05 (–4.56, 4.46) Year 6: 2.81 (–2.29, 7.91) Verbal IQ Year 4: –0.19 (–4.50, 4.12) Year 6: 2.67 (–2.56, 7.90) No other statistically significant associations or interactions by sex

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 unit increase in PFOS and by tertiles	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 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 composite 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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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) 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),	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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					memory retention distance (%), and memory retention time (s)		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) 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 Medium	United States, Recruitment: 2003–2006; Follow-up at age 8 years	Cohort	Mother-child pairs with children aged 8 years from the HOME study N = 161	Maternal serum Mean = 13.9 (SD = 7.9)	Wide Range Achievement Test 4 (WRAT-4) reading composite score	Regression coefficient per log10-unit increase in PFOS	7.0 (–2.9, 16.9)
							Confounding: Maternal age, race/ethnicity, education, household income, marital status, maternal depression, maternal serum cotinine, maternal blood lead levels, maternal fish consumption, maternal IQ, child sex, HOME score
Wang et al., 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	Cohort	Pregnant women and their children aged 3, 5, and 7	Serum Maternal: 13.0 (9.1–17.8) 3 years: 6.6 (4.6–10.2)	Basic reading, brief reading, letter word identification, passage	Regression coefficient per ln- unit increase in PFOS	Basic Reading Maternal Serum: 3.2 (–2.0, 8.3) Year 3 Serum: 1.1 (–4.8, 7.0) Brief Reading

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
	ages 3, 5, and 7 years		years from the HOME study N = 167	8 years: 3.6 (2.7–4.9)	comprehension measured by Woodcock Johnson Test of Achievement-III (WJ-III)		Maternal Serum: 2.9 (–2.2, 8.1) Year 3 Serum: 3.2 (–2.6, 9.1)
					Reading composite, word reading, sentence Comprehension measured by Wide Range Achievement Test 4 (WRAT-4)		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) 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 et al., 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	OR per log2-unit increase in PFOS and for ≥90 th percentile	HFHI OR (per doubling): 0.96 (0.85, 1.10)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					(HFHI and LFHI)	vs. <90 th percentile	OR (90 th percentiles): 1.31 (0.75, 2.27) LFHI OR (per doubling): 0.87 (0.73, 1.03) OR (90 th 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 C8 Health Project N = 21,024	Serum Range = 0.25–759.2	Memory impairment (self-reported)	OR per doubling log-unit PFOS and by quintiles	0.93 (0.90, 0.96) Q2: 0.96 (0.87, 1.07) Q3: 0.86 (0.78, 0.96) Q4: 0.87 (0.78, 0.96) Q5: 0.85 (0.76, 0.94) p-trend < 0.001
							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)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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 adjacent to Hudson River N = 126	Serum 33.7 (23.3–50.8)	Affective state: Beck Depression Inventory (BDI) total score, State-Trait Anxiety Inventory state and trait t-scores Attention: Trail making test Part A (ln- transformed time to complete) Executive function: Stroop color word test t-score, Trail making test part B (ln- transformed time to complete),	Regression coefficient per IQR increase in ln-unit PFOS	Depression: 0.25 (–0.77, 1.26), p-value = 0.63 CVLT-Total score: –0.14 (–0.59, 0.31) Wisconsin card-sorting test Perseverative Error: –0.14 (–0.30, 0.02), p-value = 0.09 Perseverative Response: –0.16 (–0.34, 0.01), p-value = 0.07 Wechsler Memory Scale Logical Memory Immediate Recall: –0.7 (–1.92, 0.52), p-value = 0.26 Delayed Recall: –0.14 (–1.29, 1.01), p-value = 0.81 Visual Reproduction Immediate Recall: 0.56 (–0.16, 1.29), p-value = 0.13 Delayed Recall: 0.79 (0.03, 1.55), p-value = 0.04 No statistically significant associations: State-Trait Anxiety

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					Wisconsin card sorting test preservative ln- transformed error and response		Inventory, Stroop color word test, trail-making tests, motor function outcomes, visuospatial outcomes
					Memory and learning: California Verbal Learning Test total and subscores, Wechsler Memory Scale logical memory and visual reproduction immediate and delayed recall scores		
					Motor function (dominant and non-dominant hands): finger tapping test average scores, grooved pegboard test ln-transformed times to completion, static motor steadiness test ln-transformed		

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					total numbers of contacts and times touching		
					Dominant hand reaction time		
					Visuospatial function: Wechsler Adult Intelligence Scale-Revised total scores for 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							

ADHD = Attention deficit hyperactivity disorder; ASD = Autism spectrum disorder; BRIEF = Behavior Rating Inventory of Executive Function; ASQ-3 = Ages and Stages Questionnaire-3; CDI = Comprehensive Developmental Inventory; ID = Intellectual disability; HFHI = High frequency hearing impairment; LFHI = Low frequency hearing impairment; SDQ = Strengths and Difficulties Questionnaire; PFOS = Perfluorooctane sulfonic acid; BMI = Body mass index; OR = Odds ratio; RR = Risk ratio; IQR = Interquartile range; GM = Geometric mean; NHANES = National Health and Nutrition Examination Survey; HOME = Health Outcomes and Measures of the Environment; ALSPAC = Avon Longitudinal Study of Parents and Children; EMA = Early Markers for Autism; LINC = Linking Maternal Nutrition to Child Health; CHARGE = Childhood Autism Risk from Genetics and Environment; MoBa = Mother, Father, and Child Cohort Study; DaFO88 = Danish Fetal Origins 1988; TMICS = Taiwan Maternal and Infant Cohort Study; HUMIS = Human Milk Study.

^aExposure levels are reported as median unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval), unless otherwise noted.^cConfounding indicates factors the models presented adjusted for.

C.9 Renal

Table C-18. Associations Between PFOS Exposure and Renal Effects in the General Population

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 Method, 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. 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 (μ mol/L), creatinine (μ mol/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- μ g/mL), creatinine in urine (log10-	Regression coefficient per log10-unit increase in PFOS, or	Albumin in urine Per log10-unit increase: –0.08 p-value <0.01 Negative associations across eGFR stages

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
					mg/dL), albumin-to- creatinine ratio in urine (log10- mg/g), albumin in serum (log10- mg/dL), creatinine in serum (log10- mg/dL)	percent change per 10% increase in PFOS	<p>Percent change per 10% increase: –0.75, p-value <0.05 p-value for gender and race/ethnicity interaction = 0.10</p> <p>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</p> <p>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</p> <p>Albumin in serum Per log10-unit increase: 0.01 p-value <0.01 Positive associations across eGFR stages Percent change per 10% increase: 0.11 p-value <0.05</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							p-value for gender and race/ethnicity interaction = 0.68
							Creatinine in serum Per log10-unit increase: 0.01 p-value = 0.01 Positive associations in GF-1, GF-2, GF-3A Negative association in GF-3B/4 Percent change per 10% increase: 0.11 p-value <0.05 p-value for gender and race/ethnicity interaction <0.01
							GF Stages: GF-1: GFR ≥ 90 mL/min/1.73m ² ; GF-2: GFR between 60 and 90 mL/min/1.73m ² ; GF- 3A: GFR between 45 and 60 mL/min/1.73m ² ; GF- 3B/4: GFR between 15 and 45 mL/min/1.73m ² .
							Confounding: Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), poverty income ration, NHANES survey period
Jain et al., 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: $60 < \text{eGFR} \leq 90$ mL/min per 1.73 m ² ; GF-3A: $45 < \text{eGFR} \leq 60$ mL/min per 1.73 m ² ; GF-3B/4: $15 < \text{eGFR} \leq 45$ 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), p-value = 0.2.05 Q2: 1.19 (0.67, 2.09) Q3: 1.42 (0.82, 2.47)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			N = 1612 (males = 1204, females = 408)			in PFOS, or by quartiles	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
Outcome: Hyperuricemia defined as serum uric acid levels >7.0 mg/dL in males or >6.0 mg/dL in females.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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	<p>Uric acid</p> <p>Overall population</p> <p>Q2: 0.13 (0.01, 0.24)</p> <p>Q3: 0.21 (0.05, 0.37)</p> <p>Q4: 0.29 (0.14, 0.44)</p> <p>p-value for trend = 0.003</p> <p>Participants with CKD</p> <p>Q2: 0.6 (0.15, 1.05)</p> <p>Q3: 0.31 (–0.02, 0.7)</p> <p>Q4: 0.38 (0.06, 0.83)</p> <p>p-value for trend = 0.08</p> <p>Participants without CKD</p> <p>Q2: 0.03 (–0.1, 0.15)</p> <p>Q3: 0.13 (–0.02, 0.28)</p> <p>Q4: 0.2 (0.06, 0.34)</p> <p>p-value for trend = 0.02</p> <p>Hyperuricemia</p> <p>Overall population</p> <p>Q2: 1.1 (0.84, 1.45)</p> <p>Q3: 1.27 (0.92, 1.76)</p> <p>Q4: 1.45 (1.03, 2.03)</p> <p>p-value for trend = 0.15</p> <p>Participants with CKD</p> <p>Q2: 1.93 (0.91, 4.06)</p> <p>Q3: 0.85 (0.4, 1.77)</p> <p>Q4: 1.15 (0.53, 2.5)</p> <p>p-value for trend = 0.12</p> <p>Participants without CKD</p> <p>Q2: 0.94 (0.68, 1.3)</p> <p>Q3: 1.26 (0.89, 1.79)</p> <p>Q4: 1.35 (0.92, 1.99)</p> <p>p-value for trend = 0.19</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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 <60mL/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
Outcome: Hyperuricemia defined as serum uric acid levels ≥6 mg/dL.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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. Results: Lowest quartile used as the reference group.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Confounding: Age, gender, BMI, parental education level, exercise, environmental tobacco smoke exposure, and serum creatinine							
Khalil et al., 2018, 4238547 Low	United States 2016	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)
Confounding: Age, sex, race							
Pregnant Women							
Nielsen et al., 2020, 6833687 Low	Sweden, 2009–2014	Cohort	Pregnant women, PONCH study N = 73	Serum Early pregnancy: 5.6 (5 th –95 th percentile = 2.6–11.5) Late pregnancy: 4.8 (5 th –95 th 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.							
Confounding: Number of days between sampling, pregnancy-induced change in BMI							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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

FCC = Fernald Community Cohort; YOTA = Young Taiwanese Cohort Study; GBCA = Genetic Biomarkers Study for Childhood Asthma; eGFR = estimated glomerular filtration rate (mL/min per 1.73 m²); GF = glomerular filtration; CKD = chronic kidney disease; BMI = body mass index; GM = geometric mean; OR = odds ratio; SD = standard deviation; SE = standard error; NHANES = National Health and Nutrition Examination Survey; PONCH = Pregnancy Obesity Nutrition and Child Health study; LMrev = Lund Malmö Revised; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration study; CAPA = Caucasian Asian Pediatric Adult; AFFF = aqueous film-forming foam.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

C.10 Hematological

Table C-19. Associations Between PFOS Exposure and Hematological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
General Population							
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 PFOA: Vitamin D deficiency POR: 1.05 (0.97, 1.13) 25-hydroxy Vitamin D –0.9 (–1.5, –0.2) Q5: –2.8 (–4.7, –0.8) 60+ years: –1.7 (–2.9, –0.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
							No other statistically significant associations or trends
Results: Lowest quintile used as reference group. Confounding: Gender, race/ethnicity, age, 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) anemic females: GM = 5.0 (95% CI: 4.4, 5.8)	Whole blood hemoglobin (WBHGB) (log10-g/dL)	Regression coefficient per log10-unit increase in PFOA	Non-anemic males: 0.009, p-value <0.01 Non-anemic females: 0.006, p-value <0.01 Anemic males: 0.023, p-value <0.01 Anemic females: 0.024, p-value <0.01
Confounding: Age, BMI, poverty income ratio, serum cotinine, survey year, daily alcohol intake							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
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							

aPTT = activated partial thromboplastin time. HIV = human immunodeficiency virus. PPT = prothrombin time; GM = geometric mean; BMI = body mass index; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

C.11 Respiratory

Table C-20. Associations Between PFOS Exposure and Respiratory Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 (min = 0.60, max = 27.80) WTCHR group: median = 3.72 (min = 1.01, max = 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
Confounding: Sex, race/ ethnicity, age, BMI, tobacco smoke exposure							
Impinen et al., 2018, 4238440 Medium	Norway 1992–2002	Cohort	Infants followed up at 2 years and 10, N = 641	Cord blood, Median = 5.2 (4.0, 6.6)	Oslo Severity Score (1–5 vs. 0) Oslo Severity Score (6–12 vs. 0) Reduced lung function at birth	OR per log2-unit increase in PFOS	1.71 (1.16, 2.53), p-value = 0.007 1.15 (0.71, 1.84), p-value = 0.576 0.86 (0.43, 1.72), p-value = 0.680
Outcome: Reduced lung function at birth: Lung function (tPTEF/tE) with standardized z-score, and binary variable of decreased lung function (cutoff <0.20). Confounding: Sex							
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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							

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; WTCR = World Trade Center Health Registry; BMI = body mass index.

^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.

^bResults reported as effect estimate (95% confidence interval), unless otherwise noted.

^cConfounding indicates factors the models presented adjusted for.

C.12 Musculoskeletal

Table C-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),	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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
					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)		
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
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	Serum Controls: 0.82 (0.4–1.3)	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)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
			N = 100 (50 controls, 50 exposed)	Exposed: 1.11 (0.8–1.3) Semen Controls: 0.11 (0.08–0.13) Exposed: 0.11 (0.01–0.14)			Adjusted p-value for comparison of medians = 0.738
Results: Values for each outcome are reported as median (25 th –75 th 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 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 recreational 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,	Serum GM = 15.32 (SD = 17.58)	Total BMD (g/cm ²) in hip or lumbar spine; fractures in hip,	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

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
			Females N = 842, Females in menopause N = 305		wrist, spine, or all types		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	Adults from NHANES, Ages 12–80, Males N = 956, Females N = 958	Serum Mean = 12.7 (SE = 1.20)	BMD (g/cm ²) of total femur, femoral neck, lumbar spine; Osteoporosis among females	BMD: Regression coefficient per ln-unit increase in PFOS and by quartiles Osteoporosis: OR per ln-unit increase in PFOS and by quartiles	Total femur Females: −0.018 (−0.034, −0.002), p-value <0.05 Q2: −0.007 (−0.038, 0.023) Q3: −0.009 (−0.037, 0.019) Q4: −0.044 (−0.074, −0.014), p-value <0.05 Males: Not statistically significant Femoral neck Females: −0.016 (−0.029, −0.002), p-value <0.05 Q2: 0.001 (−0.019, 0.019) Q3: −0.001 (−0.025, 0.025) Q4: −0.034 (−0.059, −0.009), p-value <0.05 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							

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
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 Δ BMD (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 Δ BMD: -0.005 (-0.009 , -0.001), p-value <0.05 Hip intertrochanteric area BMD analyses 2-yr Δ BMD: -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							

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.

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

^cConfounding indicates factors the models presented adjusted for.

C.13 Gastrointestinal

Table C-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 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;	Cohort	Children and adults from CHEF	Blood	Inflammatory bowel disease	Incidence rate ratio for highest vs. lowest tertile	0.30 (0.08, 1.07)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
	follow-up until 2017		N = 2,843	Low exposure: GM = 2.33 (1.93–2.90) High exposure: GM = 26.88 (21.90–32.24)		of PFOS exposure	
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							

1 PFOS = perfluorooctane sulfonate; RR = risk ratio; BMI = body mass index; RCT = randomized controlled trial; CHEF = Children's Health and the Environment in the Faroes.

2 ^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

3 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

4 ^cConfounding indicates factors the models presented adjusted for.

C.14 Dental

Table C-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							

PFOS = perfluorooctane sulfonate; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; CI = confidence interval; IQR = interquartile range.

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults are reported as effect estimate (95% confidence interval).

^cConfounding indicates factors the models presented adjusted for.

C.15 Ocular

Table C-24. Associations Between PFOS Exposure and Ocular Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Zeeshan et al., 2020, 6315698 Low	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

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

PFOS = perfluorooctane sulfonate; BMI = body mass index.

^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

^bResults are reported as effect estimate (95% confidence interval).

^cConfounding indicates factors the models presented adjusted for.

C.16 Dermal

Table C-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							

PFOS = perfluorooctane sulfonate; DNBC = Danish National Birth Cohort.

^aExposure levels reported as median (10th–90th percentile).

^bResults reported as effect estimate (95% confidence interval).

^cConfounding indicates factors the models presented adjusted for.

1 C.17 Cancer

2 Table C-26. Associations Between PFOS Exposure and Cancer in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
Ducatman et al., 2015, 3859843 Medium	United States 2005–2006	Cross-sectional	Men from C8 settlement, Age 20–49, 9,169; Age 50–69, 3,819	Serum Mean = 22.18	Prostate-specific antigen (PSA) level	Regression coefficient (β) and geometric mean ratio (GMR) (PSA < 4.0 ng/mL vs. PSA \geq 4.0 ng/mL)	Age 20–49 β = 1, p-value = 0.71; GMR = 0.95 (0.71, 1.28) Age 50–69 β = 1, p-value = 0.99; GMR = 1.1 (0.98, 1.23)
Confounding: Age, smoking status, average alcohol intake, and body mass index ^c							
Ghisari et al., 2017, 3860243 Medium	Denmark 1996–2002	Nested case-control	Adult women, 283	Serum Median = 27.80 (cases), 4.90 (controls)	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							
Wielsoe et al., 2017, 3858479 Medium	Greenland 2000–2003, 2011–2014	Case-control	Adult women, 158	Serum Median=35.50 (cases), 18.2 (controls)	Breast cancer	OR per unit increase in PFOS or by tertiles	T2: 3.13 (1.2, 8.15), p-value = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							T3: 5.5 (2.19, 13.84), p-value <0.001 Per increase: 1.02 (1.01, 1.03), p-value = 0.005
							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 = 6.695 (cases), 6.950 (controls)	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 change: 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 Median = 30.5 (cases), 32.1 (controls)	Breast cancer	OR per log2-unit increase in PFOS	0.3 (0.1, 0.9), p-value = 0.02
							Confounding: Maternal: cholesterol, age at pregnancy, history of breast cancer, primiparity, overweight at first prenatal visit, serum levels of DDTs and metabolite DDE, African-American status, whether daughter was breastfed
Mancini et al., 2019, 5381529 Medium	France 1990–2013	Nested case-control	Postmenopausal women, 40–65 in 1990, 388	Serum Median = 17.51	Breast cancer	ORs by quartiles ORs 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							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 Median = 38.4	Renal cell carcinoma	ORs per log2-unit increase in PFOS or by quartiles (total cohort only)	Q2: 1.67 (0.84, 3.3) Q3: 0.92 (0.45, 1.88) Q4: 2.51 (1.28, 4.92) p-trend = 0.009 Per doubling increase: 1.39 (1.04, 1.86)
			Results: Lowest quartile used as the reference group Confounding: BMI, smoking, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, calendar year of blood draw; sex, race and ethnicity, study year of blood draw, study center				
Fry and Power, 2017, 4181820 Medium	US NHANES 2003–2006	Cohort	Adults 60+, 1,036	Serum Median = 4.3 ng/g	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, 50+, 154	Serum Median = 19.00	Cancer (any)	OR per unit increase of PFOS	0.99 (0.96, 1.01)
			Confounding: Age, BMI, work status, alcohol consumption				
Lin et al., 2020, 6835434	China 2014–2017	Case-control	Children, <16, 84	Serum Median = 4.47	Germ cell tumors	OR per unit increase in PFOS	1.08 (0.96, 1.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
Low	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 = 5.64	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							

OR = odds ratio; DDT = dichloro-diphenyl-trichloroethane; DDE = dichlorodiphenyldichloroethylene

^aExposure levels reported as median (10th–90th percentile).

^bResults reported as effect estimate (95% confidence interval).

^cConfounding indicates factors the models presented adjusted for.

Appendix D. Detailed Toxicokinetics

D.1 Absorption

D.1.1 Cellular Uptake

Lipid binding may influence PFOS accumulation in various cell types relevant to absorption as well as distribution. Sanchez-Garcia (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 D-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 (CHIAM7.4). 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 D-1. Cellular Accumulation and Retention Relative to Lipophilicity and Phospholipidicity

Chemical	Cellular Accumulation and Retention		Lipophilicity		
	Accumulation in Lung Epithelium (%) (AZI)	Retention in Lung Epithelium	Phospholipid Binding (CHIAM7.4)	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

AZI = azithromycin-dihydrate; PFOS = perfluorooctane sulfate; PFOA = perfluorooctanoic acid; 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 uptake 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 D.4.2.1). Ebert et al. (2020, 6505873) determined membrane/water partition coefficients ($K_{\text{mem/w}}$) for PFOS and examined possible 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 ($K_{\text{mem/w}}/[L/kg]$) for PFOS was 4.69, similar to the experimentally determined value of 4.89 ± 0.30 . $K_{\text{mem/w}}$ 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 $K_{\text{mem/w}}$.

values. The predicted anionic permeability ($\log P_{\text{ion}}/[\text{cm/s}]$) 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.

D.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 $9.08 \pm 0.51\%$ of the total PFOS- ^{14}C dose was recovered across digestive tract, feces, or urine, while the carcass retained $94.2 \pm 5.1\%$, indicating that the PFOS was largely absorbed.

D.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.

D.1.4 Dermal Exposure

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

D.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 D.2.3.

D.1.6 Bioavailability

Toxicokinetic parameters informing absorption were derived by comparing oral to 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 D-2. Both studies found very high ($\geq 100\%$) 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 $\geq 100\%$ 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 D-2. PFOS Parameters from Toxicokinetic Studies Informing Bioavailability in Sprague-Dawley Rats

Study	Dose (mg/kg)	Route	Sex	C_{\max} ($\mu\text{g/mL}$) ^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

PFOS = perfluorooctane sulfonate; C_{\max} = maximum serum concentration; T_{\max} = time to C_{\max} ; IV = intravenous; NA = not applicable.

^aConverted published C_{\max} (mM) to C_{\max} ($\mu\text{g/mL}$) for Huang et al. 2019 (5387170).

^bConverted published T_{\max} (days) to T_{\max} (hours) for Kim et al. 2016 (3749289).

D.2 Distribution

D.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 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 $2.2 \times 10^4 \text{ M}^{-1}$ 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 B₂ (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, 10, and 200 μ M 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 μ M. 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 $49 \pm 8 \mu\text{M}$ for serum binding proteins, $38 \pm 5 \mu\text{M}$ for albumin, and $81 \pm 7 \mu\text{M}$ 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 PFOA to maternal serum albumin can reduce the free PFOA available to move across the barrier through passive diffusion. Similarly, higher fetal albumin levels will lead to less free PFOA 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 PFOA 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 and $3.3 \pm 0.1 \mu\text{mol}$, 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 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).

D.2.2 Tissue Distribution

D.2.2.1 Human Studies

Human blood is a known site of PFOS accumulation. A recent example measured PFAS in blood samples from 344 Wilmington, NC residents (289 adults and 55 children) exposed to contaminated drinking water from release of PFAS chemicals into the Cape Fear River between 1980 and 2017. The mean serum PFOS concentration was 9.4 ng/mL in adults and 5.1 ng/mL in children (Kotlarz et al., 2020, 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., VLDL, LDL and 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 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 PFASs 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) {Kärmen, 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 {Poonthong., 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, 4.77 and 2.85 ng/mL, respectively. Similar to other studies, PFOS preferentially accumulated in plasma relative to other blood fractions and also suggest 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.

D.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, Kärman 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 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 $\mu\text{g/mL}$) and in nine amniotic fluid samples (0.0002–0.0018 $\mu\text{g/mL}$). 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 (CSF) and serum {Harada, 2007, 2919450; Wang, 2018, 5080654}. In both studies, PFOS levels in CSF 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}. Kärman 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.

D.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.

D.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, 0.03, 0.15, 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, and 0.75 mg/kg/day groups. Serum PFOS measurements demonstrated a linear increase with dosing duration in the 0.03 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 and 100 µg/mL on day 113 and 50, respectively. Male and female animals in the high dose group reached peak PFOS serum levels of 160–165 µg/mL on day 365. Consistent with the previous study, no sex differences were found. At the end of the experiment, the animals were reported to have a 1:1 PFOS liver:serum ratio, while the previous Seacat et al. (2002, 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).

D.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 , 229 ± 10 , and 401 ± 21 µg/g after 1, 3, or 5 daily doses, respectively. Mean serum concentrations were 23 ± 2.8 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, 0.312, 0.625, 1.25, 2.5, 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 ($\mu\text{M}/\text{mmol}/\text{kg}/\text{day}$) 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 and $318.2 \pm 8.87 \mu\text{g}/\text{mL}$ at the lowest and highest doses, respectively. In females, these values were 30.53 ± 0.92 and $413.56 \pm 8.07 \mu\text{g}/\text{mL}$ 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 D-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 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 D-3. Concentrations of PFOS in Various Tissues of Male Sprague-Dawley Rats Exposed to PFOS by Gavage for 28 Days

Tissue ^a	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day
Blood ($\mu\text{g}/\text{mL}$)	ND	72.0 ± 25.7	No sample ^b
Liver ($\mu\text{g}/\text{g}$)	ND	345 ± 40	648 ± 17

Tissue^a	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day
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

PFOS = perfluorooctane sulfonate; ND = not detected.

^aData are presented as mean ± standard deviation.

^bAnimal 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, 2, 20, 50, or 100 mg/kg/day via feed for 28 days (Table D-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 and 20 mg/kg/day; however, this linearity was lost between the 20, 50, 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 D-4. Concentrations of PFOS in Various Tissues of Male and Female Sprague-Dawley Rats Exposed to PFOS by Feed for 28 Days

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

PFOS = perfluorooctane sulfonate.

^aData are presented as mean ± standard deviation.

Iwabuchi et al. (2017, 3859701) exposed male Wistar rats to PFOS in drinking water at 0, 0.077, 0.38, 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 D-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 D-5. Distribution of PFOS in Male Wistar Rats Exposed via Drinking Water for 1 or 3 Months

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

PFOS = perfluorooctane sulfonate.

^aData are presented as mean values.

A combined chronic toxicity/carcinogenicity good laboratory practice (GLP) study was performed in male and female Sprague-Dawley CrI:CD (SD)IGS BR rats administered 0, 0.5, 2, 5, or 20 ppm PFOS (equivalent to 0, 0.018–0.023, 0.072–0.099, 0.184–0.247 and 0.765–1.1 mg/kg/day, respectively) for 104 weeks (Thomford et al., 2002, 5432392; Butenhoff et al., 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 D-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 and 20 ppm doses.

Table D-6. PFOS Levels in the Serum and Liver of Male and Female Sprague-Dawley Rats Exposed to PFOS in Feed for 2 Years

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

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

PFOS = perfluorooctane sulfonate; LOQ = limit of quantification.

^aLOQ = 0.00910 pg/mL.

^bLOQ = 0.0457 pg/mL.

^cSamples were obtained from the recovery group administered 20 ppm for 52 weeks and then observed until necropsy at 106 weeks.

D.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 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). In a study conducted by Li et al. (2017, 4238518) PFOA concentrations in both liver and serum increased with dose in BALB/c mice following exposure for 28 days, with PFOA concentrations being generally higher in the liver than the serum in both males and females.

Bogdanska et al. (2011, 2919253) performed a radioisotope distribution study in adult C57BL/6 male mice using ³⁵S-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 ³⁵S-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 $\mu\text{g/mL}$, 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, 10, 50, or 200 mg/kg single dose) and female (0, 20, 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, 0.25, 2.5, 25, 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, 5, 40, 240, and 300 $\mu\text{g/mL}$ and PFOS levels in the brain were approximately 0, 2, 5, 30, and 70 $\mu\text{g/g}$ for the 0, 0.25, 2.5, 25, and 50 mg/kg/day dose groups, respectively. These data indicated that PFOS levels in serum and in brain deposits are dose-dependent and that brain levels were much lower (100-fold less than that observed in blood and liver). These authors also conducted in vitro studies showing that PFOS significantly decreased the expression of tight junction-related proteins (e.g., ZO-1, Claudin-5, Claudin-11, Occludin) in endothelial cells. These findings suggest that exposure to PFOS may also disrupt the blood-brain barrier, that in turn could lead to increased accumulation of PFOS in brain.

Qui et al. (2013, 2850956) exposed ICR mice orally to PFOS at 0, 0.25, 2.5, 25, 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.

D.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 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, 0.2, 1, 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 and 3 mg/kg/day groups, respectively. No effect with treatment was observed on OATp1.

Transporters responsible for PFOA transport across the placenta are not well understood. Kummur 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 $12.9 \pm 1.5\%$ and $14.4 \pm 3.9\%$, respectively. Immunoblot analysis of OAT4 and ABCG2 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 (FR α), 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, FR α , 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.

D.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.

D.2.3.1 Human Studies

T. 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% versus 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 ($p < 0.001$).

D.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).

T. 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 (Table D-7). 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 (Table D-7). 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 T. 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.

T. 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 (Table D-7). 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.

Table D-7. PFOS Concentrations in Human Placenta, Maternal Blood, and Transplacental Transfer Ratios (RPM)

Study	Gestational Age (weeks)	Isomer	Placenta(ng/g), SD	Maternal Serum (ng/mL), SD	Placenta: maternal serum ratios (RPM) ^a
Chen et al, 2017, 3859806	38.9 ± 1.6	Total PFOS	2.842 ± 2.023	8.670 ± 5.266	0.330
		Linear PFOS	2.484	6.971	0.360
		Iso PFOS	0.151	0.490	0.317
		(3+5)m PFOS	0.388	0.466	0.698
		4m PFOS	0.147	0.157	0.676
		1m PFOS	0.148	0.136	0.749

Study	Gestational Age (weeks)	Isomer	Placenta(ng/g), SD	Maternal Serum (ng/mL), SD	Placenta: maternal serum ratios (RPM) ^a
Chen et al, 2017, 3981340	38.9 ± 1.6		0.42 ± 0.30	8.670 ± 5.27	0.048
T. Zhang et al., 2013, 3859792	40.3 ± 2.3		8.18 ± 3.03	14.6 ± 4.98	0.59

SD = standard deviation.

^aRPM values were reported by authors as the ratio of the concentration in placenta to the concentration in maternal serum.

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 PFASs 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.

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 0.55–29.85 ng/mL) in maternal serum and 1.33 ng/mL (range 0.12–5.89 ng/mL) in cord serum. PFOS concentrations in maternal serum were strongly correlated to concentrations in cord blood ($r = 0.745$).

Linear and branched PFOS have been detected in both maternal and cord serum {Cai, 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 D-8). 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 D-8. 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 D-8. PFOS concentrations in Human Cord Blood, Maternal Blood, and Transplacental Transfer Ratios (RCM)

Study	Country, Cohort	Number of Maternal-Infant Pairs ^a	Mean Gestational Age (weeks) ^b	PFOS Measurement	Cord Serum (ng/mL) ^c	Maternal Serum (ng/mL) ^c	Cord: Maternal serum ratios (RCM) ^d
Manzano-Salgado et al., 2015, 3448674	Sabadell and Valencia, Spain 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.	53	NR	total PFOS	1.86	6.99	0.29
Chen et al, 2017, 3981340 and Chen et al, 2017, 3859806	Wuhan, China Note: PFOS detected in 100% of maternal and cord samples except for m-PFOS in cord samples, where the detection rate of 96.87%. PFOS isomers were reported in Chen 2017a and total PFOS was reported in Chen 2017b.	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
				Cariou et al., 2015, 3859840	Toulouse, France	94	NR
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	Faroe 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
	Faroe 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
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, 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, L. L.; Hays, S. M.; Kirman, C. R.; Marchitti, S. A.; Kenneke, J. F.; English, C.; Mattison, D. R.; Becker, R. A. Relationships of chemical concentrations in maternal and cord blood: a review of available data. J. Toxicol. Environ. Health, Part B 2014, 17 (3), 175–203.							

NR = not reported, LOD = Level of Detection, NA= Not Applicable, IQR = Interquartile Range.

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

^b Gestational age reported as mean \pm 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.

D.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. The authors reported a positive correlation between PFOA in amniotic fluid and maternal blood ($r = 0.621$, $p < 0.01$) and cord blood ($r = 0.664$, $p < 0.01$), adding to the evidence that PFOA levels in amniotic fluid is a potential biomarker for fetal exposure during pregnancy,

Table D-9 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 et al., 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 D-9. 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: 0.42ng/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

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
	Maternal serum T2 Mean: 13.0 ng/mL Median: 13.20 ng/mL IQR: 7.62–20.38 ng/mL		NR	NR	NR
	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 (Faroe 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	NR	NR	NR
	Cohort 5 mean: 8.82 ng/mL SD: 0.51 ng/mL IQR: 6.94–11.6ng/mL	Whole cord blood: Mean: 4.90 ng/mL SD: 0.26 ng/mL IQR: 3.33–6.94 ng/mL Cohort 5 Cord serum: mean: 3.09 ng/mL SD: 0.22 ng/mL IQR: 2.31–4.42 ng/mL	NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
		Whole cord blood: mean: 1.60 ng/mL 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: 2.94–6.34ng/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: 2.31ng/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 Whole blood Median: 5.79 ng/mL Mean: 6.11 ng/mL Range: 3.61–8.38 ng/mL	Plasma Median: 4.11 ng/mL Mean: 3.93 ng/mL Range: 1.75–6.27ng/mL Whole blood Median: 1.88 ng/mL Mean: 1.92 ng/mL Range: 0.49–3.89 ng/mL	NR NR	NR 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 Plasma median: 0.23 ng/mL	NR	NR	NR	NR
		NR	NR	NR	NR

Study (Location of Study)	Maternal Blood	Cord Blood	Infant Blood	Placenta	Amniotic Fluid
	mean: 0.33 ng/mL 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 T2 serum Mean: 3.87 ng/mL SD: 1.99 ng/mL Median: 3.43 ng/mL Range: 1.04–8.19ng/mL 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	NR NR	Mean: 1.43 ng/g SD: 0.63 ng/g Median: 1.35 ng/g Range: 0.65–3.09 ng/g Mean: 1.23 ng/g SD: 0.60 ng/g Median: 1.08 ng/g Range: 0.63–2.33 ng/g Mean: 1.53 ng/g SD: 0.90 ng/g Median: 1.42 ng/g Range: 0.45–3.87 ng/g	NR 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			

D.2.3.1.3 Distribution in Fetal Tissues

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), 1.0 (0.3–2.6), 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 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 (2003). This reduction results in increased flow of cord blood through the liver, thus increasing levels of PFOA and PFOS during the second trimester. Furthermore, Mamsen et al. (2019, 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.

D.2.3.1.4 Partitioning to Infants

Four studies shown in Table D-10 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) versus non-breastfeeding mothers (13.48 ng/mL). Conversely, breastfed infants had higher GM serum PFOS (13.54 ng/mL) than infants who were never breastfed (12.65 ng/mL).

Cariou et al. (2015, 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 D-10, 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 D-10. Summary of Human PFOS Concentrations in Maternal Serum, Breast Milk, and Infant Serum

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Mondal, 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
Morgensen 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: <u>median</u> : 6.0 ng/mL (IQR 5.2, 7.2) 11 months: <u>median</u> : 23.2 ng/mL (IQR 14.9, 34.7) 18 months: <u>median</u> : 24.0 ng/mL (IQR 20.2, 29.1) 60 months: <u>median</u> : 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	Primiparae 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

GM = geometric mean; CI = confidence interval; IQR = interquartile range; SD = standard deviation; PFAA = perfluoroalkyl acid; NR = not reported.

^aNeonatal 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, L. L.; Grandjean, P.; Heinzow, B.; Jorgensen, P. J.; Nielsen, F.; Patterson, D. G., Jr.; Sjodin, A.; Turner, W. E.; Weihe, P. Partition of environmental chemicals between maternal and fetal blood and tissues. *Environ. Sci. Technol.* 2011, 45 (3), 1121–1126.

Mondal et al. (2014, 2850916) also examined the change in maternal and infant PFOS levels with duration of breastfeeding (Table D-11). 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 D-12), 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 versus 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 D-11. Percent Change in PFOS Ratios in Human Maternal Serum and Breast Milk and Breast Milk and Infant Serum by Infant Age

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 D-12. Percent Change in Human PFOS Serum Concentration by Exclusive, Mixed or No Breastfeeding Per Month

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 PFAS-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.

D.2.3.2 Animal Studies

D.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 20 {Luebker, 2005, 1276160}. PFOS levels were analyzed in serum, liver, urine, and feces samples in dams and fetuses as indicated in Table D-13. 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 D-13. Liver, Serum, Urine, and Feces PFOS Concentrations in Pregnant Sprague-Dawley Dams and Fetuses

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	–

Parameter	Dose (mg/kg/day)	GD 1	GD 7	GD 15	GD 21	
		Dams	Dams	Dams	Dams	Fetuses
	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	–

GD = gestation day.

^aData presented in mean ± standard deviation (µg/mL)

^bData presented in mean ± standard deviation (µg/g)

This same study also included a subset of dams allowed to litter naturally and dosed through LD4. 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, 0.1, 0.3, or 1.0 mg/kg/day potassium PFOS by gavage from GD0 through PND20. 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 section 3.2.6 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 D-14).

Table D-14. Serum, Liver, and Brain Tissue PFOS Concentrations of Sprague-Dawley Dams and Offspring

Time	Dose (mg/kg)	Serum PFOS ^a		Liver PFOS ^b		Brain PFOS ^b	
		Dam	Offspring	Dam	Offspring	Dam	Offspring
GD20 ^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
PND4 ^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
PND21	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)	NS	5.980 ± 0.614 (M)	NS	0.220 ± 0.014 (M)
			1.771 ± 0.076 (F)		5.278 ± 0.174 (F)		0.229 ± 0.011 (F)
	0.3	8.981 ± 0.275	5.048 ± 0.108 (M)	NS	14.780 ± 0.832 (M)	NS	0.649 ± 0.053 (M)
5.246 ± 0.138 (F)			13.550 ± 0.298 (F)		0.735 ± 0.039 (F)		
1.0	30.480 ± 1.294	18.611 ± 1.011 (M)	NS	44.890 ± 2.637 (M)	NS	2.619 ± 0.165 (M)	
		18.010 ± 0.744 (F)		41.230 ± 2.295 (F)		2.700 ± 0.187 (F)	
PND72	Control	NA	< LLOQ (M/F)	NA	< LLOQ (M/F)	NA	NS (M/F)
	0.1	NA	0.042 ± 0.004 (M)	NA	0.981± 0.091 (M)	NA	NS (M/F)
			0.207 ± 0.042 (F)		0.801 ± 0.082 (F)		
	0.3	NA	0.120 ± 0.009 (M)	NA	2.464 ± 0.073 (M)	NA	NS (M/F)
0.556 ± 0.062 (F)			2.252 ± 0.095 (F)				
1.0	NA	0.560 ± 0.105 (M)	NA	7.170 ± 0.382 (M)	NA	NS – M/F	
		1.993 ± 0.293 (F)		7.204 ± 0.414 (F)			

< LLOQ = sample less than lower limit of quantification; GD = gestation day; PND = postnatal day; NS = no sample obtained; NA = not applicable; M = male; F = female.

^aData presented as mean ± standard deviation (µg/mL).

^bData presented as mean ± standard deviation (µg/g).

^cData are from samples pooled by litters in the fetuses/pups.

On GD20, 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 GD20, 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 GD20. 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 PND4 and continued to drop through PND72. However, based on the concentrations still present in the neonate serum, lactational transfer of PFOS was occurring. At PND72, 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 PND72. Liver values were similar at all timepoints between males and females. On GD20, the brain levels for the pups were tenfold higher than those for the dam. The levels in pup brains gradually declined between PND4 and PND21.

Ishida et al. (2017, 3981472) also examined distribution to livers and brains in Wistar rat dams and pups on PND4. Tissue-to-plasma partition coefficients (K_p s) 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, 0.1, 0.6, or 2.0 mg/kg/day of PFOS by oral gavage in 0.5% Tween 80 from GD2 to GD21. On GD21, 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 PND21, 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 PND21 compared to PND0 (Table D-15).

Table D-15. Serum, Hippocampus, and Cortex PFOS Concentrations of Sprague-Dawley Rat Pups

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***

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.

^aData presented as mean ± standard deviation (µg/mL).

^bData 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 GD1 to GD21 at 0, 0.1, or 2.0 mg/kg/day. There was a postnatal decline in the serum and brain PFOS levels between PND0 and PND21. PFOS concentrations were higher in the serum when compared to the lung in offspring on both PND0 and 21 {Chen, 2012, 1276152} (Table D-16).

Table D-16. Serum and Lung PFOS Concentration of Sprague-Dawley Rat Pups

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**

ND = not detected; PND = postnatal day.

*p < 0.05 compared with control.

**p < 0.01 compared with control.

^aData presented as mean ± standard deviation (µg/mL).

^bData presented as mean ± standard deviation (µg/g).

D.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 GD16 to C57Bl/6 dams. Using whole-body autoradiography and liquid scintillation, counting distribution of PFOS was determined for the dams/fetuses (GD18 and 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 GD18 (3 times higher than maternal levels). In the fetuses on GD18, values in the lungs were similar to the maternal lungs, and this value increased by GD20. 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 PND1 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 PND1 were similar to their respective dams despite being higher in fetuses on GD18. 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 D-17.

Table D-17. Concentration Ratios of ³⁵S-PFOS Maternal Serum to Various Organs of C57BL/6 Mouse Dams, Fetuses, and Pups

Group	[³⁵ S-PFOS] _{organ} /[³⁵ S-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 GD18	2.6 ^{**} ± 0.8	2.1 [*] ± 0.6	2.8 ^{**} ± 0.3	1.2 ± 0.3	2.3
Fetuses on GD20	2.4 ^{**} ± 0.5	2.5 ^{**} ± 0.4	1.4 ± 0.2	0.9 ± 0.1	1.1 ± 0.04
Pups on PND1	2.4 [*] ± 0.4	3.0 ^{**} ± 0.5	1.0 ± 0.5	0.9 ± 0.2	1.7 ^{**} ± 0.3

³⁵S-PFOS = ³⁵S-radioisotope perfluorooctance sulfonic acid; GD = gestation day; PND = postnatal day.

^{*}Statistically-significant (p ≤ 0.01) in comparison to maternal blood.

^{**}Statistically-significant (p ≤ 0.001) in comparison to maternal blood.

^aData presented as mean ± standard deviation (μg/g).

^bData presented as mean ± standard deviation (μg/mL).

Male and female KM mice were administered PFOS by subcutaneous injection one time on PNDs 7, 14, 21, 28, or 35 at concentrations of 0 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 D-18. The distribution shows that, beyond PND14, the levels in the liver are approximately 2–4 times greater than those found on PND7.

Table D-18. Percent Distribution of PFOS in Male and Female KM Mice After 50 mg/kg Subcutaneous Injection

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 ^{**}

PFOS = perfluorooctance sulfonic acid; PND = postnatal day

^{**}Statistically significant from PND 7 (p < 0.01).

^aData presented as mean percentage ± standard deviation (μg/mL).

^bData presented as mean percentage ± standard deviation (μg/g).

D.2.4 Volume of Distribution

D.2.4.1 Human Studies

None of the available studies provide data for calibration of V_d of PFOS in humans. However, several researchers have attempted to characterize PFOS exposure and intake in humans (Thompson, 2010, 2919278; Egeghy and Lorber, 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 and Lorber, 2011, 723765). The models developed were designed to estimate intakes of PFOS by young children and adults (Egeghy and Lorber 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 D-19. 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 fractions.

Table D-19. 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 mL/kg	Reports an average of human and animal V _d values	Authors note that due to declining values in U.S. and Australian populations, steady state was not achieved.

V_d = volume of distribution; AUC = area under the curve; GM = geometric mean.

D.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 200–300 mL/kg.

Two recent studies in rats (Kim et al., 2016, 3749289; Huang et al., 2019, 5387170) measured toxicokinetic parameters including V_d (Table D-20). In the Kim et al. (2016, 3749289) study, V_d values were calculated as $\text{Dose} \times \text{AUMC} / (\text{AUC}_{0-\infty})^2$, 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 than those observed in the liver for both males and females.

Huang et al. (2019, 5387170) calculated the apparent volume of central (V1) and peripheral (V2) 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 D-20, 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 (244–280 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 $BD/\text{elimination rate constant (ke)} \times \text{plasma concentration (AUC)}$. 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 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_d s 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.

Unlike the sex differences observed in rats, V_d calculations were similar in male and female monkeys as shown in Table D-21 {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 (11–17.2 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 D-20. 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 \times AUMC/(AUC0- ∞) ²	IV	2 mg/kg	Sprague-Dawley	8–12 weeks	Males	382.55 \pm 17.59 mL/kg	Blood Plasma	AUC: 216.47 \pm 8.63 μ g day/mL	5.23 \pm 0.24 μ g/mL	NR
						Females	351.50 \pm 19.20 mL/kg	Blood Plasma	AUC: 203.60 \pm 8.42 μ g day/mL	5.69 \pm 0.33 μ g/mL	NR
		Oral	2 mg/kg	Sprague-Dawley	8–12 weeks	Males	279.81 \pm 16.71 mL/kg	Blood plasma	AUC: 272.69 \pm 20.39 μ g day/mL	6.71 \pm 0.30 μ g/mL	NR
						Females	288.97 \pm 15.59 mL/kg	Blood Plasma	AUC: 234.61 \pm 10.05 μ g day/mL	6.66 \pm 0.29 μ g/mL	NR
Huang et al., 2019, 5387170	Standard equations Gabrielsson et al., 2000, 9642135	IV	2 mg/kg	Sprague-Dawley	8 weeks	Males	417 \pm 31 mL/kg	Central	AUC: 7.32 \pm 0.42 μ M-hr	0.01 \pm 0.01 mM	NR
							264 \pm 71 mL/kg	Peripheral	AUC: 7.32 \pm 0.42 μ M-hr	0.01 \pm 0.01 mM	NR
						Females	297 \pm 43 mL/kg	Central	AUC: 10.72 \pm 0.78 μ M-hr	0.01 \pm 0.01 mM	NR
							124 \pm 62 mL/kg	Peripheral	AUC: 10.72 \pm 0.78 μ M-hr	0.01 \pm 0.01 mM	NR
		Oral	2mg/kg	Sprague-Dawley	8 weeks	Males	280 \pm 48 mL/kg	Central	AUC: 9.86 \pm 0.74 μ M-hr	0.01 \pm 0.01 mM	NR
							244 \pm 81 mL/kg	Peripheral	AUC: 9.86 \pm 0.74 μ M-hr	0.01 \pm 0.01 mM	NR
						Females	222 \pm 84 mL/kg	Central	AUC: 17.74 \pm 1.02 μ M-hr	0.02 \pm 0.01 mM	NR
							93.4 \pm 93 mL/kg	Peripheral	AUC: 17.74 \pm 1.02 μ M-hr	0.02 \pm 0.01 mM	NR
			2 mg/kg (x5d)	Sprague-Dawley	8 weeks	Males	176 \pm 27 mL/kg	Central	AUC: 58.18 \pm 3.00 μ M-hr	0.11 \pm 0.01 mM	NR
							123 \pm 42 mL/kg	Peripheral	AUC: 58.18 \pm 3.00 μ M-hr	0.11 \pm 0.01 mM	NR
						Females	136 \pm 25 mL/kg	Central	AUC: 89.18 \pm 5.00 μ M-hr	0.14 \pm 0.02 mM	NR
							86.3 \pm 37.3 mL/kg	Peripheral	AUC: 89.18 \pm 5.00 μ M-hr	0.14 \pm 0.02 mM	NR
			20 mg/kg	Sprague-Dawley	8 weeks	Males	34.6 \pm 4.8 mL/kg	Central	AUC: 149.76 \pm 10.60 μ M-hr	AUC: 0.21 \pm 0.03 μ M-hr	NR
							43.9 \pm 7.7 mL/kg	Peripheral	AUC: 149.76 \pm 10.60 μ M-hr	AUC: 0.21 \pm 0.03 μ M-hr	NR
						Females	27.9 \pm 4.7 mL/kg	Central	AUC: 213.94 \pm 16.00 μ M-hr	AUC: 0.27 \pm 0.03 μ M-hr	NR
							27.5 \pm 6.5 mL/kg	Peripheral	AUC: 213.94 \pm 16.00 μ M-hr	AUC: 0.27 \pm 0.03 μ M-hr	NR
Iwabuchi et al., 2017, 3859701	Dose / elimination rate constant (ke) \times plasma concentration (AUC).	Oral	100 μ g/kg	Wistar	7–9 weeks at start of exposure	Males	7.9 kg tissue volume/kg BW	Brain	180 μ g/kg tissue volume - day	9.17 μ g/kg tissue volume	NR
							4.5 kg tissue volume/kg BW	Heart	380 μ g/kg tissue volume - day	27.7 μ g/kg tissue volume	NR
							0.043 kg tissue volume/kg BW	Liver	240000 μ g/kg tissue volume - day	2730 μ g/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

V_d = volume of distribution; AUC = area under the curve; C_{max} = Maximum concentration achieved; BW = body weight; NR = not reported.

Table D-21. Pharmacokinetic Parameters After Acute PFOS Exposure in Cynomolgus Monkeys^a

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

T_{1/2} = half-life (time); K_{e1} = elimination rate per day, Cl = clearance, V_d = volume of distribution; AUC/dose = area under the curve per dose.

^aData presented in mean ± standard deviation.

D.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.

D.4 Excretion

D.4.1 Urinary and Fecal Excretion

D.4.1.1 Human Studies

Three major studies highlight the urinary excretion of PFOS in humans. T. 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 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:

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

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.

D.4.1.2 Animals 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 ($76.13 \pm 16.83 \mu\text{g}$) and feces ($61.65 \pm 7.29 \mu\text{g}$) were similar after oral administration. After intravenous dosing, urine levels in males ($103.04 \pm 21.56 \mu\text{g}$) were more than 2-fold higher than fecal levels ($43.73 \pm 5.29 \mu\text{g}$). Females also excreted higher levels in urine compared to feces by both dosing routes. After oral administration, urine and fecal levels were $95.42 \pm 22.14 \mu\text{g}$ and $53.29 \pm 8.64 \mu\text{g}$, respectively. Similar values in urine ($88.29 \pm 14.91 \mu\text{g}$) and feces ($48.37 \pm 4.98 \mu\text{g}$) 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, 5, 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, 0.05, 0.5, 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, 0.5, 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. (2005b, 1276160), in which five groups of 16 dams each 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 GD14 or 20. Urine and feces were collected overnight from dams on the eve of cohabitation day 1 and during GDs 6–7, 14–15, and 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.

D.4.2 Physiological and Mechanistic Factors Impacting Excretion

D.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.

Y. Zhang et al. (2013, 3859849) determined half-lives for PFOA 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.

D.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 [¹⁴C]PFOS. CSM is a bile acid sequestrant, and its facilitation of PFOS gastrointestinal 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 gastrointestinal (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 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 D-22 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. NTCP transported PFSA's 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 versus long chained perfluoroalkyl sulfonates. While these in vitro studies demonstrate that PFOS is a substrate of enterohepatic transporters found in the livers and intestines of humans and rats, it is as unknown whether and to what extent these transporters function in vivo.

Table D-22. Enterohepatic Transporters of PFOS

Human Transporters			Rat Transporters	
Organ	Liver	Intestine	Liver	Intestine
Cell type	Hepatocyte	Enterocyte	Hepatocyte	Enterocyte
Sodium-dependent (Zhao et al., 2015, 3856550)	NTCP	ASBT	NTCP	
Sodium- independent (Zhao et al., 2017, 3856461)	OATP1B1 ^a	OATP2B1 ^a	OATP1A1 ^a	OATP1A5
	OATP1B3 ^a		OATP1B2	OATP2B1
	OATP2B1 ^a		OATP2B1	

NTCP = Na⁺/taurocholate cotransporting polypeptide; ASBT = human apical sodium-dependent bile salt transporter; OATP = organic anion transporting polypeptide.

^aTransporter examined in transfection studies; PFOA also shown to be a substrate of these transporters in HEK293 cells transiently transfected with cDNA constructs encoding these transporters (Zhao 2015, Zhao 2017a).

D.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 D.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 D.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 PFASs in 39 Danish women who underwent legal termination of pregnancy before gestational week 12 {Mamsen, 2017, 3858487}. All PFASs 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, 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 PFOA 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 36.4–63.8 ng/L) and the median concentration for all PFAS chemicals measured was 151 ng/L (range of 105–212 ng/L). Pooled breast milk samples were measured to follow the time course of PFOS in breast milk after birth. Concentrations in breast milk measured 30 days after birth were significantly higher than those measured prior to 7 days after birth. These findings are contrast with results of Thomsen et al. (2010, 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 to –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 Toulouse, 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 sex differences observed for blood and urinary levels of PFOA by sex and age.

D.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 et al. (2015, 2850226) suggested a need to consider the nonblood 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 et al. (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 from both groups showed lower PFOA and PFOS concentrations than predicted based on blood loss. Estimated concentrations based on a 1-compartment models were consistent with measured concentrations. These authors suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may 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, 0.05, 0.5, 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 to 2086 ng/g in 0.05 and 5 mg/L treatment groups, respectively. Notably, the PFOS concentration in hair was significantly higher than the levels of PFOA (3.31–444 ng/g) and PFNA (14.2–1,604 ng/g) at 0.05 to 5 mg/L doses. Unlike PFOA and PFNA which showed a sexual 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 and 5 mg/L dose.

Gao also measured the composition of the mixture excreted in urine, feces, and hair after administration of 0.5 or 0.05 mg/mL of a mixture of PFAS (Table D-23). At the lower dose of 0.05 mg/mL, 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 D-23. Estimated Percentage of the Sum of PFOS, PFNA, and PFOA in Excreta and Serum^a

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

PFOS = perfluorooctane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; ND = not detected.

^aDose of 0.05 mg/kg.

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 sequesterants. With the exception of PHxS, 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 PFOA excretion by all possible routes, and to establish factors that influence the proportion of PFOA excreted through urine versus other excreta matrices.

D.4.5 Half-life Data

D.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 BW changes). The calculation of PFOA half-lives reported in the literature vary considerably posing 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 PFOA to be reabsorbed via renal and enterhepatic routes of excretion and binding affinities to relevant transporters including OATs, OATPs, MRPs, and sodium-dependent transporters involved in bile acid transport including sodium/taurocholate cotransporting polypeptide (NTCP) and the apical sodium-dependent bile acid transporter (ASBT). Exposures to high levels of PFOA 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 enterhepatic 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 PFOA 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 PFOA 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 PFOA 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}.

D.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 Company, 2000, 8568548; Burris, 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: 0.037–1.740 µg/mL). Semi-log graphs of concentration versus time for each of the 26 individuals were created, and individual serum elimination half-lives were determined using first-order elimination. The arithmetic and geometric mean serum elimination half-lives of PFOS were 5.4 years (95% confidence interval [CI]: 3.9–6.9 years) and 4.8 years (95% CI: 4.1–5.4 years), respectively.

The rate of serum PFOS decline was measured in residents of two communities exposed to contaminated municipal drinking water contaminated in Bleking county, Sweden in 2013 {Li, 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 year) 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 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} to 0.54 mL/kg/day (Geometric mean of 0.010 mL/kg/day).

Half-lives were calculated by $\ln 2/k$ 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 PFOA 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, the Agency for Toxic Substances and Disease Registry (ATSDR) collected blood samples from subjects and followed up with blood and urine measurements in 2016. Biological half-lives were estimated for PFOA, PFOS using a one-compartment pharmacokinetic model.

Geometric mean serum PFOA concentrations were significantly higher in subjects in both 2010 (16.3 µg/L) and 2016 (11.7 µg/L) relative to national averages reported by NHANES (3.07 µg/L in 2009-2010 and 1.94 µg/L in 2013-2014). Interestingly, the authors observed a non-significant relationship between PFOA serum and urine concentrations in women (n = 23, Pearson's r = 0.35) and a significant strong linear relationship in men (n = 22, Pearson's r = 0.75).

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 $\pm 20\%$, 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 compounds 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 contaminated water was 0.62 for linear PFOS and 0.64 for branched PFOS. Specific gravity adjusted urine levels for PFOS were generally below detectable limits for linear and branched forms of PFOS with respective median 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 (linear) and 100 (branched). PFOS levels measured in paired serum and urine samples obtained from the second to the fifth sampling was 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). 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 PFOA half-lives along with measured intake and serum and urine PFOA concentrations {Xu, 2020, 6781357; Worley, 2017, 3859800; Fu, 2016, 3859819; Zhang et al., 2013, 2639569} (Table 24. PFOA half-life values among these 4 studies varied from 1.7 in Xu et al. (2020, 6781357) to 4.7 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 females which may, at least in part, relate to menstruation as routes of elimination {Zhang, 2013, 3859849}. Second, blood and urine concentrations varied by several orders of magnitude across these 4 studies. While blood and urine PFOA concentrations varied by two orders of magnitude across these studies, half-life estimates were similar, ranging from 1.77 to 4.70 years. This variability in serum and urine

concentrations may reflect the role of non-urinary routes of excretion and the difficulty in measuring renal resorption. Finally, only two studies estimated PFOA intake in subjects {Xu, 2020, 6781357}{Worley, 2017, 3859800}. Altogether, there is insufficient data to correlate PFOA 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 PFOA half-life estimates in humans.

Table D-24. 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, 6315709	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	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • PFOS was detected in 45.7% of samples. LOD was 0.02 μg/L • Estimate intake rate for PFOS was 6 ng/h , based on PFOS drinking water concentration of 0.12 μg/L, Volume of distribution of PFOS was reported as 230 mL/kg body weight. • Clearance rate was not reported

Study	Number of Subjects	Age Range	Primary Exposure Route	Intake	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half Life (y)	Considerations
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 5624 ng/mL Median 1725 ng/mL (50.3–118000 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	<ul style="list-style-type: none"> • 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 47ng/g creatinine Median 28 ng/g creatinine (range 2.8–232 ng/g creatinine)	Young females: 6.2 Males and older females: 27	<ul style="list-style-type: none"> • All participants had paired (whole blood/serum and urine) • For young females menstrual clearance was estimated and added to renal clearance. • Renal clearance rate for total PFOS: mean 0.050 mg/kg/day (young female), 0.037 mg/kg/day (male and older female)

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

All human PFOS half-life values identified in the literature review are provided in Table D-25. 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 isoforms exhibit longer half-lives than branched isoforms {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 (2.3y) 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 to 0.00241 µg/mL. The study grouped the blood spots by two different time-points; those collected in 1999–2000 and in 2003–2004, which corresponded to the intervals reported by NHANES. The PFOS concentrations decreased with a mean value of 0.00243 µg/mL reported in 1999–2000 and 0.00174 µg/mL in 2003–2004. The study authors determined the half-life of PFOS using the regression slopes for natural log blood concentrations versus the year 2000 and after. The calculated half-life for PFOS was 4.1 years.

Table D-25. Summary of Human PFOS Half-Life Values

Study	Number of Subjects	Age Range	Estimated Half-Life (y)	Subjects
Burris et al., 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, 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 the National Health and Nutrition Survey (NHANES) from the USA. 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
Spiltehoff 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, 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 the US National Health and Nutrition Examination Survey. (NHANES). Data from age-stratified biomonitoring data for PFOS extracted from US 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	Subjects in Arvidsjaur, Sweden exposed to contaminated drinking water occupationally

Study	Number of Subjects	Age Range	Estimated Half-Life (y)	Subjects
			2/6m-PFOS: 1.04	(working at the airport) and through residential drinking water
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	Σ 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 Σ m2-PFOS young females: 5.1 males and older females: 14	Healthy volunteers in Shijiazhuang and Handan, Hebei province, China, in April–May 2010

PFOS = perfluorooctane sulfonic acid; PFAA = Per- and polyfluoroalkyl substances; ATSDR = Agency for Toxic Substances and Disease Registry.

D.4.5.3 Animal Studies

D.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 versus females. Serum elimination half-lives ranged 122–146 days in male monkeys and 88–138 days in females. Mean values are shown in Table D-26. 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.

D.4.5.3.2 Rats and mice

Half-lives rodents are very short relative to those observed in humans and primates (Table D-26). 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 2mg/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.

1 **Table D-26. 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-PFOS: 11.1
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
Huang et al., 2019, 5387170	Rat, Sprague-Dawley	IV	8 weeks		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
				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
Kim et al., 2016, 3749289	Rat, Sprague-Dawley	IV	8–12 weeks	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
		Oral	8–12 weeks	Male	2 mg/kg	28.70 ± 1.85
				Female	2 mg/kg	24.80 ± 1.52
				Male	2 mg/kg	26.44 ± 2.77
				Female	2 mg/kg	23.50 ± 1.75

1 IV = intravenous; NR = not reported.

2 ^aData reported in mean days ± standard deviation.

Appendix E. Pharmacokinetic Modeling

E.1 Comparison of Fits to Training Datasets Used in Wambaugh et al., 2013

The following figures show comparisons of the model predicted serum concentrations to the data used for model training. Fits also presented in supplemental material of {Wambaugh, 2013, 2850932}.

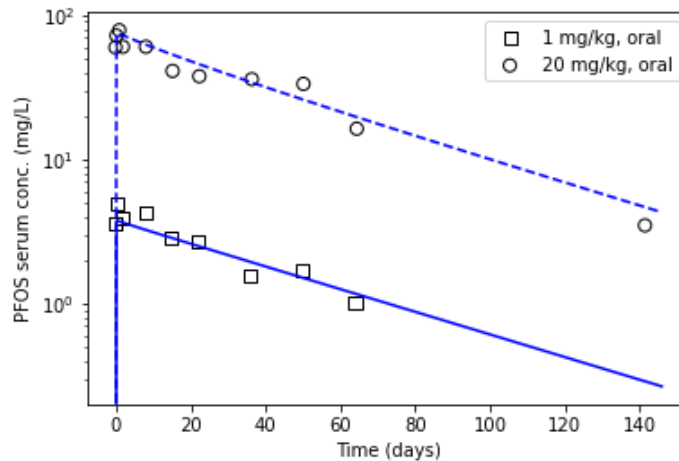


Figure E-1. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Female CD1 Mice^a

^a 1 mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

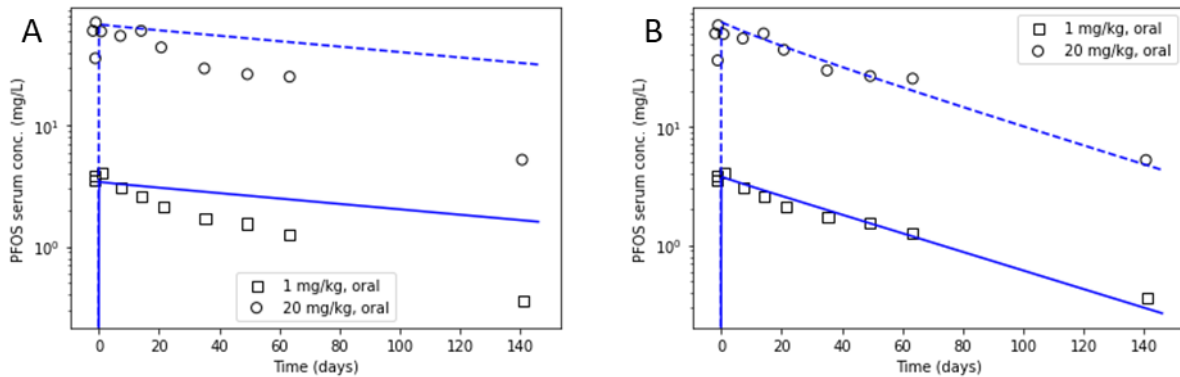


Figure E-2. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single Oral Dose of 1 or 20 mg/kg PFOS to Male CD1 Mice^a

A) Fits to observed male data using male-specific model; B) Fits to observed male data using female-specific model parameters.

^a 1 mg/kg represented by the squares and solid line; 20 mg/kg represented by the circles and dashed line.

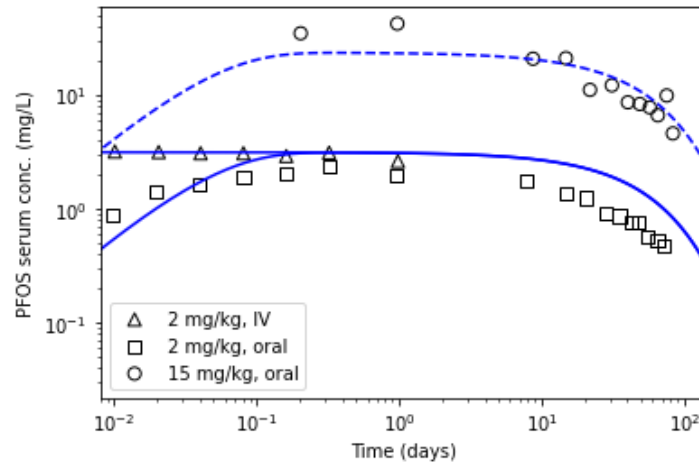


Figure E-3. Experimentally Observed Serum Concentrations {Chang, 2012, 1289832} and Median Prediction for a Single IV Dose of 2 mg/kg or a Single Oral Dose of 2 or 15 mg/kg PFOS to Male Sprague-Dawley Rats^a

^a 2 mg/kg 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.

E.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, 2013, 2850932} parameterization.

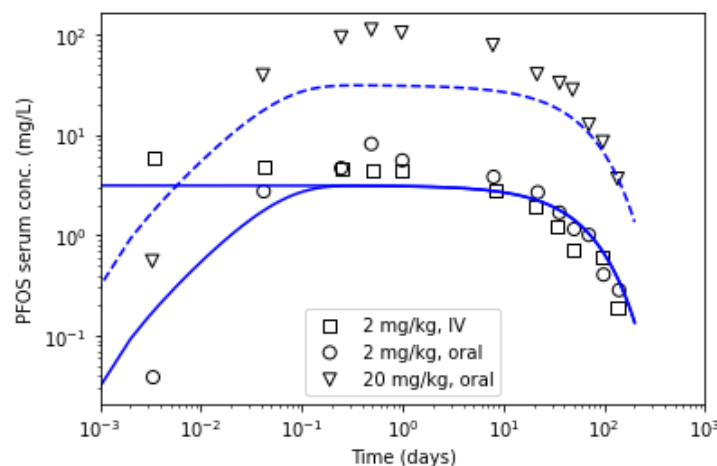


Figure E-4. Experimentally Observed Serum Concentrations {Huang, 2019, 7410147} and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Male Sprague-Dawley Rats^a

^a 2 mg/kg 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.

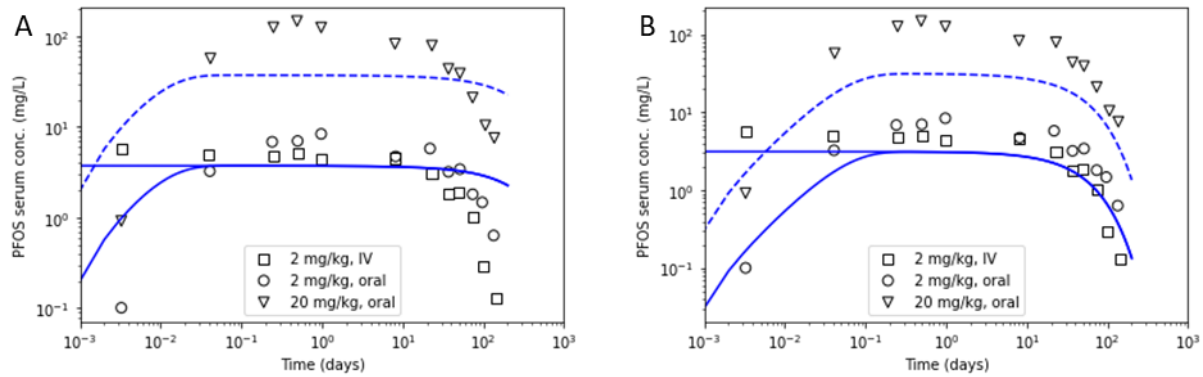


Figure E-5. Experimentally Observed Serum Concentrations {Huang, 2019, 7410147} and Median Predictions for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 or 20 mg/kg PFOS to Female Sprague-Dawley Rats^a

A) Fits to observed female data using female-specific model parameters; B) Fits to observed female data using male-specific model parameters.

^a 2 mg/kg 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.

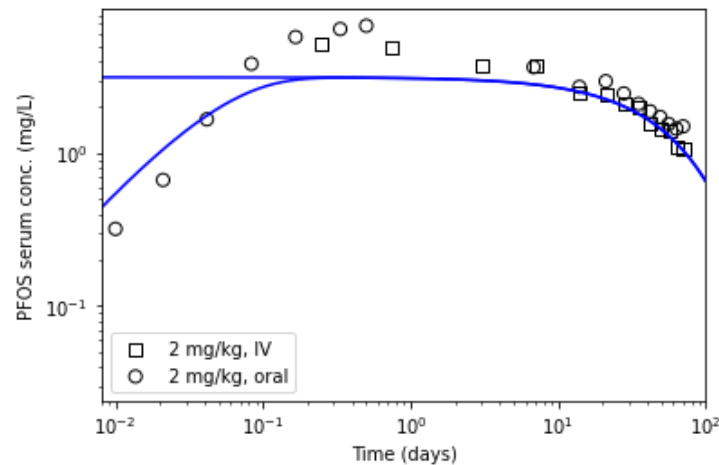


Figure E-6. Experimentally Observed Serum Concentrations {Kim, 2016, 3749289} and Median Prediction for a Single IV Dose of 2 mg/kg or an Oral Dose of 2 mg/kg PFOS to Male Sprague-Dawley Rats^a

^a 2 mg/kg IV dose represented by the squares; 2 mg/kg oral dose represented by the circles.

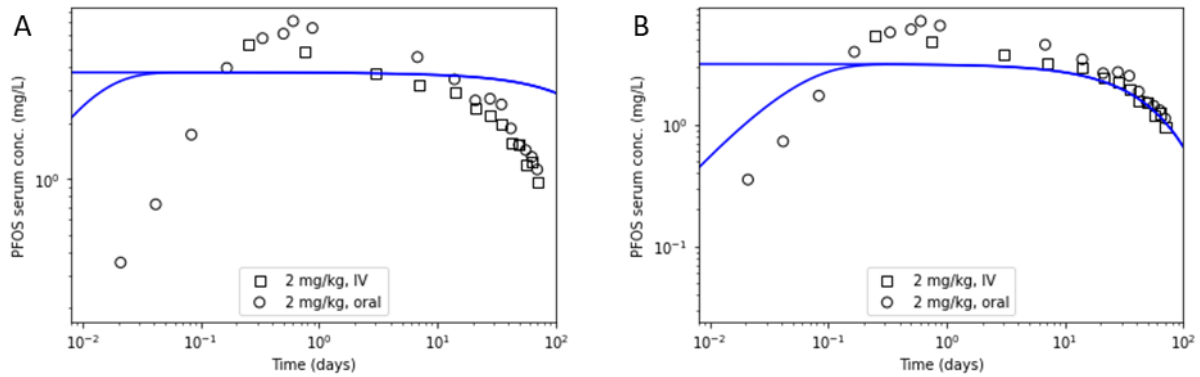


Figure E-7. Experimentally Observed Serum Concentrations {Kim, 2016, 3749289} and Median Prediction for a Single IV Dose of 2 mg/kg an Oral Dose of 2 mg/kg PFOS to Female Sprague-Dawley Rats

A) Fits to observed female data using female-specific model parameters; B) Fits to observed female data using male-specific model parameters.

^a 2 mg/kg IV dose represented by the squares; 2 mg/kg oral dose represented by the circles.